Contamination of game carcasses during harvesting and slaughter operations at a South African abattoir

by

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: December 2015

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Dedication

This study is dedicated to:

- my late father, Lawrence Shange
- my late brother, Sifiso Shange

Summary

The consumption of game meat and its by-products is increasing locally and internationally. The increase in consumption requires research that is focused on the microbiological quality of game meat. The harvesting and slaughter process of springbok carcasses revealed the presence of bacterial contamination. Swab samples taken after skinning portrayed a presence of *Escherichia coli* (*E. coli*) and *Enterobacteriaceae*. Springbok carcasses swabbed after chilling indicated aerobic bacteria, *Clostridium spp*. and lactic acid bacteria. In contrast, swab samples taken at the evisceration's incision area tend to be lower in counts when compared to swab samples taken after skinning and after chilling. Bacterial contamination was linked to poor hygienic practices during the harvesting and slaughter process. Results showed a need for the investigation of the slaughter process. To evaluate the slaughter process's impact on the microbial quality of game carcasses, black wildebeest (*Connochaetes gnou*) carcasses were sampled throughout the slaughter process. Before skinning, aerobic bacteria, *Enterobacteriaceae*, and *E. coli* were enumerated from hide samples, counts ranged from 0.92 to 7.84 log cfu/g. after skinning, bacterial counts ranged from 0.93 to 6.12 log cfu/g and further decreased after chilling. *Clostridium* spp. counts increased after skinning, however, statistical analysis detected no significant differences between counts. *Salmonella* spp. was not detected. The results indicate that bacterial contamination does occur during the slaughter process. Hygienic status during the production of game meat products was also determined. Bacterial counts from raw game meat ranged from 2.37 to 5.37 log cfu/g. Counts as high as 6.16 log cfu/g were enumerated from retail products. Aerobic plate counts (APC) from ≤ 2.62 log cfu/cm^2 to ≤ 6.3log cfu/cm^2 were enumerated from surfaces, hands and equipment during production. Results highlighted the inefficiency of cleaning procedures and revealed that contaminated meat can allow for bacterial contamination. To determine if muscle pH influences colour stability and microbial spoilage of game meat, normal (n=6) and dark, firm and dry (DFD) (n=6) black wildebeest *Longissimus thoracis et lumborum* (LTL) muscles were studied. pH affected colour, as initial (day 0) L*,a*,b*,C* and H_{ab} values from Normal pH samples were significantly higher than values reported for DFD samples. Initial APC and *Enterobacteriaceae* counts from samples with Normal pH were not significantly different from counts reported for DFD samples. Initial contamination was linked to the harvesting and slaughter process. Further refrigeration (5±1ºC) for 12 days in an aerobic environment and analyses of samples every third day revealed that pH did not affect lightness and brownness as L* and b* values for DFD samples did not significantly differ overtime, the same trend was seen for samples with Normal pH. Normal pH samples showed a significant increase in a* and C* values until day 12, whilst H_{ab} values decreased until the 12th day. The same trend was seen for a* and C* values for DFD samples until the 9th day as on the 12th day values increased. Similarly, H_{ab} values for DFD samples decreased until the 9th day, then increased on the 12th day. Using the microbial spoilage limit of 6 log cfu/g, it was seen that DFD meat reached this limit earlier than samples with Normal pH. Overall, the study provides baseline information on

the microbiological quality of game meat harvested in South Africa and slaughtered at a South African abattoir.

Opsomming

Die plaaslike en internasionale verbruik van wildsvleis en wildsvleisprodukte is aan't toeneem. Hierdie toename in verbuik vereis navorsing wat gefokus is op die mikrobiese kwaliteit van wildsvleis. Die oes-en slagproses van springbok karkasse het die teenwoordigheid van bakteriese kontaminasie aan die lig gebring. Monsters geneem met 'n depper na afslag van karkasse het 'n teenwoordigheid van *Escherichia coli* (*E. coli)* getoon. Springbok karkasse wat getoets is na verkoeling het hoë vlakke van die aërobiese bakterium *Clostridium spp*. en van melksuurbakterieë getoon. In teenstelling hiermee is getalle laer rondom die ontweidings insnyding. Bakteriese kontaminasie was gekoppel aan swak higiëne gedurende die oes- en slagproses. Hierdie resultate het 'n ondersoek van die slagproses aangemoedig. Om die impak van die slagproses op die mikrobiese kwaliteit van wildskarkasse te evalueer, is monsters regdeur geneem van swartwildebees (*Connochaetes gnou)*. Getalle van aërobiese bakterieë, *Enterobacteriaceae,* en *E. coli* was bepaal op vel monsters voor afslag; getalle het gewissel tussen 0.92 en 7.84 log cve/g. Getalle van bakterieë na afslag het gewissel tussen 0.93 en 6.12 log cfu/g, en het verder afgeneem na verkoeling. *Clostridum* spp*.* het toegeneem na afslag, maar statistiese analises het geen beduidende verskille getoon nie. Monsters het negatief getoets vir *Salmonella* spp*.* Die resultate toon aan dat bakteriese kontaminasie wel plaasvind gedurende die slagproses. Die higiëniese status gedurende die produksie van wildsvleis is ook vasgestel. Bakteriegetalle van rou wildsvleis het gewissel tussen 2.37 log cve/g en 5.37 log cve/g. Getalle van handelsprodukte het getalle getoon van soveel as 6.16 log cve/g. Aërobiese plaat telling tussen ≤2.62 cve/cm^2 en ≤ 6.3log cve/cm^2 is vasgestel vanaf oppervlakte, hande en toerusting gedurende produksie. Resultate beklemtoon die ondoeltreffendheid van skoonmaakprosedures en wys dat aangetaste vleis bakteriese kontaminasie kan toelaat. Om te bepaal of die kleurstabiliteit en mikrobiese bederf van wildsvleis geaffekteer word deur spiere se pH, is normale (n=6) en donker, ferm, en droë (DFD) (n=6) *Longissimus thoracis et lumborum* (LTL) spiere van die swartwildebees bestudeer. Kleur was geaffekteer deur vleis pH, siende dat die aanvanklike waardes (dag 0) vir L*, a*, b*, C* en Hab aansienlik hoër was vir monsters met normale pH as DFD monsters. Aanvanklike getalle van aërobiese plaat telling en *Enterobacteriaceae* telling van monsters met Normale pH het nie beduidend verskil van DFD monsters nie. Aanvanklike besmetting was gekoppel aan die oes- en slagproses. Verdere verkoeling (5±1^{o}C) vir 12 dae in 'n aërobiese omgewing en analise van monsters wys dat pH nie ligtheid en bruinheid affekteer nie; waardes vir L* en b* vir DFD monsters het nie beduidend verskil oor tyd nie. Dieselfde geld vir monsters met Normale pH. Monsters met Normale pH het 'n beduidende toename in a* en C* getoon tot en met dag 12, terwyl waardes vir Hab afgeneem het tot en met dag 12. Dieselfde patroon is waargeneem by waardes vir a* en C* vir DFD monsters tot en met dag 9, terwyl dit toegeneem het op die 12de dag. Soortgelyk het Hab waardes vir DFD monsters afgeneem tot n met dag 9, en toegeneem op die 12de dag. Dit is ook gevind dat DFD vleis die limiet vir mikrobiese bederf (6 log cve/g) vroeër bereik as monsters met Normale pH. Die studie voorsien basis inligting

oor die mikrobiese kwaliteit van wildsvleis wat geoes is in Suid Afrika, en geslag is by Suid Afrikaanse slagpale.

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List of abbreviations

<	Less than
>	More than
˚C	Degrees Celsius
ANOVA	Analysis of variance
APC	Aerobic Plate Counts
cfu/cm^2	Colony forming unit per centimetre
cfu/g	Colony forming unit per gram
DFD	Dark, Firm and Dry
EEC	European Economic Community
EFSA	European Food Safety Authority
EHEC	Enterohemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
et al	And others
ETEC	Enterotoxigenic *Escherichia coli*
EU	European Union
FDA	Food and Drug Association
FMD	Foot and mouth disease
g	Gram
GMP	General Management Practices
GRAS	Generally Regarded As Safe
HACCP	Hazard Analysis Critical Control Point
KCl	Potassium Chloride
LAB	Lactic acid bacteria
LSD	Least significant difference
LTL	*Longissimus thoracis et lumborum*
MKTTn	Muller-Kauffmann-Tetrathionate
MSA	Meat Safety Act
MRS	De Man Rogosa Sharpe
Na-Iodoacetate	Sodium Iodoacetate
OIE	Office Internationaldes Epizooties
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
ROC	Receiver Operating Characteristic
RVS	Rappaport-Vassiliadis media with soya
S.D	Standard Deviation

S.E.	Standard Error
SOPs	Standard Operating procedures
SS	*Shigella/Salmonella*
TBX	Trypton Bile X-glucuronide
TSP	Trisodium phosphate
USA	United States of America
VPN	Veterinary Procedure Notice
VRBG	Violet Red Bile Glucose
WHO	World Health Organisation
XLD	Xylose Lysine deoxycholate

CHAPTER 1
Introduction

The term game is used to refer to animals not normally domesticated that can be hunted for human consumption (van Schalkwyk & Hoffman, 2010). Game species are found all over the world, in South Africa, the Meat Safety Act 40 of 2000 define game meat as 'blesbok (*Damaliscus dorcas philipsi*), blue wildebeest (*Connochaetes taurinus*), buffalo (*Syncerus caffer*), Burchell's zebra (*Equus burchelli*), crocodile (*Crocodylus niloticus*), eland (*Taurotragus oryx*), elephant (*Loxodonta africana*), gemsbuck (*Oryx gazela*), gray rhebok (*Pelea capreolus*), hippopotamus (*Hippopotamus amphibius*), impala (*Aepyceros melampus*), kudu (*Tragelaphus stepsiceros*), mountain reedbuck (*Redunca fulvorufula*), springbok (*Antidorcas marsupialis*) and zebra (*Diplodus trifasciatus*) (South Africa, 2000).

The consumption of game meat is increasing in South Africa and the world at large (Paulsen *et al.*, 2011). A reason for this increase in consumption is due to the fact that game products tend to be low in Kilojoules and cholesterol and are protein dense (Hoffman & Wiklund, 2006).

South Africa is a significant game meat importer, as South Africa imports up to 46% of its game meat (carcasses and/or game products) from Namibia (van Schalkwyk *et al.*, 2010). The export of game meat in South Africa is on a smaller scale. However, growth in exportation is predicted, particularly when the growth in the local industry is considered as well as the fact that the Office Internationaldes Epizooties (OIE) has lifted the ban (in 2014) on the export of game meat due to Foot-and-mouth disease (FMD). Table 1.1 gives an indication of game animals harvested in South Africa for the local and international markets. The export of zebra increased as zebra is immune to FMD.

Table 1.1 Game animals harvested in South Africa at the two major game meat abattoirs in the country for export and local market per year (Uys, 2015).

Company	T**ime period**	**Amount of species harvested per year**
Camdeboo meat processors	Prior to 2011	Springbok - 50 000
		Blesbok - 5 000
		Blue and black wildebeest – 2 000
		Kudu – 1 000
	2015	Zebra – 250 (for export)
Mosstrich	2008-2010	Zebra – 331
		Antelope – 40 336
	2011-2014	Other - 355
		Zebra – 964 (for export)

SAMIC (2009) also reported that 20% of fresh red meat consumed in South Africa during the winter is game meat alone, therefore game meat has a national and international demand.

Available research on game meat is mainly focused on the quality and nutritional value of game meat. There is an apparent need for research that is focused on assessing the microbial quality and safety of game meat. Lack of research in this area can be due to the lack of knowledge around the points of contamination from point of harvest to the actual slaughter process and during the processing of game meat products. This increasing demand for game meat directly correlates to the need of having microbiologically safe game meat and knowing more about the microbial quality of game meat. The microbiological safety of game meat depends on the microorganisms present on the hide, in the gastrointestinal tract and on the equipment used during the slaughter and harvesting process (Bell, 1997). During harvesting game animals are shot, bled out and eviscerated in the field, partially opened carcasses are then transported to a field depot. At the field depot the red offal is removed from carcasses for veterinary inspection. Lastly, carcasses are loaded and transported to a game processing facility, where they are slaughtered/processed further (van Schalkwyk & Hoffman, 2010). To ensure safe game meat, carcasses should be harvested, slaughtered and handled in a manner that decreases contamination and minimises bacterial load.

Microbial contamination is the exposure of food products (such as meat) to harmful microorganisms. Exposed/contaminated food items are deemed unsafe for human consumption, as consumption will lead to severe illnesses. Illnesses due to food contamination is a wide spread international problem according to the World Health Organisation (WHO). Epidemiological surveillance of the last two to three decades revealed an increase in Salmonellosis, Cholera and Enterohemorrhagic *E. coli* (EHEC) contamination (WHO, 1984) in developed and developing countries. Of particular concern are foodborne pathogens. Foodborne pathogens can cause severe adverse health effects when consumed (van Schalkwyk & Hoffman, 2010) whilst spoilage bacterial contamination can result in an undesirable change in sensory attributes of products as well as in the wastage of a valuable protein source. Additionally, meat with high ultimate pH, frequently found in game meat can spoil at a faster rate (Hoffman & Dicks, 2011). Assessing spoilage is important as spoilage can lead to a significant economic loss for the food industry. If game animals are not harvested or slaughtered correctly and in a hygienic manner, sterile meat can be exposed to both spoilage and pathogenic bacteria.

During the making of products, meat becomes more exposed to contamination as it undergoes frequent handling, comes into contact with surfaces and equipment. The extent of product contamination is amongst others, dependent on the hygiene levels of the processing area, hands of workers and utensils used during processing (Nel *et al.,* 2004). As most of the game harvesting occurs during the winter months in South Africa, excess meat is frequently stored frozen. During processing, thawed meat portions are frequently made into game products, namely cubes, mince and sausages; production of these products requires a high incidence of handling and contact with foreign surfaces; all potential sites for bacterial contamination. Products are then vacuum sealed.

Vacuum packaging will inhibit spoilage bacteria (Buys *et al.,* 1997) However it must be noted that if oxygen is trapped within the packaging, bacteria can use it, which can result in signs of spoilage (Nel *et al.,* 2004).

Information on these possible areas of contamination during the game meat processing/value chain is lacking. The aim of this study was therefore to firstly determine the points of contamination of game carcasses by assessing the commercial harvesting/slaughtering process. Secondly, microbial quality of game carcasses was determined throughout the slaughter to deboning process. Thirdly, the hygiene levels of utensils and personnel involved in the production of game meat products was evaluated. Lastly the spoilage of game meat was investigated by studying, the colour stability and microbial spoilage of game meat that is dark, firm and dry (DFD) and normal in pH ().. Data obtained from this study is important as it sheds light on the slaughter process of game meat and the overall microbiological quality of game meat harvested, slaughtered and processed in a South African abattoir.

The study had some limitations. Firstly, samples were collected from only one commercial game abattoir. Secondly, even though the use of molecular methods (such as Polymerase Chain Reaction) in detecting the presence of microorganisms is growing within the food industry, this study only made use of conventional methods, due to the number of samples gathered per trial and available laboratory equipment and budget constraints.

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CHAPTER 2

Literature review

2.1 Introduction

Game species are found all over the world including South Africa, in various ecological niches including places such as the Karoo (Graaff-Reinet). Graaff-Reinet is considered to be the heart of the Karoo, antelopes found and harvested in the Karoo for human consumption are as follows: springbok (*Antidorcas marsupialis*), impala (*Aepyceros melampus*), kudu (*Tragelaphus stepsiceros*), eland (*Taurotragus oryx*), oryx (*Oryx gazela*), red hartebeest (*Alcelaphus caama*), blesbok (*Damaliscus dorcas philipsi*) and blue/black wildebeest (*Connochaetes taurinus/gnou*) (Anon, 2012). Hoffman *et al.* (2005) revealed that consumers from the formal market as well as tourists are interested in eating game meat. Game meat appeals to consumers as it is rich in protein and taste (Hoffman & Wiklund, 2006). Furthermore, game meat tends to be leaner than meat from conventional livestock (Hoffman & Cawthorn, 2013). For health conscious consumers, game meat can be seen as a healthy alternative to other red meat (Hoffman *et al.*, 2004). At least 73% of the consumers interviewed from the Western Cape (South African province) have eaten game meat (Hoffman, 2003), furthermore, game meat products are liked by tourists as the consumption of game meat is seen as a South African experience (Hoffman & Wiklund, 2006). Game farming in South Africa is the fastest growing agricultural industry with Cloete (2007) reporting that many cattle farmers are shifting to game farming; at present it is estimated that at least a third of South Africa's surface area has some form of wildlife management on it (van der Merwe & Saayman, 2014). This world-wide increase in interest towards game meat requires attention to be focused on the quality and safety of game meat.

Within this literature review a brief description of the game harvesting process will be described, a more detailed description can be found in van Schalkwyk & Hoffman (2010a). The overall aim of this literature review is to assess the availability of information on microbial quality of game meat. Within this section potential sources of contamination from the point of harvest to the processing facility/abattoir will be discussed. Very little information about abattoir hygiene in relation to game meat has been published. Therefore, this section will focus on studies that have been done on other types of meat.

Meat pH will be discussed as pH is recognised as an important intrinsic factor that can influence microbial growth on meat. A limited amount of Information on the pathogenic contamination and bacterial spoilage of game meat has been published. However, the general characteristics and environmental needs of microorganisms that have been associated with game meat will be reviewed. Furthermore, the advantages and disadvantages of both conventional and molecular methods for the enumeration and detection of pathogenic microorganisms will be discussed.

Lastly, some of the decontamination techniques (vacuum steam, lactic and other organic acids, hot water washes) used in the meat industry will be reviewed. Evidence of their efficacy and possible disadvantages, will be presented.

2.2 Cropping system

Cropping refers to the harvesting of game animals in their natural habitat. A suitable cropping/harvesting method must be implemented. A suitable method will aim to decrease the ante-mortem stress, bullet damage and wounding of animals. The cropping system used should also be practical and economically viable (van Schalkwyk & Hoffman, 2010b).

The commercial cropping system implemented will aim to deliver partially dressed carcasses that are unstressed, unsoiled and uninjured/wounded to the game meat handling facilities. Different cropping systems that are used in the industry exist, namely: day cropping, boma cropping and night cropping and on occasion, cropping from a helicopter. The information presented below will only focus on night cropping as it is the most economical cropping method that is most often used in the large scale harvesting of plains game animals (Hoffman & Wiklund, 2006). Figure 2.1 gives an indication of the harvesting system used for game meat, as well as possible points of contamination (which are discussed later in this section).

Game animals are usually harvested at night and during the winter season. Night culling results in better meat quality due to less stress (Hoffman & Laubscher, 2009) although it has been shown that when trained marksman are used, there is no difference in ante-mortem stress (Hoffman & Laubscher, 2009; van Schalkwyk *et al.*, 2011). The latter argue that this is due to the fact that trained marksman are able to shoot animals at distances that fall outside the flight/fight zone of the animals. The harvesting period is determined by the mating and birth seasons which tend to be species specific although they often fall within the traditional South African winter hunting season. Mating and birth seasons for most African ungulates are between February to March and October to November, respectively (van Schalkwyk & Hoffman, 2010c). Furthermore, harvesting in winter months lowers the impact of temperature (ambient day temperature 12°C and lower) and flies (Hoffman & Bigalke, 1999).

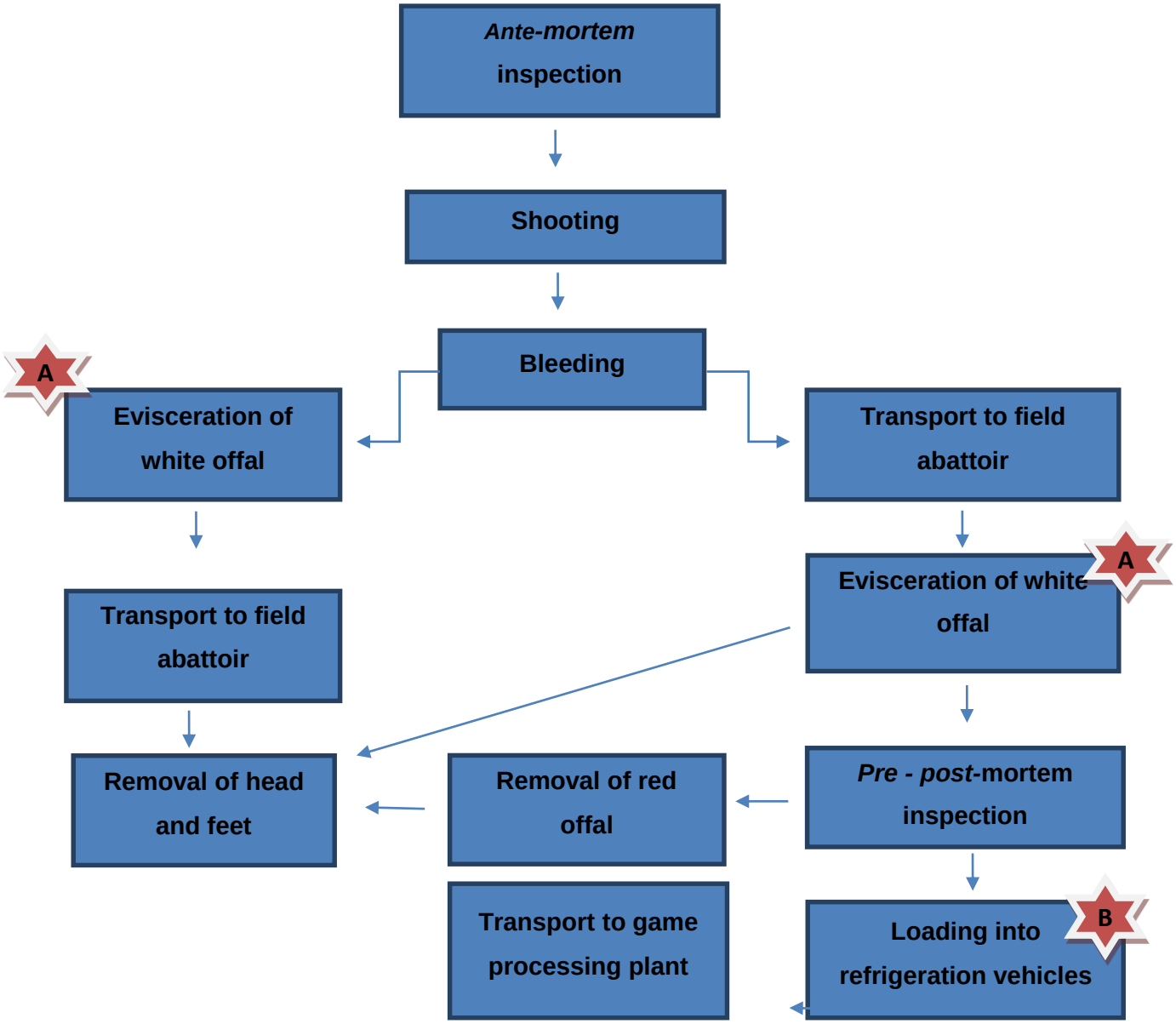

Figure 2.1 Ideal harvesting system with indicated possible points of contamination, adapted from van Schalkwyk & Hoffman (2010a).

Game animals such as springbok, black wildebeest, impala, etc. are spotted at night using a spot light from open vehicles (Figure 2.2). The spot light will help to immobilise the animals allowing a shot to be fired from at least 25 to 100 m away (van Schalkwyk & Hoffman, 2010b). At least four different placements of shots can occur during a hunt; head and neck shots are preferred as they will result in instantaneous death for the animal and the least amount of damage to the carcass/meat. Body and thoracic shots are not preferred as carcass can be contaminated with intestinal content and blood, respectively (van Schalkwyk & Hoffman, 2010b). Furthermore work by van der Merwe *et al.* (2014) proved that thoracic shots can lead to poor bleeding and high pH values (the effect of pH on meat quality will be discussed in section 2.1.4). The animals are normally hunted indiscriminately, unless the land owners have specifically indicated that a certain group of animals/gender, etc. may

not be culled. The only “selection” criteria applied by the marksmen is that only healthy animals are shot.

Once the animals have been shot, circulating blood flow is stopped by exsanguination by means of firstly, cutting through the skin with a sterilised clean knife. Secondly, the jugular vein and carotid artery found on the sides of the neck are properly severed with another clean and sterilised knife (Figure 2.3) (van Schalkwyk & Hoffman, 2010b). As pointed out by van Schalkwyk & Hoffman (2010d), knives used during this stage should be clean and sterilised, which means, knives should be cleaned with hot water (82˚C) or any other suitable steriliser after each cut to decrease chance of cross contamination (the concept of cross contamination from skin to carcass through slaughter equipment will be discussed in section 2.1.3).

The carcasses are then hung onto the side of the harvesting vehicle (Figure 2.4), which helps facilitate bleeding out. Game animals intended for commercial use should be bled out within 10 minutes of being shot. During this stage the marksmen in the vehicle continues looking for more game animals to harvest.

After this, the animals are either transported back to the field depot or eviscerated in the field in a hygienic manner. It should be noted that evisceration (marked as point B in Figure 2.1) is a point of concern as gastrointestinal content can result in bacterial contamination of carcasses; this notion will be discussed in section 2.1.2. The rough offal (such as stomach and intestines) is removed first. Rough offal evisceration should be removed within 30 minutes after exsanguination (van Schalkwyk & Hoffman, 2010b). Point of evisceration can depend on the size of the animal, as large game animals are usually eviscerated as soon as possible to decrease the carrying load on the hunting vehicle (Figure 2.2).

Partially opened carcasses are hung (Figure 2.4) or transported at a minimum of 20° angle (Figure 2.2) and transported to the field depot within 2-3 hours after exsanguination. At the field depot the red offal (lungs, heart, liver and spleen) is eviscerated and undergoes a health inspection. Partially dressed carcasses are hung in a cold truck with a temperature of -2˚C (to achieve carcass temperature of <7˚C) and await transportation to the processing facility (van Schalkwyk & Hoffman, 2010e). Loading (marked as point A on Figure 2.1) of carcasses is another point of concern, as possible hide to hide and hide to exposed flesh cross contamination can occur; the subject of hide/skin as a source of contamination is discussed further in section 2.1.1.

Figure 2.2 Harvesting truck

Figure 2.3 Exsanguination (van Schalkwyk & Hoffman, 2010b)

Figure 2.4 Hanging springbok carcasses

2.2.1 Inspection of carcasses

Inspection can be defined as the examination of meat that will be sold for human consumption for the presence of any diseases that can be transferred from animal to humans (van Schalkwyk & Hoffman, 2010d; van Schalkwyk & Hoffman, 2010f). This can include the examination of live animals and carcasses. This concept of inspection has been adopted by the game industry, as both ante- and post-mortem inspection is performed on animals. The section below will present a summary of

the aims and objectives of both ante- and post-mortem inspection as reported by van Schalkwyk & Hoffman (2010d) and van Schalkwyk & Hoffman (2010f).

2.2.1.1 Ante-mortem inspection

As indicated by the name, *a*nte-mortem inspection is done whilst the animal is still alive. Time period for inspection is usually one to seven days before harvesting. At the same time the registration of the farm and suitability of erecting a field depot are also confirmed.

The aim of the inspection is to identify:

- Conditions that can cause adverse reactions in humans (zoonotic diseases listed by the OIE)
- Diseases with no clear specific signs during *post-mortem* inspection (rabies, tetanus, botulism)
- Zoonotic diseases that are transferable to humans (rabies and Foot and mouth disease (FMD))
- Animals suffering from diseases or with symptoms indicating organ failure
- Septic conditions such as wounds and abscesses on the animals

The inspection is limited to health conditions that can be seen whilst the animal is still alive, ante-mortem inspection will allow the inspector/hunter to determine if animals intended for harvest can be transformed into nutritious and safe products for humans. This can be done by using an efficient checklist. A typical inspection will include a checklist with the following points:

- General behaviour of animals in the herd
- Movement and pasture
- Skin/hide condition
- State of nutrition
- State of external features

Ante-mortem inspection will ultimately allow the inspector/hunter to determine if the animals can be harvested or not. Furthermore, ante-mortem inspection can clearly determine if the game animals to be harvested can be sold commercially or not. If injured, diseased, wounded and excessively soiled animals are seen, cropping might not commence. A secondary form of ante-mortem inspection is carried out by the marksman just before shooting the animal; he will not shoot a sick animal. Each animal shot is provided with a unique number that ensures full traceability back to Farm/Region of origin. The marksmen also maintain a full record of all important information of each individual animal (Annexure A) (van Schalkwyk & Hoffman, 2010d).

2.2.1.2 Post-mortem inspection

Post-mortem inspection will determine if the meat is suitable for human consumption. Inspection will monitor the stressed experienced by animals during harvesting, possible bruising of animals, which can be caused by poor harvesting techniques and contamination occurrences through faeces, blood

and intestinal content. Post-mortem inspection will also try to identify diseases and injuries that were not visible during ante-mortem inspection.

A full post-mortem inspection can be done at the game processing facility (also known as a field depot) after off-loading. This is the first phase of post-mortem inspection. The inspection is carried out by a qualified veterinary inspector/ trained game meat examiner. An in-depth post mortem inspection will be carried out on the:

- Head
  - The presence of foreign bodies on the mouth, tongue and throat
  - Lymph nodes of tonsils and under the ears will be examined
  - A visual inspection of lips, gums, hard palate, soft palate and nostrils to determine whether aktinonycosis (a bacterial disease that affects bone tissue) is present
  - Lesions present on the mouth, skin, bone and hoofs can be an indication of FMD
  - Foam around the mouth will be an indication of rabies
- Feet
  - Gangrene and necrosis are determined by the presence of lesions and decomposing body tissue
  - Possible signs of FMD
- Lungs (Plucks are removed allowing for the inspection of the lungs)
  - The presence of pneumonia and pericarditis
  - Content build up due to pleuritic infection, on lungs and thoracic cavity
  - lymph nodes
- Reproductive organs and lactating udders (van Schalkwyk & Hoffman, 2010d)

After this inspection, the carcasses with their red offal (also individually marked to ensure a linkage with the unique carcass number) are loaded into the cold truck and when the truck is full, it is sealed and the carcasses are transported to the breaking plant/abattoir where the carcasses are processed further. Once carcasses have arrived at the abattoir, carcasses are given a secondary inspection (Van der Merwe *et al.*, 2013). The Secondary inspection is conducted by an official veterinarian as stated in the Meat Safety Act (MSA) of 2000 (South Africa, 2000). Tasks carried out by the veterinarian include: verification of the number of carcasses, tag (unique identification) numbers and temperature maintained during the transportation of carcasses. However, this inspection will not give an indication of all possible bacterial contamination; it only gives an indication of visible (faeces, blood, etc.) contamination that could cause microbiological spoilage.

2.3 Sources of contamination

It should be noted that primary production, which includes killing, evisceration, transport and cooling of carcasses is performed at the field, this differs from the process followed for normal livestock where the animals are transported alive to the abattoir where they are slaughtered. An exception is

in deer farming in New Zealand where live animals are transported to the abattoir (Hoffman & Wiklund, 2006). However, skinning and deboning is performed within an abattoir same as for farmed livestock, therefore the game meat hygiene status can be improved if effective Hazard Analysis Critical Control Point (HACCP) systems are developed and implemented for meat production (Gill, 1995). The challenge though is to find suitable Standard Operating Procedures (SOPs) that are robust for primary production. This section will discuss some of the sources of microbial contamination during these stages.

2.3.1 Skin/hide

The skin provides the best protection against the possibility of contamination of sterile meat (van Schalkwyk & Hoffman, 2010f). However, it can also play an important role in cross contamination if skinning of carcasses is not performed correctly. It should be noted though that skinning does not occur in the field when game animals are harvested, partly due to the fact that the field depot is outside and water is very limited in the field. Therefore, unskinned carcasses are transported to an abattoir, where they are skinned. One of the problems encountered is that the skins/hides are removed from cold carcasses rather than warm carcasses, a more difficult process in the former which could more readily lead to cross contamination. A more descriptive summary of the harvesting process is described in section 2.2

Skin contamination is aided by the environment the animal is exposed to. In their natural habitat animals are readily contaminated by dirt, soil and water (Loretz *et al.,* 2011). Bacteria in soil can contaminate the outside of the animal through direct contact or by dust (also known as aerosol contact) (Bell, 1997). Soil bacteria can colonise on the skin of the animal. Van Schalkwyk & Hoffman (2010a) reported that excessively soiled game animals are not normally harvested. The marksman who does the culling normally does a preliminary inspection (during ante-mortem inspection) and where possible, will not harvest excessively contaminated animals.

Animal hides can be contaminated through direct or indirect contact with faecal material (Bell, 1997). Faecal contamination of hides can result in the transference of pathogenic bacteria onto the surface of carcasses.

Microorganisms can be expected to be present on the hides/skin and the hooves of the animals. Microorganisms on the skin/hide are expected to be at least 3.5-10 log cfu/cm^2 (Paulsen, 2011). Only a small percentage of these microorganisms are transferred onto the muscle of the carcass if due diligence is followed during the various processing steps described. However, a number of the transferred microorganisms can be of a pathogenic nature, therefore contamination is still a significant problem.

During skinning, the hide which was once protecting the flesh can turn into a major source of contamination. This was confirmed when Bell (1997) investigated the distribution and sources of contamination for beef carcasses and reported that the hide and faecal matter on the hide were the

major sources of contamination. Bell (1997) also noted that sampling sites that came into contact with faecal matter had both a higher *E. coli* and aerobic count.

2.3.2 Gastro intestinal tract

Contamination of meat with intestinal microbial flora has been said to be possible, due to the intestinal lymphatic vessel's permeability towards bacteria sized particles. Due to their size, bacteria can migrate from intestines to other body tissue. This assumption has led to the removal of animal (pigs, cattle and sheep) viscera, immediately after death to decrease contamination. However, Barnes & Shrimpton (1957) reported sterility of stored poultry carcasses, when viscera were not removed. Several experiments were conducted by Gill *et al.* (1976) to settle the discrepancies. Firstly, guinea pig carcasses were injected with ^{14}C- labelled bacteria. Inoculation procedure occurred as follows: guinea pigs were killed with chloroform vapour, once deceased the abdomen was opened and 1 ml of bacterial suspension was injected carefully and slowly into the small intestine or the colon. After 24 hours, migration of the radioactive material through the lymphatic vessels was observed. Radioactive material appeared in the muscles of the hind legs, and in the guinea pig tissues (lung, liver, lymph nodes and spleen). The migration of the ^{14}C- labelled bacteria took approximately 15 minutes. Secondly, to determine if viable bacteria found in the intestine can actually reach body tissues after death another set of guinea pigs were inoculated again. Deceased guinea pigs were inoculated with^{14}C- labelled bacteria, live unlabelled *E. coli* and *Clostridium pefringens.* Previous trials with the ^{14}C- labelled bacteria allowed an expectation of at least 10^3 viable bacteria/g. However, this was found not to be so as samples from lung, liver, lymph nodes and spleen tissues showed no bacterial growth. Thirdly, the effect of delayed evisceration was investigated. Six skinned lamb carcasses with intact viscera were hung at room temperature. After 24 hours muscle and lymph node samples were gathered and bacteria were enumerated using nutrient agar. There was no bacterial growth observed. It was suggested that the lack of enumerated bacteria was due to the rate of migration by the bacteria; migration was said to be too slow or the duration of the experiment was too short. Another reason was said to be, that the death of an animal does not necessarily impair immunity mechanisms; these mechanisms can possibly kill any bacteria rapidly reaching the lymphatic system. Gill *et al.* (1975) did conclude that the act of immediate evisceration is unnecessary as proved by the study.

However, the experiments of Gill and co-workers (1975) were conducted on hanging carcasses in a controlled environment, whilst game carcasses are frequently transported through bumpy terrain and the effect that this "shaking" may have on the bacteria's possible migration needs quantification. Winkelmayer *et al. (*2008) recommended evisceration within three hours; a realistic and ideal time period that still limits microbial spread, therefore contamination during evisceration is accidental in nature. It should also be noted that as the time before evisceration increases, bloating of the intestinal tract occurs; this bloating increases the possibility of the intestines being raptured during evisceration which could lead to carcass contamination.

Partially opened carcasses for the removal of offal can allow for accidental spillage and contamination of gastrointestinal tract contents (Humphrey & Jørgensen, 2006; Galland, 1997). Another area of contamination is through leakage of intestinal fluids out of the oesophagus due to the exsanguination cut. It is therefore recommended that the evisceration should be seen as a point of concern; proper and hygienic execution of this step is advised (Humphrey & Jørgensen, 2006; Gill, 2007) to lower cross contamination.

Hudson *et al.* (1981) reported that the hygienic condition of carcasses during the dressing stage can differ from carcass to carcass as the contamination or bacterial loading onto carcasses is amongst others, dependent on the skill of the worker responsible for dressing the carcasses. During the investigation of the bacteriological status of beef carcasses at a commercial abattoir before and after slaughter line improvements, Hudson *et al.* (1981) reported that the dressing stage can be improved by simultaneously upgrading the dressing line and worker's practises. The same rationale can be adopted for the improvement of field tasks such as the removal of offal in the field. Similarly, during the investigation of hygiene practices of three systems of game meat production in South Africa, van der Merwe *et al.* (2011) found that the quantity of bacteria transferred onto the carcass can be dependent on the adherence of hygiene practices. For this study aerobic bacterial counts enumerated from game carcasses indicated that the level of bacterial contamination can possibly be due to the dressing process.

2.3.3 Equipment and personnel

During meat processing, equipment such as hand held knives are frequently used. Peel & Simmons (1978) investigated factors involved in the spread of *Salmonella* in meat working plants. Cutting equipment such as knives, were reported to be responsible for contamination: knives used for cutting the hide had a higher *Salmonella* count than knives used for other cutting operations. To combat contamination hygienic practices for cutting equipment can include immersing or spraying equipment with water at 82ºC for at least 10 seconds, or using the two knife principle; one knife for cutting through the skin/hide and the second for skinning. However, Abdalla *et al.* (2010) reported that hand held equipment is hardly ever held under water for more than two seconds by workers and hands are hardly washed. However, if done correctly, washing and sterilising of equipment can significantly decrease total viable counts; results reported by Abdalla *et al.* (2010) indicated a 44% reduction in bacterial counts.

Larger equipment used for meat processing can be sprayed with hot water for longer periods to ensure cleanliness. However, spraying can lead to rapid cooling of water. Cooling occurs, as water has to travel from the spray head to the targeted equipment. It has been noted that large volumes of water need to be used in order to achieve the desired level of hygiene (Peel & Simmons, 1978), this results in additional costs to the processing plant as systems need to be implemented to process the water, before and after use. This is also a huge problem at the field depot with South African game species as the quantity of water is always limited.

Bell (1997) also investigated bacterial counts on personnel hands and knives whilst attempting to identify the distribution and sources of contamination for beef carcasses. Hands before and after cutting the hind legs open were sampled (after cutting, hands and knives were rinsed using a combined hand rinse - knife steriliser). Aerobic bacteria was enumerated on the hands and knife blades sampled, counts were as high as 4.74 log cfu/cm^2 and 3.61 log cfu/cm^2, respectively. Bacterial counts showed an increase after cutting. It was reported that contamination found on the hands was similar to that found on the hide, as hand and hide contact is unavoidable during slaughtering. Furthermore, knife blades carried at least a tenth of the bacteria found on the hands. When the game slaughtering process is considered, game animals are skinned after being chilled for at least 24 hours. That means cold carcasses are skinned whilst for species such as cattle/sheep, warm carcasses are skinned making the skinning process easy and possibly minimising cross contamination. This is an aspect that warrants further research: the effect of hot or cold skinning on carcass contamination.

At a deboning plant (also known as a processing facility) carcasses are broken into primal cuts. It has been reported that during the breaking of carcasses, pathogenic bacteria can transfer from contaminated equipment or the bodies of personnel to the meat. Many abattoirs have intervened by lowering temperatures of deboning facilities to <10ºC, as low temperatures can inhibit bacterial growth. However, these low temperatures lead to worker discomfort. Additionally, abattoirs have employed routine cleaning and hygienic practices by personnel (Gill, 1995).

Furthermore, handling of carcasses can result in redistribution of bacteria by personnel. Gill (1995) reported that General Management Practices (GMP) to enforce requirements for personnel hygiene have been noted to decrease contamination during the deboning stage. Additionally, having requirements for the cleanliness of fixed deboning equipment, can increase hygiene levels. The efficacy of cleaning programmes can be monitored regularly through obtaining samples for microbial testing (Gill, 1995).

Nel *et al*. (2004a) investigated bacterial populations associated with meat from the deboning room which consisted of collecting beef samples at the deboning room. *Enterobacteriaceae*, *E. coli* and aerobic bacteria were found to be present. *Enterobacteriaceae* and aerobic bacteria counts reached 2.1x10^4 and 5.7 x10^7 cfu/g, respectively. *E. coli* counts ranged from 3.7x10^3 to 1x10^6 cfu/g. It should be noted that *E. coli* counts found during the study were above 10 cfu/g; a limit set by the South African Department of Health (Ne l*et al.,* 2004a). Contamination of *E. coli, Enterobacteriaceae* and aerobic bacteria was due to faecal contamination and poor sanitary procedures during the deboning process. Furthermore, high *E. coli* counts can be an indication of possible cross faecal contamination between meat handlers and retail meat cuts (Ne l*et al.,* 2004a). The personal and general hygiene practices in the deboning room were also investigated by means of a survey; workers who partake in the deboning of carcasses indicated that they often come into contact with faecal matter. Frequency was indicated to be weekly for some workers (Nel *et al.,* 2004b).

During the study, Nel *et al*. (2004a) recommended cleaning carcasses during the slaughter process, in order to decrease contamination. Risk reduction interventions/ decontamination techniques will be discussed further in section 2.4.

2.3.4 pH of the meat

One of the intrinsic parameters that determine the microbiological quality of food is pH. pH in meat is a measurement of the amount of hydrogen ions in meat and is closely correlated to the concentration of lactic acid produced during anaerobic glycolysis

Normal muscle pH is usually around 7 and as the muscle changes into meat post-mortem, the pH decreases until an ultimate pH of ~5.6 is attained around 24 hours *post*-mortem, although this time may differ between different species. The rate of pH change and ultimate pH attained is influenced by numerous factors such as ante-mortem stress, temperature and post-mortem interventions. For example, the ultimate pH can be higher if the animal is stressed ante-mortem (Kritzinger *et al.*, 2003; Hoffman & Dicks, 2011; Van der Merwe *et al.,* 2013a). Ante-mortem stress in animals can result in the depletion of glycogen in muscles and the low glycogen content will lead to low production of lactic acid anaerobically post-mortem and high ultimate muscle/meat pH (>6). A higher pH can ease the growth of many microorganisms as bacteria prefer a higher pH range for growth (Magwedere *et al.,* 2013a; van Schalkwyk & Hoffman, 2010). Game meat with a high ultimate pH will be dark, firm and dry (DFD). It is important to note that ante-mortem stress can be caused by poor cropping techniques. However, post-mortem inspections should be performed efficiently enough to determine poor cropping techniques (Van Schalkwyk& Hoffman, 2010d).

Magwedere *et al.* (2013a) found the pH of springbok carcasses to vary from 5.73 to 6.00. At the higher pH level it can be expected that the shelf life of springbok meat products will be shorter than that typically found for domesticated livestock (Magwedere *et al.*, 2013a) as this pH is close to the optimum growth pH of both spoilage and pathogenic bacteria. Therefore, it is important to ensure that a minimum amount of microbiological cross contamination occurs as there are a number of inherent pathogenic microorganisms found in game animals.

2.4 Inherent microorganisms

2.4.1 Enterobacteriaceae

2.4.1.1 General

Enterobacteriaceae is a large family of rod shaped gram negative and facultative anaerobic bacteria. The species within the *Enterobacteriaceae* family range from harmless, spoilage to those with a more pathogenic nature. Pathogenic microorganisms such as *Salmonella* spp., *E. coli* and coliforms are found in this family. Most *Enterobacteriaceae* are found in the gut flora of animals and humans (Magwedere *et al.*, 2013b).

This family of bacteria is found in warm temperatures (Ercolini *et al*., 2008), an optimum environment can allow for growth that can lead to spoilage of food products. Microbial loads from 10^7 cfu/cm^2 can result in off odours. Off odours will have a cheesy, buttery odour. As the *Enterobacteriaceae* count increases to levels around 10^9 cfu/cm^2, the odour can change to a fruitier odour and further increase will result in a putrid odour (Ercolini *et al*., 2008).

Enterobacteriaceae can use amino acids as a carbon source. During the utilisation of amino acids off odours and flavours can occur. Compounds such as hydrogen sulphide can be produced; hydrogen sulphide can lead to a green discolouration of food products (Ercolini *et al*., 2008).

Enterobacteriaceae counts can be a reflection of environmental hygiene levels and good manufacturing practices (GMP) (Magwedere *et al.*, 2013b). Magwedere *et al.* (2013b) conducted a microbiological analysis on springbok carcasses where samples were collected from 2009 to 2010. Contamination of carcasses was classified into three different levels: Acceptable, marginal and unsatisfactory (acceptable range ≤1.5 log cfu/cm²; marginal range 1.5-2.5 log cfu/cm²; unacceptable range >2.5 log cfu/cm², limits were as set out in the Commission Regulation No 1441/2007/EC). In 2010, the *Enterobacteriaceae* contamination reached an unacceptable level, ~71 % of the springbok carcasses that were analysed were unsatisfactory according to the regulation set by the European Union (EU) (Commission Regulation No 1441/2007/EC). Counts were as high as 2.93 ±1.50 log cfu/cm^2. Magwedere *et al.* (2013a) concluded that the high *Enterobacteriaceae* count in 2010 was due to a poor processing and/or harvesting process of springbok carcasses.

2.4.1.2 *Escherichia coli* (*E. coli*)

E. coli strains are gram negative, facultative anaerobic, non-spore forming bacteria. As mentioned, *E. coli* is part of the *Enterobacteriaceae* family. *E. coli* strains represent a small percentage of gut flora found in mammals and birds (Jay, 2005a). Therefore, the presence of *E. coli* in game meat is an indication of possible faecal contamination.

E. coli can grow in an environment with a temperature range of 7-46ºC, whilst temperatures above 60ºC can kill the organism (Dunn *et al.,* 2004). Furthermore, *E. coli* can grow within the pH range of 4.5 to 7.4 (optimum pH of 4.5). *E. coli* has the ability to survive but not grow in hostile conditions, for example at low refrigerator temperatures (-20 ºC to 4ºC). *E. coli* can also survive in low pH environments, by triggering the production of amino acid carboxylases that aid *E. coli* in controlling and maintaining internal pH.

Table 2.1 is a list of the five classes of *E. coli* that cause diarrheal diseases. For this literature review Enterohemorrhagic *E. coli* (EHEC) will be discussed in more detail. EHEC is characterised by the unique production of a shiga toxin therefore, also referred to as shiga toxin *E. coli* (STEC). When EHEC contaminated food is consumed, EHEC can cause haemorrhagic colitis (bloody diarrhoea), uraemic syndrome and thrombotic-thrombocytopenic purpura in humans (Bartels & Bulte, 2011). Table 2.1 also lists the infectious doses for each *E. coli* class. A low infectious dose is

required to trigger the symptoms mentioned above. The onset of infection and symptoms will approximately take 48 hours to show (Adams & Motarjemi, 1999).

Table 2.1 infectious dose of *E. coli* needed to initiate an infection (adapted from Adams & Motarjemi, 1999).

E. coli	**Infectious dose (cells)**
Enteropathogenic (EPEC)	10^6-10^{10}
Enterotoxigenic (ETEC)	10^6-10^8
Enteroinvasive (EIEC)	10^8-10^{10}
Enterohaemorrhagic (EHEC)	10^1-10^3

One of the most recognised serotypes in the EHEC class is *E. coli* O157:H1. For the past 15 years, *E. coli* O157:H1 has been seen as a major food pathogen (Bartels & Bulte, 2011). In 1982, *E. coli* was first identified in insufficiently heated hamburgers. The consumption of contaminated hamburgers led to severe illnesses and deaths. Ground beef was recognised as a major vehicle for the transmission of *E. coli* O157:H7. *E. coli* O157:H7 contaminated the surfaces of beef carcasses during the slaughtering process. These chilled carcasses were cut into smaller portions for sale and during cutting, trimmings such as subcutaneous fat were used in making the ground beef. The grinding helped to distribute the bacteria throughout the ground beef (Tompkin, 2002). Since1982, *E. coli* O157:H7 has been a steadily increasing cause of food borne illnesses.

Bandick & Hensel (2011) were involved in reviewing the risk of consuming game meat with an emphasis on European hunted meat. Risk was said to be associated with the lack of hygiene during processing and unrecognised zoonotic diseases that can be transferred to humans. One of the zoonotic disease causing microorganisms that were evaluated was EHEC. Since 2001, 1100 EHEC infections were recorded in humans. Beef was reported to be the primary transport of infection. However, in more recent studies by the reference laboratory for zoonotic diseases (at the German Federal Institute of Risk Assessment) in 2002, 3% of game samples analysed were contaminated with EHEC and in 2005, 14.8% of game samples analysed were contaminated. During the year of 2005, contaminated game samples were higher than beef samples. The increase of contamination could possibly be due to poor hygienic procedures during wild game processing.

Published data on the microbial analysis of game carcasses is limited. However, Magwedere *et al.* (2013c) conducted a study that included the detection of STEC serotypes of bison, rabbit, deer and boar. Game products were purchased from a local retail supermarket in the United States of America (USA). Figure 2.5 displays the different STEC serotypes that were found, and the different samples that were positive for the serotypes. From the results obtained *E. coli* O157:H1 was only present in bison. A similar finding was reported by Dunn *et al.* (2004) for white tailed deer (Louisiana, USA). Faecal samples (n = 55) collected from white tailed deer showed low prevalence of *E. coli*

O157:H7, only one sample yielded a positive result. Dunn *et al.* (2004) concluded that deer were not carriers of the pathogen.

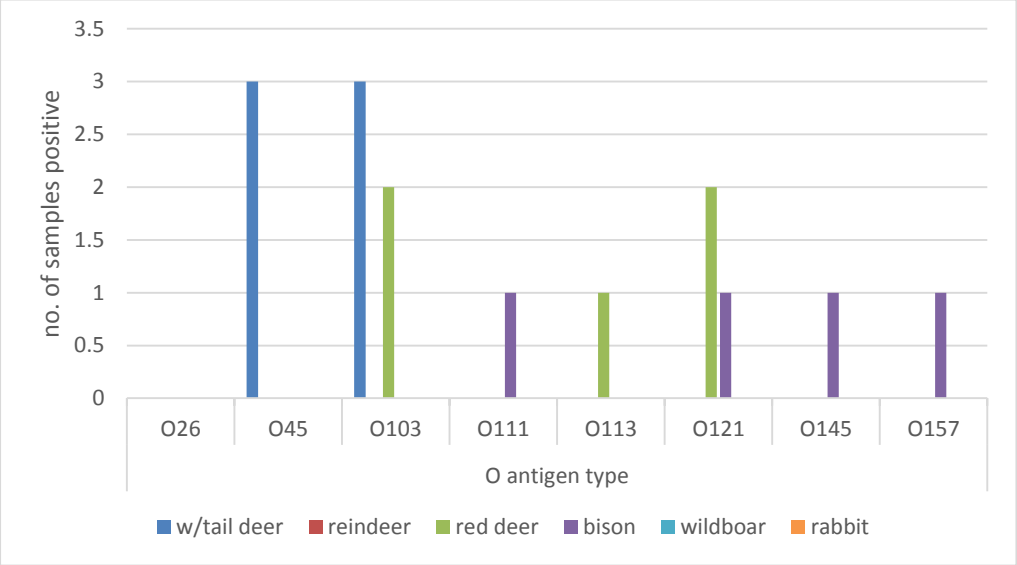

Figure 2.5 Number of game samples positive for STEC serotypes as adapted from Magwedere *et al*. (2013c)

2.4.1.3 *Salmonella* spp.

Salmonella spp. are gram negative, motile, non-spore forming, rod-shaped and facultative anaerobic microorganisms. Similar to *E. coli*, *Salmonella* spp. also belong to the *Enterobacteriaceae* family. *Salmonella* spp. can be found in the environment as it is excreted by humans, pets and farmed animals. *Salmonella* spp. can colonise in soil, water, insects and seafood found in the environment (Ohtsuka *et al.*, 2005).

Salmonella spp. have a wide growth temperature range starting from 5°C to 47°C, however, the optimum growth temperature is 35°C; *Salmonella* spp. can be inhibited through heating at 75°C for 10 minutes. A pH range from 4 to 9 can allow growth, where optimum pH of growth is between 6.5 and 7.5 (Ercolini *et al.,* 2008).

Some *Salmonella* spp. strains are major foodborne pathogens that have been associated with poultry, eggs, meat and dairy products (Ohtsuka *et al.*, 2005). Salmonellosis is an illness that occurs when contaminated food by *Salmonella* spp. is consumed. If a healthy individual ingests 100 to 1000 cells of pathogenic *Salmonella* spp., salmonellosis can occur. Once ingested, symptoms will only show between 6 to 48 hours after ingestion and infection can last for 1 to 4 days. Symptoms include nausea, diarrhoea, abdominal pain, fever, and headache. Severe Salmonellosis cases can lead to pneumonia and meningitis.

Very little information has been published on the prevalence of pathogenic *Salmonella* spp. strains in game meat. However, Viera-Pinto *et al.* (2011) reported on *Salmonella* spp. in wild boar. Additionally, in 2007 the European Food Safety Authority (EFSA) reported at least 170 000 cases of Salmonellosis in the EU. *Salmonella* spp. has been classified as one of the 'main food-borne pathogens'.

Paulsen *et al*. (2011) reported that free range game animals might have lower levels of *Salmonella* spp. The reason being, farming of domestic livestock allows crowded living spaces which can result in cross contamination of *Salmonella* spp. Furthermore, *Salmonella* spp. can be harboured by humans and farming would mean more contact between humans and animals. Therefore, more transference of the organism through contact can occur (Paulsen *et al.,* 2011*).*

Magwedere *et al.* (2013b) investigated the presence/absence of *Salmonella* spp. on springbok carcasses. Swab samples were gathered from springbok carcasses over two years (2009 and 2010). For this experiment *Salmonella* spp. was not detected on the springbok carcasses (Magwedere *et al.,* 2013b). However, the absence of *Salmonella* spp. does not prove that the microorganism is not harboured by these animals. Magwedere *et al.* (2013a) recommended the use of more diagnostic methods for the detection of *Salmonella* spp. and conducting a larger survey.

2.4.2 Staphylococcus aureus (*S. aureus*)

Staphylococcus aureus (*S. aureus*) is a cocci shaped gram-positive micro-organism that belongs to the genus Staphylococcus. *S. aureus* can exist as single units, pairs, tetrads, short chains or irregular grape like clusters (Jay, 2005). *S. aureus* is also a facultative anaerobic microorganism.

Most humans carry this microbe in their throat, nasal passages, hair and skin. Mammals and birds harbour the micro-organism in the nasopharyngeal region. It is transmitted through sneezing, coughing, and hand contact. *S. aureus* can make its way into food by colonising in food equipment, water, dust and environmental surfaces (Jay, 2005b).

S. aureus can grow in a wide range of temperatures; Schmitt *et al.* (1990) reported a temperature range from 6.5 - 48.5°C with an optimum growth temperature of 37°C. *S. aureus* can grow at a pH range from 4.2 to 9.3; optimum pH as recorded by Ho (1989) would be 7-7.5. Furthermore, *S. aureus* is non-spore forming; however, *S. aureus* produces a heat stable toxin (Bhatia & Zahoor, 2007). Ingestion of the heat stable enterotoxin causes severe abdominal cramps, vomiting and diarrhoea. Enterotoxin production is triggered when cell counts exceed 100 000 cfu/g. The enterotoxin is impressively stable, as it cannot be broken down by proteolytic enzymes (pepsin and trypsin) found in the digestive system (Jay, 2005b). If the enterotoxin is ingested, symptoms can show within 38 hours and the symptoms can last up to two days.

Richards *et al*. (2011) investigated the microbial quality of venison meat in the United Kingdom; the microbial quality was in relation to production practices and processes. *S. aureus* count for all the samples was <2 log cfu/g (Richards *et al*., 2011). The amount of *S. aureus* did not correlate to the environmental nor faecal counts. Therefore, contamination was due to handling of carcasses during the harvesting process (Richard *et al*., 2011). Another, recent study was conducted by van der Merwe *et al.* (2011) on game carcasses in South Africa that reported no presence of *S. aureus*. The lack of enumeration was attributed to the maintained cold chain from the point of harvest. The low temperatures were reported to stunt the growth and viability of the microorganism.

2.4.3 *Clostridium* spp.

Clostridium spp. is a genus of gram positive rod shaped, obligate anaerobic bacteria (a few strains are aero-tolerant and obligate anaerobes). Bacteria that belong to this genus can form endospores (Jay, 2005; Engelkirk & Duben -Engelkirk, 2008). Clostridial spores can survive in adverse environmental conditions, heat, light, refrigerator temperatures, dry environments and radiation (van Schalkwyk & Hoffman, 2010f). When favourable conditions arise, spores can then proceed with germination (Engelkirk & Duben-Engelkirk, 2008).

Clostridium spp. (specifically *Clostridium botulinum*) can be associated with food poisoning of humans and animals. The genus has also been associated with a phenomenon known as bone taint. Bone taint usually occurs in deep muscle tissues (such as hip joints and shoulders) of heavy and fat game carcasses (typically eland and zebra) where cooling occurs very slowly. Bone taint is also frequent in game animals that are stressed. Bone taint is characterised by a sewage, sweet/sour odour. Riemer & Rueter (1979) investigated deep tissue contamination in wild deer carcasses. Microbiological analyses indicated that microorganisms were at levels of 2 log cfu/g in half of the carcasses analysed. On the other hand, a more recent study by Paulsen *et al.* (2011) also analysed deep tissue contamination in wild deer. This study yielded a lower microbial count (<2 cfu/g). The lack of consistency between the two studies could be due to the methods used for sample collection or due to inherent variation between animals. Gill (2007) also noted that methods used for sample collection can easily be contaminated by environmental microorganisms therefore microbial levels might appear to be higher.

Clostridium botulinum strains are gram negative, facultative anaerobes that belong to the *Clostridium* genus. *C. botulinum* is widely distributed in both soil and water (Jays, 2005c). Botulism is a disease caused by a highly toxic exotoxin produced by *C. botulinum*. Seven toxin types (type: A, B, C, D, E, F and G) are known. Types A, B, E, F and G have been reported to cause botulism in humans. Food borne botulism occurs when ingestion of toxin contaminated food occurs. Symptoms can appear in a few hours or in a couple of days. Symptoms such as weakness and fatigue will appear first and will be followed by blurred vision, speech impairment, difficulty swallowing and respiratory failure which can lead to death (Jays, 2005c). Botulism is one of the major pathological diseases that are checked for during the *ante-mortem* on-farm inspection (van Schalkwyk & Hoffman, 2010f) as *C. botulinum* remains a major food pathogen.

Again, very little information has been published on the prevalence of *C. botulinum* in game meat. However, C*. botulinum* has been isolated from dried venison jerky samples from the United States (Midura *et al*., 1972). With further analyses it was determined that *C. botulinum* type F was causing the outbreak. At least three out of the twenty people that consumed the home made venison jerky developed botulism.

Cross contamination from the environment to agricultural products by this organism can be low. However, one must keep in mind the large quantities of agricultural products that are produced

every year as large scale agricultural production increases the likelihood of *C. botulinum* contamination and other major pathogens.

2.5 Decontamination techniques

There are a number of decontamination techniques that could become applicable for minimising the microbiological load of game carcasses under South African conditions. These decontamination techniques can be subdivided into three types, namely: physical (hot water, steam, steam vacuuming), chemical (organic acids, polyphosphates, and chlorine) and biological (bacteriophages, bacteriocins). Physical interventions are seen as traditional, whilst the biological methods are viewed as new emerging techniques (Hugas & Tsigarida, 2008). This section will give the general characteristics, advantages, disadvantages and evidence of efficiency in combating microbial loads on meat carcasses for the more frequently used methods in the meat industry (physical and chemical interventions). The significance of this section is to ultimately be able to suggest decontamination techniques that can be implemented in the field during the harvesting of game meat, in order to combat the initial stages of contamination at points A and B as indicated in Figure 2.1.

2.5.1 Hot water wash

Hot water treatment (also known as hot water pasteurisation) has proven to be an effective heat treatment, especially when used in combination with other interventions such as a lactic acid treatment. Hot water treatments can decrease microbial loads and help in removing debris from carcasses such as soil, which can aid cross contamination (section 2.3). To be effective, hot water is required to be at temperatures above 75°C. Hot water treatments can be administered on the hide or the surface of the carcass. The hide of animal can be washed, usually regarded as a pre-slaughter treatment for normal livestock such as cattle. Hide washing is seen as an effective treatment in the reduction of hide to carcass cross-contamination as hides can harbour a significant amount of microorganisms (Small, 2005, Loretz *et al.,* 2011). A hide wash would entail the spraying of hides with a power hose for a significant amount of time; Mies *et al.,* (2004) showed that washing of cattle hides using a power hose, for 1 minute and 2 minutes did not result in reduction of aerobic bacterial, *E. coli* and coliform counts. However, Byrne *et al.,* (2000) showed that a 3 minute wash resulted in reduction of 3.4 log cfu/cm^2 in *E. coli,* when the method was administered on cattle hides that were inoculated with *E.* coli. Another option is a post-slaughter wash of the carcass, after skinning. Gill *et al*. (1995) reported a 2.5 log *E. coli* reduction, using hot water at a temperature of 85°C for 20 seconds. Sofos & Smith (1998) reported a general bacterial log reduction of at least 1-3 in pathogens, when hot water is used to treat carcasses.

The efficacy of hide/skin washing has been reported, however the washing of the hide may be seen as a form of re-distribution of bacteria and not as effective as washing of carcass surfaces. Hot water treatments are effective, but they can be disadvantageous to the carcass as treatment can lead to heat damage of carcass surfaces (Pipek *et al*., 2005). If sprayed, hot water treatments can

easily lose heat through evaporation and free water droplets can form. These free water droplets bounce from the walls and floors and can land on clean carcasses resulting in cross contamination (Hoffman *et al.,* 2010). Therefore, careful use of equipment is suggested (Sofos & Smith, 1998). The increase in moisture on carcasses can aid the growth of microorganisms. Furthermore, hot water treatments can result in visible changes on the carcasses even when used for only a couple of seconds, therefore it is important to consider the use of a warm wash instead of a hot wash. A warm wash can result in less physical changes. However; the treatment will need to be applied for longer periods.

2.5.2 Steam

Steam vacuum systems are widely used in the meat industry as they can be effective in removing contamination and inactivating surface contamination through the use of a hand held device. These hand held devices can deliver hot water of 82-88°C and steam to the surface of the carcass. Dorsa *et al.* (1996) reported that vacuum steam reduced total bacterial, coliform and *E. coli* counts by at least 50%. The effectiveness of this vacuum steam was also tested on pork carcasses with visible faecal contamination. The aim was to find an alternative method to trimming infected hide surfaces (Le Roux, 2008). When vacuum steam was used, aerobic bacteria, *Salmonella* spp. and *Enterobacteriaceae* were reduced.

Steam pasteurisation can be used as an alternative treatment to hot water treatments. Steam pasteurisation increases the carcass surface temperature which can result in thermal destruction of the bacteria (Huffman, 2002). James *et al.* (2000) investigated three treatments to most effectively reduce bacterial contamination of carcasses. These included the immersion of carcasses in 90°C water, chlorinated water (90°C) as well as steaming (100°C). The steam treatment was more effective at removing bacteria from the rough and porous carcass surfaces. McEvoy *et al*. (2003) investigated the best decontamination technique for bovine hides. The aim was to see if the use of a sub atmospheric pressure steamer would be effective in the reduction of pathogenic bacteria. This device heated the hide samples to 75°C or 80°C and the treatment was applied for one, ten and twenty seconds. These treatments resulted in noticeable decrease in the aerobic bacteria and *E. coli* counts. Five years later, Small *et al. (*2005) conducted a similar experiment, where the use of steam (condensing at sub atmospheric pressures) on cattle hides was investigated. Steam proved to be even more effective in the reduction of *E. coli* counts as the faecal matter was solid rather than liquid.

When using steam, physical changes can be observed on the carcass. This can be rectified but by decreasing the intensity of the steam device (Le Roux, 2008). Steam at 100°C has been reported to have a greater heat capacity than water at the same temperature of 100°C, making steam more effective at penetrating cavities and hair follicles (James, *et al*., 2007).

2.5.3 Chemical agents

Acetic and lactic acid are frequently used chemical decontaminants. Acetic acid is a monocarboxylic acid that is a by-product of ethanol oxidation by *Acetobacter*, *Gluconobacter* and heterofermentive lactic acid bacterial strains. Acetic acid has a strong odour and a pungent flavour. Acetic acid is a weak acid that can be used as an antimicrobial agent when it is in its non-dissociated form (Lingham *et al.,* 2012). Lactic acid can be characterised as a clear, water soluble liquid that is hygroscopic.

The non-dissociated form of lactic and acetic acid promotes antimicrobial activity. In this form lactic and acetic acid is fat soluble, therefore they can diffuse through the lipid membranes of microorganisms. Once inside, the organic acids reduces the internal pH of cells and reduce the rate of metabolic activities (Holzapfel *et al*., 1995).

Jasass (2008) evaluated the effectiveness of trisodium phosphate (TSP), lactic acid and acetic acid on reducing the microbial load of chicken carcasses and showed that both lactic acid and acetic acid had the ability to reduce both *E. coli* and aerobic counts better than TSP. Furthermore, Jasass (2008) concluded that lactic acid was the more effective organic acid in the reduction of *E. coli* and aerobic total counts when compared to acetic acid. Lactic acid will not completely eradicate pathogenic organisms but it can lower bacterial counts found on the carcasses (Jasass, 2008).

Pipek *et al*. (2005) investigated carcass decontamination using lactic acid. The aim was to prove that lactic acid is capable of decreasing microbial counts on beef carcasses. A 2% lactic acid solution was used for both trials at a temperature of 45°C and 15°C. Results showed that both temperatures were effective in decreasing microbial populations on infected carcasses, however, the warmer the solution the more effective the antimicrobial action.

Lactic acid has little sensory impact on the products being decontaminated. Lactic acid can be used as a hide or carcass wash as part of a sanitizer for animal carcasses (Dickson & Anderson, 1992) or as a cold carcass organic acid wash. Even though organic acids are effective and commonly used, their efficiency can be altered by different types of meat tissue, microbial load on animal carcasses, the concentration of the solution and the temperature of the solution.

In addition to organic acids other chemical decontaminating agents can be used to combat microbial contamination and include peroxy acid, chloride dioxide and trisodium phosphate. Unlike organic acids, peroxy acid and chlorine dioxide's antimicrobial efficacy is due to its strong oxidising activity which can disrupt cell membrane permeability and its ability to penetrate cell walls to disrupt protein synthesis (Thiessen *et al.,* 1984). Another microbial agent that shows promise is trisodium phosphate, unlike the agents mentioned above, its antimicrobial activity is not fully understood yet, however an indication of detergent like action in removing fat films found on the cell membrane of microbial cells which ultimately disrupts cell membrane integrity has been reported (Capita *et al.,* 2002).

A chemical decontamination technique can only be used if it is "Generally Regarded As Safe" (GRAS) by the Food and Drug Administration (FDA) (Raftari *et al*., 2011). Therefore a number of

international studies that study the efficacy of chemical antimicrobial agents exist However, in South African, regulations state that the application of substances that will ultimately prevent the proliferation of bacteria of a spoilage or pathogenic nature only applies to substances that comply with requirements of the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972) furthermore, chemical antimicrobial substances to be used should have been approved per protocol by a provincial executive officer.

2.6 Detection and enumeration of microorganisms

Routine microbiological meat analysis can be essential in determining the rejection/acceptance of meat products, the microbial condition of meat products and the evaluation of hazard based management strategies (Gill, 2007). This section will aim to discuss the advantages and disadvantages of both conventional and molecular methods.

Conventional methods are based on the idea of enumerating bacteria in a food matrix (agar), a pre-enrichment stage and the isolation of presumptive colonies on solid agar. Conventional methods provide a sense of familiarity among users, are easy to use, have a high level of acceptance by food processors and are still cost effective (Gracias & McKillip, 2004). Familiarity of these methods is still one of the main reasons why traditional methods are still being used in most food microbiology laboratories today. However, conventional methods can be limited by time: pre-enrichment of the food sample to increase viability of bacteria requires at least 18-24 hours. A pre-enrichment stage is done before enumeration of the bacteria. Furthermore, conventional methods tend to be labour intensive (Gracias & McKillip, 2004).

Molecular based methods allow for the detecting of bacteria through DNA amplification by polymerase chain reaction (PCR). PCR is a molecular method that will allow for rapid detection, is sensitive and allows for the specific identification of bacteria. However, molecular methods such as PCR have some limitations; PCR cannot quantify the amount of bacteria present in food samples and it sometimes gives false negative/positive results and the accuracy of the method can be influenced by the equipment and reagents used for the method (Gracias & McKillip, 2004).

2.7 Conclusion

Microbial quality on game carcasses can be influenced by the environmental contaminants, which introduce bacteria to the skin of the animal. Cross contamination from the hide to the carcass can result in both pathogenic and spoilage bacteria being present in game meat. Personnel and equipment used during processing can aid further contamination of carcasses. Once contaminated, food safety and shelf-life of meat products becomes a concern. The challenge of supplying game meat that is safe and has a minimal bacterial load can prove to be difficult as certain aspects of game production need to be considered (harvesting in the field, then transporting partially dressed carcasses to meat processing facilities). If contamination does occur during the harvesting/slaughtering of game meat, pathogenic microorganisms such as *E. coli* can be

enumerated during microbial analysis. Very little research has been published on spoilage bacteria in relation to game meat under South Africa conditions; therefore further research in this area is warranted. However, spoilage species within the *Enterobacteriaceae* family can be expected to be part of the game meat's natural microbial load.

As discussed, there are many contaminants that could aid cross contamination as well as further contamination of carcasses and include wind, dust and poor execution of field tasks such as evisceration. It is then important that control measures such as inspections (ante- and post-mortem inspection), are executed efficiently and that everyone associated with game meat production (from point of harvest to deboning and processing of game meat products) is appropriately trained to be aware of hygiene and food safety.

Contamination of game meat should ideally be controlled from the harvesting site. An implementation of suitable intervention/decontamination techniques can play a significant role in decreasing contamination. Decontamination techniques presented above can be seen as viable options in decreasing bacterial loads on game carcasses. However, lack of electricity, hot water, poor working conditions (weak light in the field), understanding of how bacterial contamination actually occurs in the field, may make the implementation process difficult. Furthermore, the practicality and value of washing game carcasses in the field or washing of carcasses at arrival at the abattoir need further investigation. It can be concluded that the use of a chemical intervention would require the least amount of electricity and water. Therefore, the use of chemical treatments such as organic acids could be an option. Therefore, further investigations in determining the lowest efficient temperature for lactic acid, the mode of application and concentration of the lactic acid solution is warranted.

The use of both conventional and molecular methods to identify and quantify bacteria involved in contamination can prove to be better than using just one method, as very little information has been published about the microorganisms found on game meat from South Africa.

The next four chapters will focus on the hygiene status of game meat from the point of harvest to the production of retail game meat products. Additionally, factors that may cause a proliferation of bacterial growth (such as meat pH) need to be evaluated so as to see whether these are indeed points of concern that warrants interventions, whilst giving special consideration to quality characteristics such as meat colour, as colour plays a significant role in consumer acceptability of fresh meat.

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CHAPTER 3

Microbial quality of springbok (*Antidorcas marsupialis*) meat in relation to harvesting and production process

Abstract

Prevalent bacteria on springbok (*Antidorcas marsupialis*) carcasses were investigated. Twelve Springbok carcasses were swabbed around the incision area after in-field evisceration; carcasses were swabbed again after skinning and after chilling at the deboning area. Swab samples taken after skinning portrayed a presence of *Escherichia coli* (*E. coli*) (< 2.7 log cfu/cm^2) and *Enterobacteriaceae* counts of <2.5 log cfu/cm^2. Springbok carcasses swabbed after chilling indicated a high level of aerobic bacteria (<3.5 log cfu/cm^2), *Clostridium spp*. (<3 log cfu/cm^2) and lactic acid bacteria (LAB) (<2.8 log cfu/cm^2). In contrast, swab samples taken at the incision area tend to be lower in counts than samples taken at the processing plant. *Clostridium spp*. and LAB counts increased after springbok carcasses had been kept at 2˚C for 24 hours. Further investigation in the abattoir is warranted, as results obtained during the study indicate that contamination may be due to poor processing and hygiene practice in the processing plant.

3.1 Introduction

The consumption of game meat is increasing in South Africa and the world at large (Paulsen *et al.,* 2011, Hoffman, 2004). South Africa has a significant role in game meat importation as it imports up to 46% of its game meat from Namibia (van Schalkwyk *et al.*, 2010). SAMIC (2009) reported that 20% of fresh red meat consumed in South Africa during the winter is game meat. This increasing demand directly correlates to the need of having microbiologically safe game meat.

Animal carcasses can remain sterile when protected by the skin (Gill, 2007) however, the incision made for evisceration can expose the carcass to bacteria. Microbial load on carcasses can also be increased by cross contamination between the hide and carcass. The possibility of these contaminations occurring is exasperated in southern African harvested game species as these first steps occur in the field (Hoffman *et al.,* 2004, van Schalkwyk *et al.,* 2011). Furthermore, during the deboning process, carcasses can come into contact with dirty equipment and hands. The initial and contaminating microbial load can consist of both spoilage and pathogenic bacteria. Spoilage bacteria can result in undesirable changes in the fresh meat and meat products, such as off-flavours and odours. Pathogenic bacteria can result in food poisoning or food infection in humans.

This study aimed to determine bacterial loads that may be found on game carcasses from point of field harvest to deboning at a game abattoir. Selected micro-organisms were identified using ISO methods, so that major sources of contamination during processing could be identified.

3.2 Materials and methods

3.2.1 Sampling area and abattoir

Springbok carcasses were hunted at a privately owned game reserve, located 10 km away from Graaff-Reinet, South Africa. Springbok carcasses were shot in the head, bled out and eviscerated in the field (Figures 3.1 and 3.2, respectively). Incisions for evisceration on the stomach were made from the inside to the outside. Springbok carcasses were later taken to a field depot where the hooves and heads were removed (Figure 3.3) (Hoffman *et al.,* 2004, van Schalkwyk *et al.,* 2011).

Springbok carcasses were hung overnight in a cold truck to lower carcass temperature to <7°C, whilst transporting the carcasses to an abattoir. Springbok carcasses were refrigerated until skinning commenced at the abattoir/breaking plant, the following day (day 1). Springbok were skinned and visibly contaminated flesh was trimmed off, carcasses were then chilled further at <4°C for 24 hours and finally deboned in the deboning section of the plant, the next day (day 3 *post-mortem*).

Figure 3.1 Bleeding of Springbok carcasses in the field

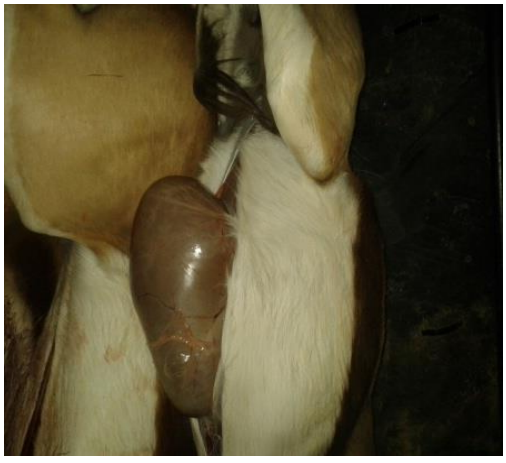

Figure 3.2 Evisceration of Springbok carcasses in the field

Figure 3.3 The removal of the head and hooves from Springbok carcasses at the field depot

3.2.2 Microbiological sampling

The springbok carcasses were sampled at the incision area (after evisceration); this included swabbing both the surrounding skin as well as the muscles exposed with the incision, after skinning and after chilling (at the deboning plant). At each stage the same 12 carcasses were swabbed. The tip of the sterile swab (Lasec, South Africa) was removed from its sterile wrapping, moistened with peptone water. The moistened tip of the swab was placed on the surface of the carcasses, primarily swabbing the rump, flank, brisket, neck, back of the carcass surface as well as around the belly where the incision was. Swabs were rotated between thumb and forefinger during swabbing. After swabbing, swabs were immediately placed back into their individual plastic holding tube. Swabs samples were collected over the 3 days were tested for the presence of *E. coli, Enterobacteriaceae, Clostridium* spp., lactic acid bacteria and aerobic bacteria.

3.2.3 Handling samples

Immediately after swabbing, swab samples were placed in sterile stomacher bags, for further protection and kept in a cooler box filled with ice. All samples were then transported to the research laboratory, where they were analysed.

3.2.4 Microbial analysis

A serial dilution was used for the enumeration of each microorganism. The following media were used: Plate count agar (PCA) (C6.500, Merck, Modderfontein, South Africa), Violet red bile glucose agar (VRBG) (CM0485, Oxoid, Hemisphere, England), Trypton Bile X-glucuronide (TBX) agar (Oxoid, CM0945), De Man Rogosa Sharp (MRS) agar (Merck, C86) and Reinforced Clostridia agar (Oxoid, CM0151) (Table 3.1). Growth media was prepared according to the specifications of the manufacturer. Media was poured carefully into pre-labelled petri dishes. Time and temperature specifications for the incubation of each microorganism were as indicated in Table 3.1. Plates were

inverted and incubated for each test. Reinforced Clostridia agar (Oxoid, CM0151) plates were incubated anaerobically using anaerobic jars and an anaerobic 3.5 L system (Oxoid, AN0035A).

Table 3.1 ISO methods, time and temperature specifications for microorganisms enumerated during microbial analysis of swabs.

Adapted ISO method	Media	Time and temperature	Microorganism
ISO 4833:1991	APC	48 hour at 30°C	Aerobic counts
ISO 21528-2:2004	VRBG	24 hour at 37°C	*Enterobacteriaceae*
ISO 16654:2001	TBX	24 hour at 44.5°C	*E. coli*
ISO 15214:1998	MRS	72 hour at 37°C	Lactic acid bacteria (LAB)
ISO 15213:2003	Reinforced Clostridia agar	48 hour at 37°C	*Clostridium* spp.

After the incubation period, colonies on plates were counted using a Suntex colony-counter 570 (Lasec, South Africa). Plates were examined under the light to avoid mistaking particles of matter for colonies.

3.3 Statistical analysis

Bacterial counts were converted to their logarithmic form for statistical analysis. Mean bacterial counts for each carcass at each sampling point of the slaughter process were determined. Mean bacterial counts were used to generate bar graphs, to visually show bacterial load from the 12 carcasses that were sampled at each sampling point of the slaughter process. Whiskers on graphs indicate standard error (S.E) of the means. A Shapiro-Wilks test for normality was done to determine the distribution of the data against a null hypothesis that the data is normally distributed. All parameters tested had normally distributed data, which warranted the use of parametric testing. An ANOVA was done and where significant differences ($p < 0.05$) were found, a Bonferroni test was used to determine variation between sampling stages (Figures 3.5, 3.7, 3.9 and 3.11). The statistical program, Statistica version 12 was used.

3.4 Results

3.4.1 Bacterial counts detected on springbok carcasses

3.4.1.1 *Enterobacteriaceae* counts

As seen in Figure 3.4 the *Enterobacteriaceae* counts found on the swabs used for the incision area ranged from 0.5 to >2.5 log cfu/cm^2 for the carcasses. After skinning, carcasses had higher *Enterobacteriaceae* counts than counts found for the incision area. After the carcasses were chilled in the cold room for 24 hours, 50% of carcasses had counts as high as 2.5 log cfu/cm^2. It was apparent that *Enterobacteriaceae* counts increased after skinning and after chilling (at the deboning plant). Therefore, *Enterobacteriaceae* contamination increased as the carcasses reached the processing plant. Due to high S.E in Figure 3.5, significant differences between sites were not prominent.

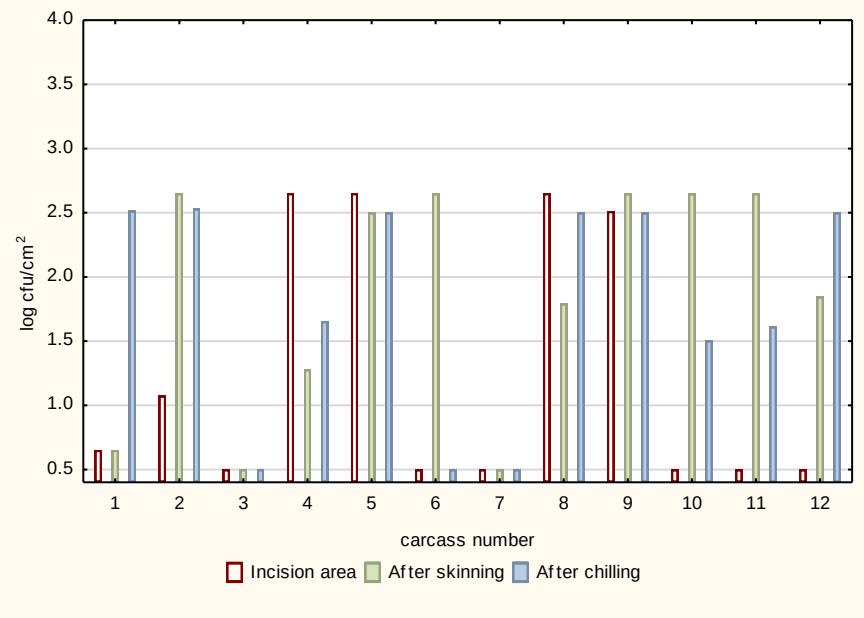

Figure 3.4 Mean *Enterobacteriaceae* counts in springbok carcass swab samples collected from the incision area at the field, after skinning and after chilling

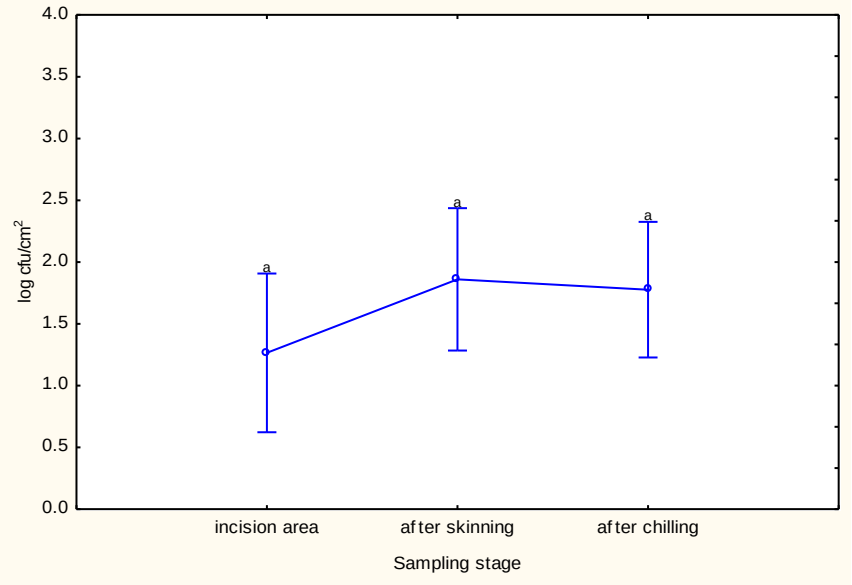

Figure 3.5 Mean *Enterobacteriaceae* counts enumerated from springbok carcass swab samples collected from the incision area at the field, after skinning and after chilling. (Whiskers indicate S.E. of the means and Letters on whiskers indicate significance differences $p < 0.05$ between sampling stages).

3.4.1.2 Aerobic plate counts (APC)

Aerobic growth was witnessed for the three sampling points, for all 12 springbok carcasses (Figure 3.6 and Figure 3.7). Incision area swabs and after skinning swabs had significantly lower counts when compared to counts obtained after chilling. Swabs taken after chilling at the deboning plant showed a significant increase in bacterial count.

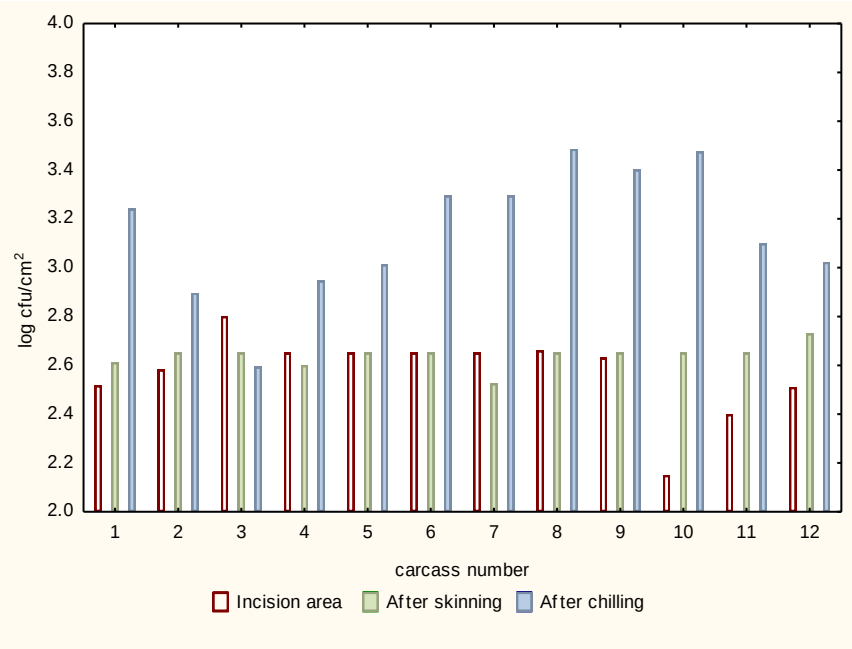

Figure 3.6 Mean aerobic plate counts in springbok carcass swab samples collected from the incision area at the field, after skinning and after chilling

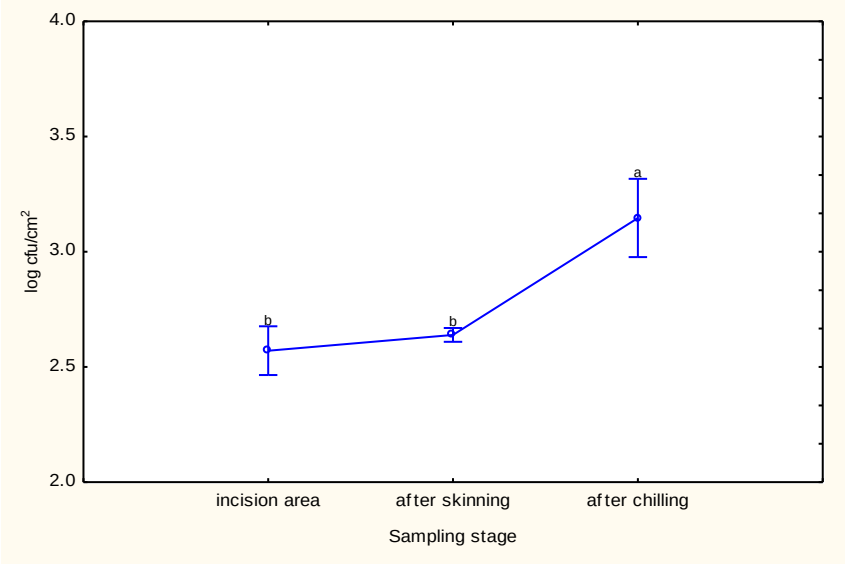

Figure 3.7 Mean aerobic plate counts enumerated from springbok carcass swab samples collected from the incision area at the field, after skinning and after chilling. (Whiskers indicate S.E. of the means and Letters on whiskers indicate significance differences $p < 0.05$ between sampling stages).

3.4.1.3 *Clostridium* spp.

An undeniable presence of *Clostridium* spp. was found on all 12 Springbok carcasses at all three stages (Figure 3.8). *Clostridium* spp. counts found from swab samples of the incision area and after skinning reached log counts of <2.7 log cfu/cm^2. However, samples taken after chilling at the deboning plant exhibited a significant increase in *Clostridium* spp. levels, as seen in Figure 3.9. The highest count at this sampling stage was ~2.9 log cfu/cm^2.

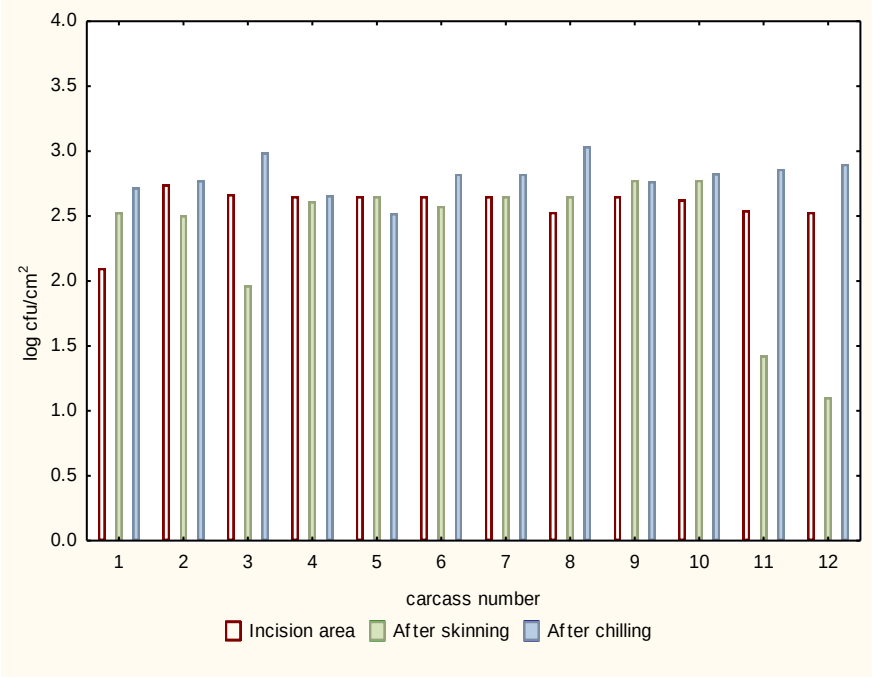

Figure 3.8 Mean *Clostridium* spp. counts in springbok carcass swab samples collected from the incision area at the field, after skinning and after chilling

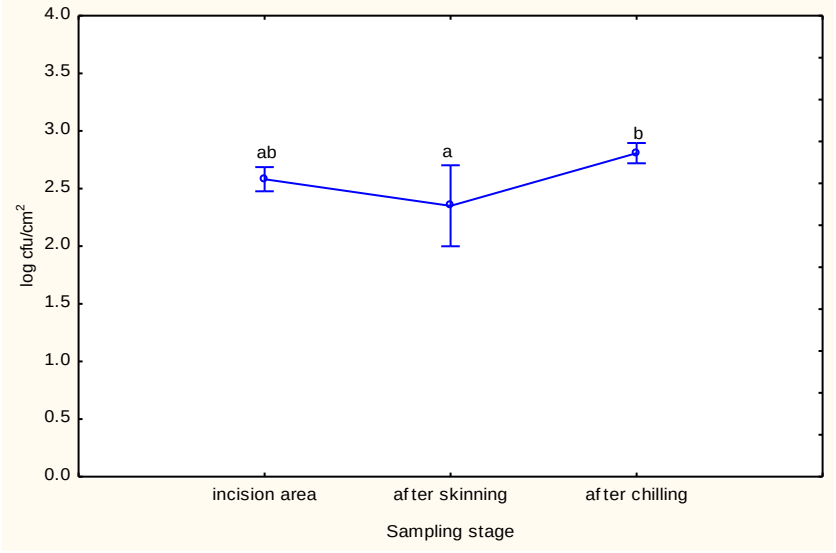

Figure 3.9 Mean *Clostridium* spp**.** counts enumerated from springbok carcass swab samples collected from the incision area at the field, after skinning and after chilling. (Whiskers indicate S.E. of the means and Letters on whiskers indicate significance differences $p < 0.05$ between sampling stages).

3.4.1.4 Lactic acid bacteria counts

Lactic acid bacteria was found on the springbok carcasses for the three sampling points (Figure 3.10). LAB counts found from swab samples of the incision area ranged from 1.5 log cfu/cm^2 to 2.8 log cfu/cm^2. Swab samples taken after skinning were also contaminated with LAB, with LAB levels ranging from 2.1 log cfu/cm^2 to 2.6 log cfu/cm^2. However, samples taken after chilling at the deboning plant exhibit a significant increase in LAB levels (Figure 3.11), where counts reached ~2.9 log cfu/cm^2.

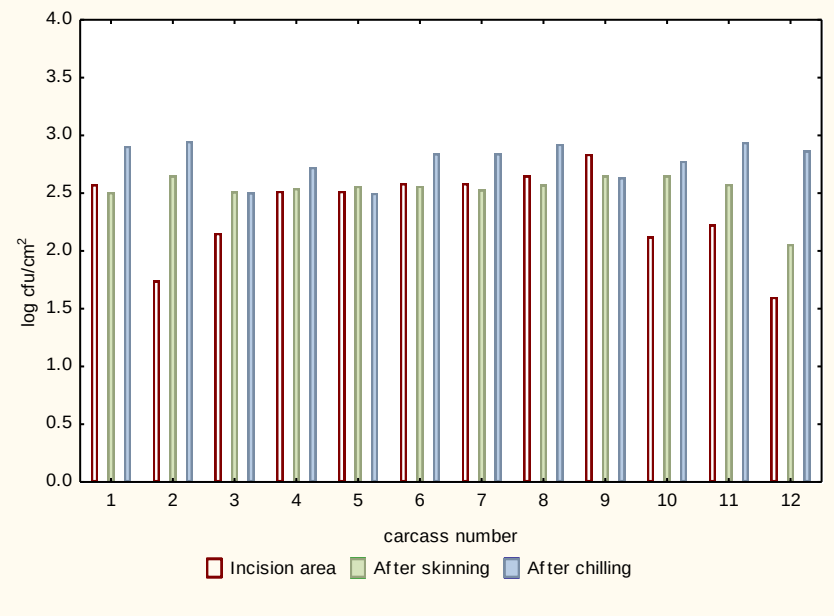

Figure 3.10 Mean LAB counts in springbok carcass swab samples collected from the incision area at the field, after skinning and after chilling

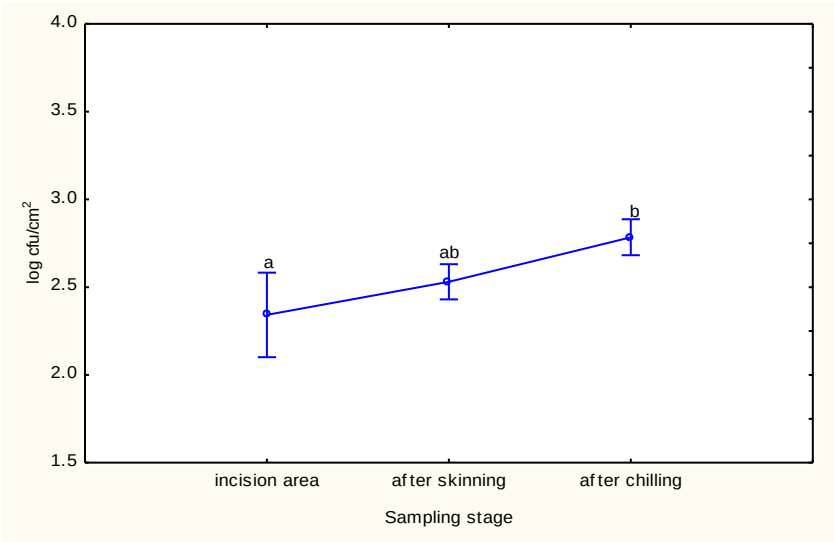

Figure 3.11 Mean LAB counts enumerated from springbok carcass swab samples collected from the incision area at the field, after skinning and after chilling. (Whiskers indicate S.E. of the means and Letters on whiskers indicate significance differences $p < 0.05$ between sampling stages).

3.4.1.5 *E. coli* counts

E. coli contamination was found on the Springbok carcasses for the last sampling point (Figure 3.8), at least 42% of carcasses sampled were contaminated with *E. coli* after skinning. *E. coli* was detected only at the deboning plant after chilling, where counts for some of the carcasses reached >2.5 log cfu/cm^2.

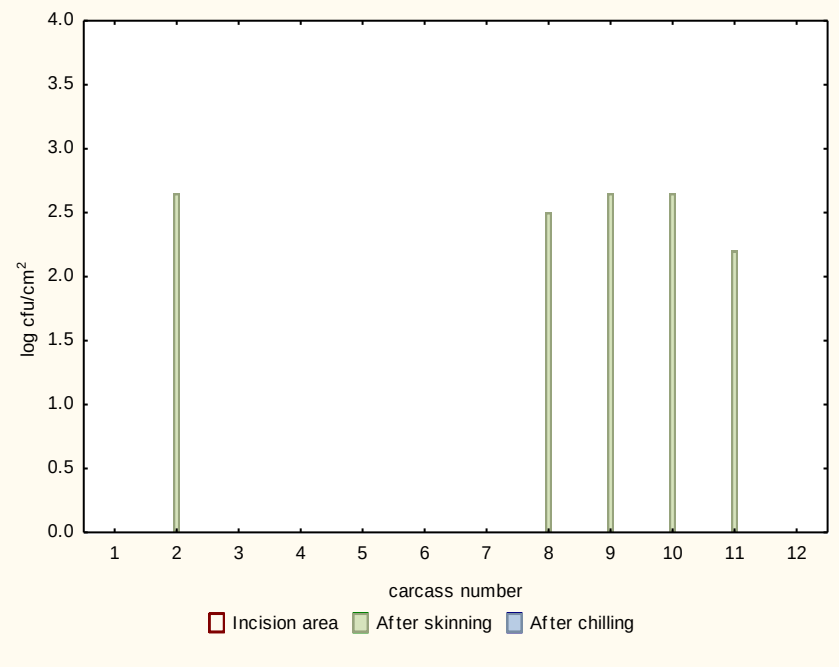

Figure 3.12 Mean *E. coli* counts in springbok carcass swab samples collected from the incision area at the field, after skinning and after chilling.

3.5 Discussion

The objective of this study was to determine the bacteria and bacterial counts that can be enumerated from game carcasses from the point of harvest. Therefore, this study was the first step in obtaining a practical view of the possible contamination during harvesting and slaughter process of game animals.

The flesh of a slaughtered animal is expected to be free of contamination, provided that the animal is healthy (Gill, 1997). However, once slaughter commences contamination can occur. The standard of harvesting and slaughter techniques can determine the extent of contamination. Like other livestock, as soon as the outermost protective barrier; the skin, is opened, contamination can occur.

From samples gathered after evisceration, at the incision area, *Enterobacteriaceae, Clostridium* spp., LAB and aerobic bacteria were enumerated. The presence of these microorganisms can be attributed to possible contamination from the skin, during the making of the incision, for evisceration of the white offal. Skin contamination can be from various factors such as

animals coming into contact with their own or other animals' faeces (typically as found during allo-grooming commonly practised by ungulates), lying in their own faeces to contamination being aided by dust in the field created by wild angulates moving in herds (Bell, 1997). It must also be noted that the enumeration of *Enterobacteriaceae* was irregular, and sometimes as low as 0.5 log cfu/cm^2. *E. coli* is part of the *Enterobacteriaceae* family therefore the lack of *E. coli* at this stage is no surprise (Cloete, 2010).

After skinning of carcasses, samples showed presence of *Enterobacteriaceae*, *Clostridium* spp., LAB, aerobic bacteria and even *E. coli*. However, contamination of carcass surfaces can be associated with improper execution of the skinning technique. Van Schalkwyk *et al.* (2011) reported that the transfer of bacteria from the hide to the surface of the carcass is a possibility during skinning. These carcasses were chilled when the skinning proceeded and it is more difficult to skin a cold carcass than a warm one. Therefore, it would seem that the skinning technique is important in determining the bacterial load on game carcass surfaces.

Contamination after chilling showed consistency and a slight increase in bacterial counts (a significant increase was seen for *Clostridium* spp. and aerobic counts). Contamination can be due to frequent exposure and handling of carcasses by the time the carcasses reach this point (Cloete, 2010). Contamination at this point can be a reflection of the adherence to hygiene practises during the slaughter process, at the abattoir.

3.6 Conclusion and Recommendations

Swab samples taken after skinning portray a presence and an increase of *E. coli* and *Enterobacteriaceae* counts. Similarly, swab samples taken after chilling portray a presence and an increase in aerobic bacteria *Clostridium* spp. and LAB. There is a clear indication of poor processing within the processing plant and possibly poor hygiene in the cold room. Therefore, further investigation of the abattoir is warranted. More specific sampling of carcass areas is needed, to provide more clarity on how the contamination occurs; this will be addressed in the next chapter which will deal with the contamination of game carcasses, namely black wildebeest carcasses, from the abattoir. Bacteria that were identified from this pilot study will be further investigated in the following chapter.

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CHAPTER 4

The microbial quality of black wildebeest (*Connochaetes gnou*) carcasses processed in a South African abattoir

Abstract

The aim of this study was to investigate the microbial quality of black wildebeest (*Connochaetes gnou*) carcasses from the point of slaughter until the deboning process, allowing for the determination of possible points of contamination during the slaughter process. Carcasses were sampled at three processing points (skin on, skin off and post chilling) at four different carcass sites (rump, flank, brisket and neck). Before skinning, aerobic bacteria, *Enterobacteriaceae*, and *E. coli* were enumerated from hide samples, counts ranged from 0.92 to 7.84 log cfu/g. After skinning the same bacteria were enumerated on the carcass, however a reduction in bacterial counts was seen as counts ranged from 0.93 to 6.12 log cfu/g. A further decrease in bacterial counts was seen after chilling of the carcasses. Significant differences between sites' bacterial loads were seen. For aerobic bacteria differences were prominent for flank (after skinning) and neck (after chilling), whilst for *Enterobacteriaceae* and *E. coli* noticeable differences between sites were noted for flank samples after chilling. *Clostridium* spp. were also enumerated from samples. *Clostridium* spp. showed an increase in counts after skinning, however, statistical analysis detected no significant differences between counts reported before and after skinning. All samples were tested for the presence of *Salmonella* spp., however samples were negative for *Salmonella* spp. The results from this study indicate that contamination does occur during the slaughter process.

4.1 Introduction

Over the years game meat has improved in popularity in South Africa. This popularity can be attributed to the fact that game meat is a nutritional source of high density protein. Hoffman (2003) indicated that at least 73% of Western Cape (South African Province) consumers interviewed had eaten game meat. Furthermore, an increase in suppliers of game meat has been recognised, where more and more game farmers are emerging in South Africa. Game farming has been deemed the fastest growing agricultural industry in South Africa. Game animals from wildlife ranches contribute to ecotourism, hunting, breeding, selling of game animals and meat production (Cloete, 2007). Van der Merwe & Saayman (2014) revealed the economic impact of game meat to be at least R2.6 billion in Limpopo province alone.

The apparent interest in game meat and the industry by both local and international market requires attention to be focused on the hygiene and microbial safety of game meat (van Schalkwyk & Hoffman, 2011). Hygiene and microbial safety of meat would normally be dependent on the slaughter process (Bell, 1997; McEvoy *et al.,* 2004; Galland, 1997). However, for game meat, hygiene status and microbial safety is dependent on both the harvesting and slaughter process. Handling and processing during harvesting and slaughtering can result in the spreading of

microorganisms. Ante- and post-mortem inspection are traditional methods aimed to detect contamination. However, visual inspection has shown short-comings along the years. Therefore, the implementation of programs based on the hazard analysis and critical control point (HACCP) approach have now become the norm in the formal meat industry (Loretz *et al*, 2011; McEvoy *et al.,* 2004). During the harvesting and slaughtering process, previously sterile muscle/meat can be contaminated by the hide and viscera (Gill, 2007). Additionally, equipment and personnel can aid the advancing of contamination. Hide and viscera can be a source of pathogenic bacteria which can be enumerated from carcasses (Bell, 1997).

The consumption of foodborne pathogenic bacteria can lead to foodborne diseases. As defined by the World Health Organisation, foodborne diseases are diseases that can be infectious or toxic in nature, which are caused by the consumption of contaminated food and/or water. The constant detection of pathogenic bacteria in food is still a major concern as contamination can cause human suffering and economic implications such as health care and production costs (Cloete, 2010).

Escherichia coli (E. coli) naturally occur in the gastro intestinal tract of animals and humans. The presence of *E. coli* in food and meat products can be an indication of faecal contamination. Contamination of carcasses with faecal matter can be traced to the slaughtering process for normal livestock such as cattle. However, for game meat, one must keep in mind that before slaughtering, animals are harvested. During harvesting, animals are eviscerated in the field, frequently under extreme environmental conditions such a strong winds, darkness, etc. Evisceration can lead to possible spillage of gastro intestinal content (Humphrey & Jørgensen, 2006), leading to the possible presence of *E. coli* on game carcasses*. E. coli*'s pathogenic ability was first experienced in 1982 (Tompkin, 2002) when insufficiently cooked hamburgers were consumed. *E. coli* contamination over the years has been associated with meat, meat products, vegetables and water contamination (Jay, 2005).

Salmonella spp*.* occurs naturally in the gut flora of mammals. *Salmonella* spp. are the main cause of Salmonellosis; an ingestion of at least 10 -1 000 cells by a healthy individual can result in the trigger of Salmonellosis (Ohtsuka *et al.*, 2005). Not all *Salmonella* strains are pathogenic however, over the years *S. enteritidis* has been reported as a major foodborne pathogen in Europe and North America (Adams & Motarjemi, 1999). *Salmonella* spp*.* has been associated with contamination of pigs, poultry and cattle carcasses. Red meat such as game meat (found in the southern part of Africa) is rarely contaminated (Magwedere *et al.,* 2013). If ingested, it has been reported that an individual can experience abdominal cramps, nausea, vomiting and diarrhoea (Ohtsuka *et al.*, 2005).

Aerobic plate counts (APC) of the slaughter process can be an indication of the hygienic care taken during the slaughter process. APC can also indicate the efficacy of the cleaning process and contamination aided by the raw products (such as meat). For game carcasses, high APC can be an indication of contamination of carcass-surfaces that typically occurred during skinning and/or evisceration (Grau, 1986).

The enumeration of *Enterobacteriaceae* and Coliforms is interchangeable within the food industry, as a high correlation between coliforms and *Enterobacteriaceae* exists as noted in a study by the European Economic Community (EEC) on poultry carcasses (Simonsen, 1989). The presence of *Enterobacteriaceae* can also be an indication of faecal contamination (Jay, 2005).

Clostridium spp. can be associated with spoilage of fresh, chilled and vacuum packed red meat. The presence of spoilage *Clostridium* spp. strains can result in a shortened shelf life of red meat products. It becomes imperative that Good Management Practices (GMP) are adhered to during harvesting, slaughter and deboning processes so as to ensure minimum spoilage of the meat and meat products (Adam & Brülisauer, 2010).

This study investigates the microbial quality of game, namely black wildebeest (*Connochaetes gnou*) carcasses from the point of slaughter until the deboning process, allowing for the determinations of possible points of contamination. Additionally, through this study the differences in bacteriological load amongst sample sites will be determined.

4.2 Materials and Method

4.2.1 Abattoir

A South African game abattoir in Graaff-Reinet received chilled black wildebeest carcasses that were partially opened, unskinned and eviscerated; these animals had been harvested commercially utilising procedures described by Hoffman & Laubscher (2004). At the abattoir, black wildebeest carcasses were processed in the following manner: partially dressed carcasses were delivered hanging by an Achilles heel in a sealed cold truck (carcass temperature ≤7°C) to the abattoir. Game carcasses are then physically offloaded and handled by staff and moved to cold storage, in order to effectively decrease carcass temperatures and slow the growth rate of bacteria. Once carcasses were effectively cooled (≤7°C) they were physically moved, hung (Achilles heel) and weighed before the skin from the hind leg was split open with a knife and loosened. Game carcasses moved along the ‘dirty area’ and the skin was loosened further working from the hind leg in a cranial (downwards) direction and from the evisceration slit in a dorsal direction. At this point, at least half of the carcass was skinned (hind leg to brisket); the loosened skin was then attached to a roller situated at the dorsal side of the carcass and the hide was then completely pulled off. The neck part of the carcass was the most exposed to potential contamination, as the heads were removed at the field abattoir, therefore visibly contaminated carcasses areas (such as the neck) were trimmed off. Carcasses were then stamped for traceability purposes and halved, to ensure that the carcasses could be stored overnight in the chillers without touching the floor of the chillers.

After chilling overnight, the skinned carcasses were moved from the chiller to the deboning room, a separate facility from the one described above. Dressed, skin-off carcasses were moved by staff to a cold truck (0 - 4°C) which transported the carcasses to the deboning plant. Carcasses were moved by staff, from the cold truck to the deboning room’s chiller (0 - 7°C). The chiller doors, opened

directly into the deboning area. Carcasses were then physically moved from the chiller to the deboning area (which is maintained at a temperature of ≤10°C). The deboning plant has a quality management system plan in place and standard hygiene practises (such as hand washing, cleaning of surfaces, etc.) that are adhered to. Along the whole slaughter line, carcasses were moved manually.

4.2.2 Black wildebeest sample collection

For this study, six carcasses were chosen to be sampled pre-skinning and post-skinning and post-chilling (post-chill samples were collected the next day, during the deboning of the carcasses), these carcass were tracked, to ensure that the same carcasses were sampled at each point. The six chosen carcasses were sampled at the demarcated positions, as shown in Figure 4.1 ((6 carcasses x 4 sampling positions) x 3 sampling process stages = 12 samples per carcass; 72 samples in total).

On the first day, pre-skinning (stage 1) and post-skinning (stage 2) sampling of the six chosen carcasses occurred. Pre-skinning samples were taken in the following manner: whilst hanging and during the skinning process, 10g of skin (an area of at least 100 mm^2 was excised to ensure that back up samples if analysis had to be repeated) per sampling position was aseptically removed at the four sampling sites shown in Figure 4.1. For post-skinning samples, the same six carcasses were sampled. Immediately after skinning, 10g of meat (a sterile cork borer of 25 mm with a surface area of 5 cm^2 was used to obtain pieces of meat) were aseptically removed from the demarcated sampling sites (Pepperll *et al.,* 2005). Post-chilling (stage 3) samples were collected in a similar manner from the same chosen carcasses; however, sampling took place on the second day, as carcasses were cooled overnight at ≤ 4°C. Once carcasses arrived at the deboning plant, pieces of meat were aseptically removed from the demarcated sampling sites (Figure 4.1).

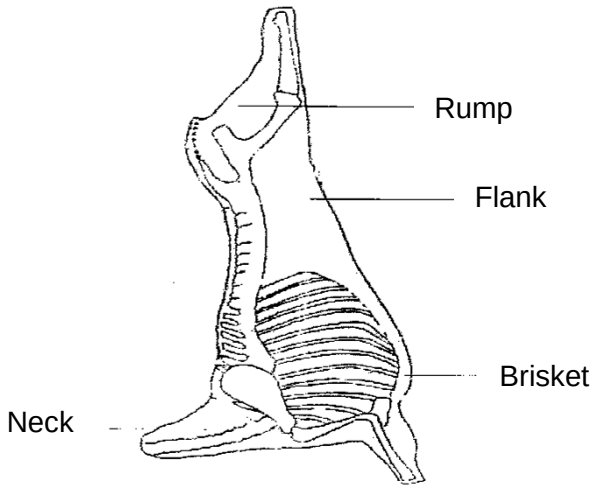

Figure 4.1 Sampling positions on large game carcasses (South Africa, 2010)

Collected samples were kept in polystyrene containers with ice to ensure that sample temperature did not exceed 2°C. On arrival at the lab samples were then frozen at -18°C until the microbial analyses could commence. Table 4.1 is a summary of the sampling procedure and microorganisms enumerated from the 72 samples.

Table 4.1 Summary of processing points, sampling positions and microorganisms tested

Sampling points/stages	Number of carcasses	Sample sites	Microorganisms enumerated
• Before skinning • Post-skinning • Post-chilling	6	• Rump • Flank • Brisket • Neck	• APC • *Enterobacteriaceae* • *E. coli* • *Salmonella* spp. • *Clostridium* spp.

4.2.3 Sample preparation

Collected skin samples (~10 g) were blended in 90 ml buffered peptone (Oxoid, CM1049) water (20% m/v) for 30 seconds using a Seward stomacher 400 (Seward Medical UAC House, United kingdom) at room temperature, prior to blending, skin samples were left to simmer in peptone for 30 minutes. Individual meat samples (~10 g) were homogenised in 90 ml Buffered peptone water (20% m/v) solution (Oxoid, CM1049) for 30 seconds using a Seward stomacher 400 (Seward Medical UAC House, United kingdom) at room temperature. Buffered peptone water was prepared according to the manufacturer's instructions: 20 g of peptone powder in 1 litre of distilled water. Buffered peptone solution was then autoclaved at 121°C for 20 minutes.

4.2.4 Conventional detection of microorganisms

4.2.4.1 Aerobic Plate Counts (APC)

Serial dilutions were prepared from the suspension made above using peptone buffered water. The pour plate method with Plate count agar (PCA) (C6.500, Merck, Modderfontein, South Africa) was used. Pour plates were duplicated. Poured plates were left to solidify at room temperature where after the plates were incubated at 30°C for 72 hours. After the incubation, colonies were counted, using a Suntex colony-counter 570 (Lasec, South Africa). Plates were examined under a light to avoid mistaking particles of matter for colonies.

4.2.4.2 *Enterobacteriaceae* Counts

A serial dilution was prepared and poured into duplicate plates, using Violet red bile agar (VRBG) (CM0455 Oxoid, Hemisphere, England). Plates were left to solidify at room temperature before a second layer of VRBG was poured and again left to solidify. Pour plates were then incubated at 37°C for 24 hours. All dark pink to red colonies were counted, using the Suntex colony-counter 570; accurate counting was assisted with a bright light.

4.2.4.3 *E. coli*

A serial dilution for the enumeration of *E. coli* was first prepared. Secondly, pour plates were made using Trypton Bile X-glucuronide (TBX) (Oxoid, CM0945) agar. Pour plates were incubated at 45-50°C for 24 hours. Colonies that were blue/green in colour were counted using the Suntex colony-counter 570.

4.2.4.4 *Clostridium* spp.

A serial dilution was prepared for the enumeration of *Clostridium* spp. Pour plates were made with reinforced clostriadial agar (Oxoid, CM0151). After solidification, an over-layer was poured where after the plates were left to solidify before incubating. Plates were incubated at 37°C for 48 hours in an anaerobic environment. A carbon dioxide rich environment was created using anaerobic jars and an anaerobic 3.5 L system (Oxoid, AN0035A). Colonies were counted using the Suntex colony-counter 570. The presence of *Clostridium* spp. was confirmed using a RapID system (Ana II) (Oxoid, R8311002) (Hoffman *et al.*, 2010). Confirmation was done in the following manner: RapID system (Ana II) vials with 2 ml inoculation (Oxoid, R8325106) fluid were inoculated with pure *Clostridium* spp. Colonies; addition was continued until turbidity matched the turbidity of the McFarland equivalent turbidity standard. Using a glass pipette, the turbid inoculation was pipetted into the RapID Ana II identification panel, which had dehydrated reagents in the wells. The dehydrated reagents allowed for biochemical identification reactions to take place. The fluid was then evenly distributed through tilting the panel to the side (tilting can also help to eliminate air bubbles), the panel was then tilted forward to fill up the wells. The panels were then incubated allowing for the dehydrated agents to dissolve and react with organisms present in the inoculation fluid. When organisms reacted with the agents, reactions that led to colour changes occurred. Visible colour changes allowed for easy interpretation. The panels were incubated for 4 hours at 37°C. After incubation RapID colour guides for Ana II RapID system were used to score each panel, scores were captured into Electronic RapID Compendium, commonly referred to as ERIC; a windows program that has the ability to process micro codes for RapID systems and give possible microorganisms.

4.2.4.5 *Salmonella* spp.

To test for *Salmonella* spp., 1 ml of the initial dilution was dispensed into a tube with 10 ml Muller-Kauffmann-Tetrathionate (MKTTn, Oxoid CM1048) broth and incubated at 35°C for 24 hours. Another 0.1 ml of the initial sample was dispensed into a second tube with 10 ml of a Rappaport-Vassiliadis media with soya (RVS, Oxoid CM0866) broth and incubated for 24 hours at 37°C and 41.5°C, respectively. After incubation, MKTTn and RVS tubes were mixed using a vortex mixer and streaked onto *Shigella/Salmonella* (SS, Oxoid CM0099) agar and Xylose Lysine deoxycholate (XLD, Oxoid CM0469) agar. SS and XLD streaked plates were incubated for 24 hours at 35°C and 37°C, respectively. XLD plates were checked for the presence of colonies that have a black centre and a lightly transparent zone of reddish colour. SS plates were checked for the presence of colonies that are black or sometimes black centred.

Table 4.2 is a summary of ISO methods and incubation requirements for each microorganism enumerated from samples gathered before skinning, post-skinning and post-chilling (sampled at the deboning plant).

Table 4.2 ISO methods, time and temperature specifications for microorganisms enumerated during microbial analysis of samples.

Adapted ISO method	Media	Time and temperature	Microorganism
ISO 4833:1991	APC	48 hour at 30°C	Aerobic counts
ISO 21528-2:2004	VRBG	24 hour at 37°C	*Enterobacteriaceae*
ISO 16654:2001	TBX	24 hour at 44.5°C	*E. coli*
ISO 15213:2003	Reinforced Clostridia agar	48 hour at 37°C	*Clostridium* spp.

4.2.5 Carcass pH measurements

The pH of carcasses was measured by inserting a glass probe in the middle of the *Longissimus dorsi* (LTL) (loin) muscle. A Testo (230) pH meter (with a probe) (Cape Instrument Services Cc) was used. The meter was calibrated using the standard buffers as provided by the manufacturer.

4.2.6 Statistical analysis

Bacterial counts were converted to logarithmic form for statistical analysis. To show highly contaminated carcass sites at each processing point, log means of rump, flank, brisket and neck were determined and subjected to an analysis of variance (ANOVA). Where significant differences were found between carcass sites (per processing point), a post-hoc Fisher's LSD test was done to determine variation between the sample sites for each operational point (Tables 4.3, 4.4 and 4.5).

To show the progression of contamination through the slaughter process, graphs were generated, log mean counts of each carcass site were plotted at stage 1, 2 and 3. An ANOVA was done and where significant differences were found, a Fisher's LSD test was used to determine variation between stage 1, 2 and 3 (Figures 4.2, 4.3 and 4.4). A Shapiro-Wilks test for normality was done to determine the distribution of the data against a null hypothesis that the data is normally distributed. All parameters tested had normally distributed data, which warranted the use of parametric testing. The statistical program, Statistica version 12 was used.

To show positive results *E. coli* and *Clostridium* spp., tables were constructed according to processing points/stages and carcass site. Percentages were determined to show increase or reduction in occurrence.

4.3 Results

In this study the microbiological status of game, specifically black wildebeest carcasses processed in a South African game abattoir was evaluated. This sampling was conducted during the winter month of October. The results below will report on aerobic bacteria, *Enterobacteriaceae*, *E. coli* and *Clostridium* spp. counts from fresh black wildebeest carcasses. Additionally, the presence/absence of *Salmonella* spp. was investigated. Tables and figures refer to the three processing points/stages, namely before skinning, post- skinning and post chilling as stage 1, 2 and 3, respectively. Some results were converted from cfu/g to cfu/cm^2 in order to allow comparison with data found from other studies. The following equation was used: log cfu/cm^2 = log cfu/g – 0.633 (Alonso-Calleja *et al.,* 2004).

4.3.1 Bacterial counts

4.3.1.1 Aerobic Plate Counts (APC)

As seen in Figure 4.2, all sites sampled showed a reduction in APC. Skin samples had the highest APC. APC decreased after skinning and a further reduction in APC was seen after cooling of carcasses. This trend was seen for all sampled sites.

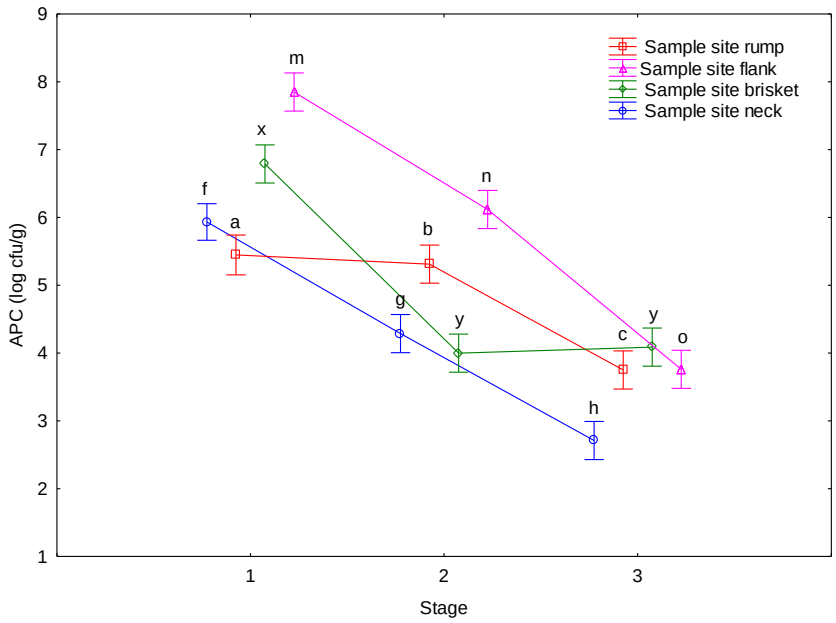

Figure 4.2 Mean Aerobic plate counts enumerated from rump, flank, brisket and neck at stage 1 (n = 24), 2 (n = 24) and 3 (n=24) (whiskers indicate SD of the means and Letters on whiskers indicate significance differences within sampling sites at $p < 0.05$).

The log means for the APC enumerated from the rump, flank, brisket and neck samples (Table 4.3) at the three different processing stages, namely, before skinning, post-skinning and post-chilling (at the deboning plant) are displayed in Table 4.3. From the table it can be seen that there were differences between sites sampled at the three stages. Differences were more prominent on the skin and after chilling, as skin samples obtained from the flank, had the highest APC and neck samples had the lowest counts after chilling.

Table 4.3 Log mean counts (log cfu/g ± SD) for aerobic bacteria enumerated from rump, flank, brisket and neck samples before skinning (n = 24), post-skinning (n = 24) and post-chilling (n = 24).

Processing stages	Rump	Flank	Brisket	Neck
Before skinning	5.45^{d} ±0.282	7.84^{a} ± 0.204	6.79^{b} ± 0.421	5.93^{c} ± 0.787
	(4.82)*	(7.21)	(6.16)	(5.30)
Post-skinning	5.3^{d} ± 0.427	6.12^{c} ± 0.576	4.00^{ef} ± 0.427	4.29^{e} ± 0.460
	(4.68)	(5.49)	(3.37)	(3.66)
Post-chilling	3.75^{f} ± 0.682	3.76^{f} ± 0.450	4.09^{ef} ± 0.450	2.7^{g} ± 0.398
	(3.12)	(3.12)	(3.46)	(2.08)

[a,b] Different superscript letters indicate that values were significantly different when compared to each other ($p < 0.05$)
*Values in brackets are mean values in log cfu/cm^2

Enterobacteriaceae

Enterobacteriaceae counts along the slaughter process for the rump, flank and neck samples are depicted in Figure 4.3. Skin samples had the highest *Enterobacteriaceae* count. After skinning *Enterobacteriaceae* counts decreased for all sampled sites. After chilling a further decrease in *Enterobacteriaceae* counts was seen for the rump, flank and neck samples. In contrast, the brisket samples showed an increase in *Enterobacteriaceae* counts after cooling.

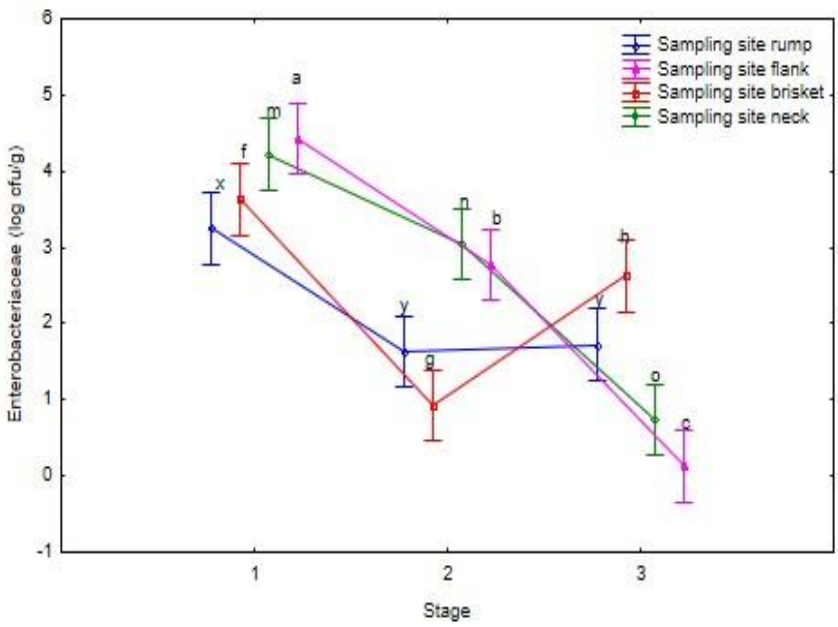

Figure 4.2 Mean *Enterobacteriaceae* counts (log cfu/g) enumerated from rump, flank, brisket and neck at stage 1 (n = 24), 2 (n = 24) and 3 (n=24) (whiskers indicate SD of the means and letters on whiskers indicate significance difference within sampling sites at $p < 0.05$).

The log means for the *Enterobacteriaceae* enumerated from the rump, flank, brisket and neck samples at three different processing points, namely, before skinning, post-skinning and post-chilling (at the deboning plant) are depicted in Table 4.4. Differences between sites were also observed for *Enterobacteriaceae* counts. These differences were more noticeable after chilling, as flank samples had lowest *Enterobacteriaceae* counts when compared to the other sites.

Table 4.4 Log mean *Enterobacteriaceae* counts (log cfu/g ± SD) enumerated from rump, flank, brisket and neck samples before skinning (n = 24), post-skinning (n = 24) and post-chilling (n = 24).

Processing stages	Rump	Flank	Brisket	Neck
Before skinning	3.25^{cd} ± 1.599 (2.62)*	4.43^{a} ± 0.426 (3.80)	3.63^{cb} ± 0.535 (3.00)	4.22^{ab} ± 0.387 (3.59)
Post-skinning	1.64^{e} ± 0.840 (1.01)	2.78^{d} ± 0.666 (2.15)	0.93^{f} ± 0.779 (0.30)	3.04^{cd} ± 0.482 (2.41)
Post-chilling	1.73^{e} ± 0.970 (1.10)	0.13^{g} ± 0.450 (0)	2.62^{d} ± 0.750 (1.99)	0.74^{fg} ± 1.121 (0.11)

^{a,b} Different superscript letters indicate that values were significantly different when compared to each other ($p < 0.05$)
*Values in brackets are mean values in log cfu/cm^2

4.3.1.2 *Clostridium* spp.

As seen in Figure 4.4, statistical differences were not detected between log means of *Clostridium* spp. counts from brisket at all three stages even though the typical trend of a low number, increasing after skinning and then decreasing again after chilling is seen. It is postulated that the means from the different sites did not differ statistically ($p > 0.05$) from each other due to the high SD within sites.

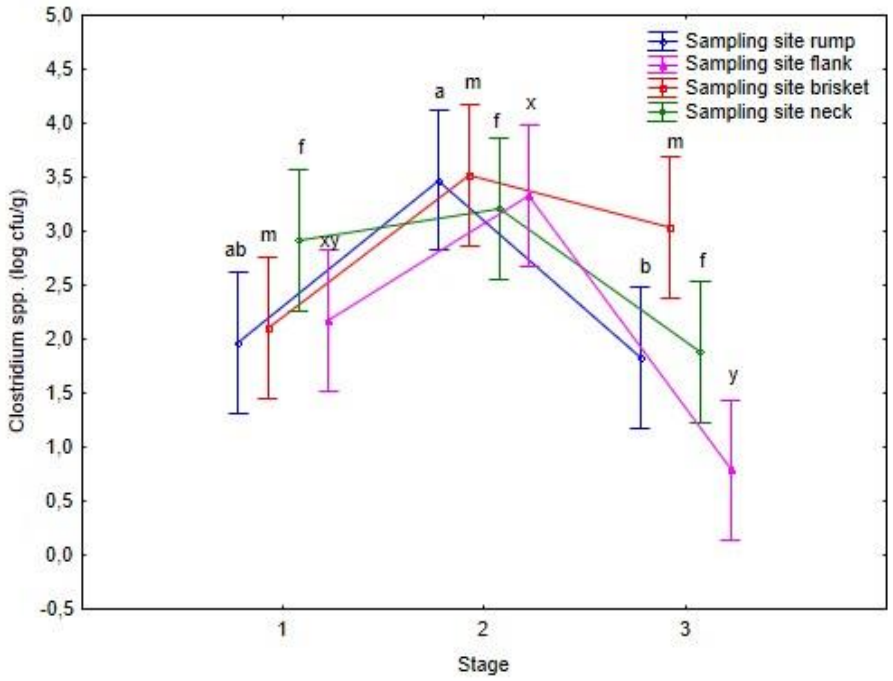

Figure 4.3 Mean *Clostridium* spp. counts (log cfu/g) enumerated from rump, flank, brisket and neck at stage 1 (n = 24), 2 (n = 24) and 3 (n=24) (whiskers indicate SD of he means and Letters on whiskers indicate significance difference).

The log means for the *Clostridium* spp. enumerated from the neck, rump, brisket and flank samples at three different processing points are reported in Table 4.3. Due to high S.D, significant differences between sites were not prominent. However, significant differences were seen for flank samples after chilling, flank samples were lowest in *Clostridium* spp. counts when compared to the other sites.

Table 4.3 Log mean counts (log cfu/g ± SD) of *Clostridium* spp. enumerated rump, flank, brisket and neck samples before skinning (n = 24), post-skinning (n = 24) and post-chilling (n = 24).

Processing stages	Rump	Flank	Brisket	Neck
Before skinning	1.97^{bc} ± 1.853 (1.34)	2.17^{bc} ± 1.435 (1.54)	2.11^{bc} ± 1.698 (1.48)	2.92^{ac} ± 1.688 (2.29)
Post-skinning	3.47^{a} ± 1.130 (2.84)	3.33^{a} ± 0.449 (2.70)	3.52^{a} ± 0.212 (2.89)	3.21^{a} ± 0.524 (2.58)
Post-chilling	1.83^{c} ± 1.178 (1.20)	0.79^{d} ± 0.979 (0.16)	3.04^{ab} ± 0.481 (2.41)	1.88^{c} ± 0.672 (1.25)

^{a,b} Different superscript letters indicate that values were significantly different when compared to each other ($p < 0.05$)
*Values in brackets are mean values in log cfu/cm^2

Table 4.4 displays the frequency of contamination at the three processing points. An increase in the number of samples contaminated can be seen after skinning, and a slight decrease in the

number of contaminated samples is observed after chilling. *Clostridium* spp. were enumerated from all samples. Through RapID tests, the presence of *Clostridium* spp. was confirmed and the type of *Clostridium* spp. was identified as *C. innocuum* and *C. subterminate*.

Table 4.4 *Clostridium* spp. occurrences for rump, flank, brisket and neck samples collected in a South African game abattoir before skinning (n = 24), post-skinning (n = 24) and post-chilling (n = 24).

Cut	Before skinning	Post-skinning	Post-chilling
Rump	100%[1] (6/6)[2]	100% (6/6)	100% (6/6)
Flank	66.7% (4/6)	83.3% (5/6)	16.7% (1/6)
Brisket	100% (6/6)	100% (6/6)	100% (6/6)
Flank	66.7% (4/6)	83.3% (5/6)	16.7% (1/6)

[1]Percentage of positive samples for each sample area and processing point
[2]Number of samples tested at each point that were positive for *Clostridium* spp.

4.3.1.3 *E. coli*

The prevalence of *E. coli* is displayed in Table 4.5. In terms of processing points, out of the 24 samples collected before skinning 83% samples were contaminated with *E. coli,* post-skinning 100% samples were contaminated and post-chilling, 83% of the samples were contaminated.

Table 4.5 *E.coli* spp. occurrences for rump, flank, brisket and neck samples collected in a South African game abattoir before skinning (n = 24), post-skinning (n = 24) and post-chilling (n = 24).

Cut	Before skinning	Post-skinning	Post-chilling
Rump	66.67%[1] (4/6)[2]	100% (6/6)	83.33% (5/6)
Flank	83.33% (5/6)	100% (6/6)	50% (3/6)
Brisket	83.3% (5/6)	100% (6/6)	100% (6/6)
Neck	100% (6/6)	100% (6/6)	100% (6/6)

[1]Percentage of positive samples for each sample area and processing point
[2]Number of samples tested at each point that were positive for *E.coli*

E. coli counts from the rump, flank, brisket, and neck samples from the three processing points, namely, before skinning, post-skinning and post-chilling (at the deboning plant) are displayed in Table 4.6. Samples showed a trend of increasing after skinning. Rump and neck samples showed a further decrease in *E. coli* counts after cooling, whilst brisket samples showed an increase after cooling, however statistical differences ($p > 0.05$) were not detected between sites, possibly due to the high S.D.

Table 4.6 Log mean *E. coli* counts (log cfu/g ± SD) enumerated from rump, flank, brisket and neck samples before skinning, post-skinning and post-chilling.

Processing stages	Rump	Flank	Brisket	Neck
Before skinning	2.53 ^{ab} ± 1.685 (1.90)	1.09 ^{cd} ± 1.520 (0.46)	2.89 ^{a} ± 0.489 (2.26)	1.08 ^{cd} ± 1.492 (0.45)
Post-skinning	1.60 ^{cd} ± 0.857 (0.97)	1.51 ^{cd} ± 1.251 (0.88)	1.19 ^{cd} ± 0.836 (0.56)	1.89 ^{cb} ± 0.690 (1.26)
Post-chilling	1.09 ^{cd} ± 1.097 (0.46)	0.08 ^{e} ± 0.289 (0)	2.48 ^{ab} ± 0.715 (1.84)	0.74 ^{ed} ± 1.121 (0.11)

^{a,b} Different superscript letters indicate that values were significantly different when compared to each other ($p < 0.05$)
*Values in brackets are mean values in log cfu/cm^2

4.3.1.4 *Salmonella* spp.

None of the samples collected during the study at the three processing points were positive for *Salmonella* spp.

4.3.2 Carcass pH measurements

For this study only four ultimate (after a minimum of 24 hrs *post mortem*) pH readings of carcasses could be obtained as this specific abattoir does not routinely measure the pH of game carcasses; pH is not seen as a critical control point. Carcass pH values for carcass 1, 2, 3 and 4 were 6.45, 5.87, 5.75 and 5.74, respectively.

4.4 Discussion

The aim of this study was to investigate the microbial quality of fresh carcasses from a South African game abattoir. Additionally, this study also allowed for the identification of hazards along the slaughter line which could ultimately lead to develop robust control measures to eliminate or reduce these microbiological hazards.

The flesh of a healthy animal is expected to be uncontaminated. For normal livestock, contamination is said to be due to the slaughtering process, however for game meat, microbial contamination can start during harvesting and then continue through the slaughter process (Humphrey & Jørgensen, 2006). More specifically, microorganisms on the skin, gastro intestinal tract

and/or the bacteria found on the muscle tissue, personnel involved in the slaughter process and processing environment may contribute to the microbiological condition of game meat (Gill, 2007). Ultimately, the accurate execution of harvesting and slaughtering techniques can determine the extent of contamination. In this study, the microbiological quality of carcasses at three processing stages was evaluated, namely before skinning, post-skinning and post-chilling at the deboning plant, a total of 72 samples were gathered from the processing points, as summarised in Table 4.1.

The presence of microorganisms on the skin/hide of slaughter animals (Paulsen, 2011) is not an unfamiliar idea, so much so that the expected bacterial count for hide/skin is expected to be from at least 3.5-10 log cfu/cm^2. Even though the range given above is generic, this study was in agreement as bacterial counts reported were within this range. Furthermore, it is important to note the type of microorganisms that were enumerated from the skin, such as *E. coli*, *Enterobacteriaceae* and aerobic bacteria. The type of bacteria enumerated was similar to bacteria noted by El-Ghareeb *et al.* (2009) (*E. coli*, *Enterobacteriaceae* and aerobic bacteria) on wild birds (pheasants and quail). However, counts obtained by El-Ghareeb *et al.* (2009) ranged from 2-3 log cfu/cm^2, much lower than those reported in this study. Higher skin counts can be attributed to environmental contamination such as soil and possible faecal contact. Bell (1997) reported that hides that have been in contact with faecal matter generally can have higher bacterial counts. It is not unreasonable to assume faecal contact with the hide, as the presence of *Enterobacteriaceae* and *E. coli* indicate possible faecal contamination.

Slaughter operations such as skinning can allow for contamination of sterile flesh (Abdalla *et al.,* 2009). Present work revealed that *Enterobacteriaceae*, *E. coli* and aerobic bacterial counts were found on the carcass after skinning, therefore the skinning process can be identified as sources of contamination (Gill, 1998; Gill, 2007). However, bacterial counts on the surface of the carcass were lower than those reported from the skin. This is in agreement with the notion that bacteria transferred from skin to carcass surfaces during skinning is only a fraction of the bacterial load found on the skin of slaughter animals (Antic *et al.,* 2010).

During the investigation of freshly shot roe deer and red deer and wild boars' microbial quality in Germany, APC mean values were 2.6, 2.9 and 3.2 log cfu/cm^2, respectively (Membré *et al.* 2011); values much lower than that obtained from this study. Similarly, *E. coli* counts (0.7-1.2 log cfu/cm^2) reported by van der Merwe *et al.* (2013) were lower than those found for this study. However, *Enterobacteriaceae* counts of 2.1 log cfu/cm^2 reported by Membré *et al.* (2011) were closer to those reported for this study. Membré *et al.* (2011) attributed high bacterial counts to the standard of techniques used during the harvesting and slaughter process. Furthermore, slaughtering of large game animals such as black wildebeest (~150 kg body weight delivering a carcass weight 74-85 kg (Hoffman *et al*. 2009)), compared to small game animals can prove to be more difficult to process (Paulsen & Winkelmayer, 2004). Furthermore, Atanassova *et al*. (2008) noted that low bacterial counts can be achieved by making sure that hunts are well organised and controlled.

The potential negative effect that night cropping has on the ability to adhere to hygiene principles has been well expressed. Poor conformance to hygiene practices during night cropping can be due to poor visibility due to inadequate lighting during evisceration in the field (van der Merwe *et al.,* 2013). Poor working conditions can allow for contamination. However, during harvest intact visceral components are removed and therefore visceral components did not contribute to the contamination; it is a SOP within this abattoir's process to ensure that any carcasses contaminated with intestinal contents be rejected during the initial inspection at the field depot. All the carcasses received in this investigation had already passed this initial inspection in the field.

Further reduction of bacterial counts was seen from samples taken post-chilling. This is in agreement with the idea that temperature does affect further growth of bacteria and bacterial counts determined (Vaarala & Korkeala, 1999). Low temperatures that are not conducive to bacterial growth can stunt the enumeration of viable bacteria from samples. However, even though bacterial counts decreased, final counts from samples collected post-chilling were still high when compared to values (0.315-2.93 log cfu/cm^2) reported by van der Merwe *et al.* (2013). Furthermore, the fact that microorganisms could be enumerated at this point shows that the hygiene practices of this slaughter process are not working towards eliminating harmful bacteria (van der Merwe *et al.,* 2013).

At least 72 samples were evaluated for the presence of *Salmonella* spp. which was not detected. Magwedere *et al.* (2013) also noted an absence of *Salmonella* spp. for springbok samples. The prevalence of *Salmonella* spp. in game meat has been reported to be rare. This was also confirmed by van der Merwe *et al.* (2013) where the analyses of 162 game meat samples from three different abattoirs showed an absence of *Salmonella* spp.

Clostridium spp. was enumerated from all samples. Through a RapID test, the presence of *Clostridium* spp. was confirmed and the type of *Clostridium* spp. was identified as *C. innocuum* and *C. subterminate*. *Clostridium* spp. are naturally found to be part of the environment and microorganisms found in the intestinal tract of animals. The occurrence of *Clostridium* spp. in game meat from South Africa has not been investigated yet. The lack of investigation can be due to the fact that regulations require the detection of only indicator microorganisms (*E. coli* and *Enterobacteriaceae* and *Salmonella* spp.) and hygiene specific microorganisms such as APC. However, the presence of these anaerobic microorganisms can result in spoilage of vacuum packed products. Spoilage can result in a significant financial loss. *Clostridium* spp. were enumerated from the skin, post-skinning and post-chilling samples. Counts statistically remained the same through the slaughter process however; log mean counts from samples after chilling were lower. This can possibly be due to an extended lag phase triggered by storing of game carcasses at a low temperature overnight (Zweifel *et al.,* 2005). A hostile growth environment can lead to an extended recovery time (during the lag phase) for the bacteria. Furthermore, exposure to an unfavourable environment can lead to reduction in viability, therefore lower enumeration of bacteria from samples (Gouws, 1998).

The distribution of aerobic bacteria, *Enterobacteriaceae*, *Clostridium* spp. and *E. coli* was also investigated. On partially opened carcasses (before skinning) aerobic bacteria, *Enterobacteriaceae*, *Clostridium* spp. and *E. coli* were enumerated on the skin at the rump, flank, brisket and neck. After skinning, it can be assumed that some bacteria were transferred onto the carcass surfaces; the neck had one of the highest *Enterobacteriaceae* and *E. coli* counts. Through visual observation, it was seen that the neck region was the bloodiest, hair deposit were also seen; this was most probably caused by in-field exsanguination. Immediately after being shot in the head, the animals are exsanguinated, usually with a two knife procedure. Visual observations agreed with Vaarala & Korkeala (1999) who reported that the neck area can be one of the dirtiest regions on a carcass. Furthermore, the carcasses are hung from the hind leg and thus the neck is the last region to be skinned, which can allow for accidental touching of the skin, due to vigorous movement of carcasses when the skin is pulled off. Additionally, as the neck was visibly contaminated, contaminated flesh was removed with a saw in the deboning plant as part of the standard operating procedures, however regular cleaning/sterilising of the saw, between each carcass was not observed.

The flank and brisket were also high in APC and *Clostridium* spp. after skinning. Through visual observations contamination of flank and brisket can occur through carcass surfaces (during skinning) coming into contact with the skin of neighbouring carcasses. Some of the skin was pulled off physically, resulting in hanging skin contaminating neighbouring carcasses. Furthermore, unwashed equipment used during skinning such as a hand held mechanical saw, knives and hands were observed to come into contact with skinned carcasses. The whole skinning procedures thus warrant further refining so as to try to minimise cross contamination.

Lastly, after chilling the brisket was high in aerobic bacteria, *Enterobacteriaceae*, *Clostridium* spp. and *E. coli*. After chilling contamination could be due to close stacking of carcasses in the chiller and/or due to the typical pushing and pulling of carcasses with the hand as it is easy to grab a carcass via the brisket to move it along the hanging rail. Also, more human contact with skinned carcasses when they were moved/carried from the chiller to the truck (during transportation to the deboning plant) could result in an increase in bacterial load.

At least one of the four carcasses measured a pH value considered to be high (>6.0). The high pH can be related to ante-mortem stress during harvesting (Hoffman & Dicks 2011; Kritzinger *et al.*, 2003, Gill; 2007). A high pH can result in the growth of both foodborne pathogens, spoilage bacteria and the occurrence of Dark Firm and Dry (DFD) meat (Hoffman & Dicks, 2011; Hoffman *et al.*, 2010). DFD meat is usually depleted in glucose, thereby forcing bacteria to exploit amino acids as the main source of nutrients. The use of amino acids can result in the production of by-products such as ammonia, which are responsible for spoilage symptoms such as off flavours and odours. However, for this study bacterial counts from the carcass with a high pH value (> 6) were not higher than those carcasses with normal pH values. Therefore, this was in agreement with van der Merwe *et al.* (2011), where pH values were found not to be associated with bacterial counts reported for

game carcasses. However, the question still remains whether meat with high pH values will deteriorate faster than meat with normal post-mortem pH readings; this aspect warrants further research.

4.5 Conclusion

This study contributes to the baseline information on the microbiological quality of game carcasses/meat from a South African abattoir. This study also indicated that there were significant bacteria deposited on the surfaces of fresh carcasses during the skinning. Skinning should therefore be executed with care so as to ensure minimum cross contamination, thereby improving the microbial quality of game carcasses. Overall, it can be concluded that the improvement and implementation of practical general hygiene practices in the field and in the abattoir are needed in order to improve the microbial quality of game carcasses. When implementing and ensuring good hygiene practices, it is important to consider the challenge of the poor working conditions faced in the field and the size of game animals received by the abattoir. Good microbial quality of carcasses will ultimately result in a better economic return for the abattoirs. In order to achieve the required results, it is important to consider the personnel directly involved in the harvesting and slaughtering of game carcasses. Personnel need to be properly trained in the basic hygiene procedures.

This study focused on only one abattoir in South Africa (although this is the largest game meat processing abattoir in the country) and contamination of large game carcasses. Therefore, further research in the microbiology of game carcasses from other abattoirs is required for a wider evaluation of the microbial quality of game meat, as the data present is still scanty; this study only provides a baseline. Furthermore, more pathogenic species in relation to game meat need to be studied. This study did not have field samples; contamination due to field tasks can only be speculated. Future research should aim to start sampling in the field, to effectively determine points of contamination and short comings of field procedures.

The next chapter will deal with hygiene levels of a game abattoir during the production of game products intended for retail.

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CHAPTER 5

Assessment of hygiene levels of raw game meat, surfaces, personnel, equipment and environmental factors during the production of game meat products

Abstract

To determine the hygienic status and compliance of hygiene practices during the production of game meat products, APC of raw game meat, retail game meat products, meat handlers (hands), surfaces, and equipment that came into contact with meat during production were determined. Air and water quality during production was also assessed. Bacterial counts from raw game meat ranged from 2.37 log cfu/g to 5.37 log cfu/g. Counts as high as 6.16 log cfu/g and 5.96 log cfu/g were enumerated from finished retail products. APC from ≤ 2.62 log cfu/cm^2 to ≤ 6.3log cfu/cm^2 were enumerated from swab samples collected from surfaces, hands and equipment during production. Environmental samples (air and water) yielded no bacterial counts. The results obtained highlight that contaminated raw meat can spread contamination to surfaces, hands and equipment. Also, cleaning and disinfecting procedures were not adequately followed as aerobic bacteria could be enumerated after cleaning. Proper adherence to hygiene practices can reduce the spread of contamination, and improve hygiene status during the production of game products.

5.1 Introduction

Game meat appeals to health conscious consumers as it is low in fat, rich in protein and has a unique taste (Hoffman & Wiklund, 2006, Bekker *et al.,* 2011). At least 73% of consumers from the Western Cape (South African province) have eaten game meat. Furthermore, game meat products are liked by tourists as the consumption of game meat is seen as a South African experience (Hoffman, 2003; Hoffman & Wiklund, 2006). Game farming in South Africa is the fastest growing agricultural industry with Cloete *et al.* (2007) reporting that many cattle farmers are shifting to game farming. This world-wide increase in interest towards game meat (Hoffman & Wiklund, 2006) requires attention to be focused on the quality and safety of game meat being delivered to consumers (Van der Merwe *et al.* 2013).

During the conversion of carcasses to retail products, unavoidable microbial contamination occurs (Eisel, *et al.,* 1997; Haileselassie *et al.,* 2013). The contamination of game meat as explored in Chapters 3 and 4 of this thesis can occur during harvesting and slaughter. During harvesting and slaughter process, previously sterile meat can be contaminated by the hide, intestinal content, the environment the animal falls upon after being shot and contaminated abattoir surfaces (Karama *et al.*, 2003, Attanassova *et al.,* 2008).

During processing, raw meat can come into contact with contaminated surfaces, equipment and worker's hands (Chen *et al.,* 2000, Gurmu & Gerbinsae, 2013). Food handlers can harbour a number of microorganisms that can affect the microbial quality of meat products. Fingernails of a food handler alone can harbour harmful bacteria such as *Salmonella* spp., *E. coli* and faecal

streptococci (Lawrie, 1998). Equipment such as hand held knives can also be a source of contamination. Gurmu & Gerbinsae (2013) evaluated the bacteriological status of knives used by butchers; the high microbial load enumerated from the knives was associated with poor hygiene practises such as the continuous use of contaminated knives. Additionally, contaminated working surfaces can be responsible for the transfer of bacteria to meat products (Newton *et al.,* 1978*)*. Furthermore, during processing, exposure to contaminated environmental sources such as air and water can allow for microbial contamination (Hoffman *et al.,* 2010).

Food handling establishments require Good Manufacturing Practises (GMP) and systematic food safety programs that can aid in the reduction and the prevention of microbial contamination (Haileselassie *et al.,* 2013). As a food handling establishment, abattoirs are required to comply with relevant South African legislation. In the subject of hygiene in abattoirs, the Veterinary Procedure Notice (VPN) 15/2010-01 would be the relevant legislation. VPN/15/2010-01 is the standard responsible for the microbiological monitoring of meat handling establishments and meat products from the establishment. Therefore, as stated by the standard, the reduction of microbial contamination requires routine monitoring of microorganisms associated with hygiene, such as aerobic bacteria. For meat samples, high Aerobic Plate Counts (APC) can be an indication of contamination of carcasses that occurred during skinning and/or evisceration (Grau, 1986, Hoffman *et al.,* 2010), and for equipment, surfaces and personnel. Aerobic plate counts can represent the ability to adhere to hygiene practices during processing (Hoffman *et al.,* 2010).

The purpose of this study was to investigate the adherence to hygiene practices during the production of game products, by determining microbial loads of: raw meat, hands (personnel, also known as the human factor), work surfaces, equipment (such as hand held knives, grinder and scales), working environment and finished retail game meat products.

5.2 Materials and Methods

5.2.1 Sample site

The study was conducted in the value adding section of a registered game abattoir. The value adding section of this abattoir is responsible for the production of game products, intended for retail. The value adding section was visited twice; visits to the abattoir were 4 weeks apart.

Swab samples were taken before production, during production and after cleaning and disinfecting. For this abattoir cleaning and disinfecting was done in the following manner: an alarm would alert the workers that surfaces, equipment and hands should be cleaned and disinfected, every 30 minutes. Personnel would proceed to clean relevant surfaces and equipment with a non-corrosive soap known as Sterirub (KemKlean, South Africa) using a brush and water from a high pressure hose. Then, surfaces and equipment would be sprayed with a chlorine based disinfectant known as Q45 (Kemclean, South Africa) and dried. The hand washing procedure was similar, as after washing the surfaces and equipment, the workers would also wash their hands with a soap

known as Steriklens (KemKlean, South Africa), where after their hands would be dried with a paper towel.

5.2.2 Swab samples

Equipment, meat handlers, and surfaces were swabbed for the purpose of bacteriological analysis, using sterile cotton wool swabs (Lasec, South Africa), which were moistened prior to use with 0.1% buffered peptone water. An area of 10 cm^2 was rubbed for at least 30 seconds. The value adding section of the abattoir is separated into three working areas according to the tasks performed as depicted in Table 5.1. Table 5.2 portrays the types of samples swabbed during the production of game meat products.

Table 5.1 A description of tasks carried out at each work area during the making of game meat products

	Area 1	Area 2	Area 3
Tasks	• Meat is sorted • Meat is cut into smaller pieces • Meat is moved to area 2	• Meat is minced /grinded • Mixed with spices, if needed • Sausage casing are filled • Meat is weighed into plastic bags.	• Meat in plastic bags is transferred into the vacuum packaging to be vacuum packed by vacuum packer (Multivac)

It should be noted that for this particular abattoir, each working area mentioned in Table 5.1 had at least 2 to 3 workers, as the value adding section of this abattoir has the smallest number of employees.

Table 5.2 Swabs taken for analyses that were derived from the hands (n = 22), surfaces (n = 23), and equipment (n =21) that came into contact with meat during production

Sample types		
Hands	**Surfaces**	**Equipment**
Personnel in contact with meat: Cutting meat into smaller pieces, weighing meat into bags.	Cutting boards	Knives, meat grinder, scooper, de-membrane machine, scale, vacuum packaging machine
1st visit, n= 12 2nd visit, n=10	1st visit, n= 11 2nd visit, n=12	1st visit, n= 10 2nd visit, n=11

5.2.3 Environmental sample

Air samples in each area were taken by placing open agar plates in each area for 10 min. Water samples from taps used by workers during processing of game meat products, were collected in specimen jars, aseptically.

5.2.4 Meat sample

Previously frozen raw meat (n=12 for 1st and 2nd visit) waiting for further processing was randomly sampled (25 g) to indicate microbial load of game meat before processing. Lastly, finished products (such as mince, sausages, cubes) (25 g) intended for the retail market were again randomly sampled (n = 6 and n = 7 for 1st and 2nd visit, respectively). All samples were collected aseptically.

5.2.5 Handling of samples

The meat samples were placed in sterile stomacher bags, identified with a permanent marker, vacuum sealed to prevent opening and stored on ice in cooler bags. Swab samples, water samples and air plates were also stored on ice in cooler bags. All the samples were transported in the coolers to the research Laboratory for further processing.

5.2.6 Microbial analysis

5.2.6.1 Sample preparation

Swab samples were homogenised in 10 ml Buffered peptone (CM1049, Oxoid, Hemisphere, England) water (20% m/v) solution (Oxoid, CM1049) for 30 seconds using a Seward stomacher 400 (Seward Medical UAC House, United kingdom) at room temperature. Buffered peptone water was prepared according to the manufacturer's instructions, which suggested 20 g of buffered peptone powder in 1 litre of distilled water.

Meat samples (~25 g) were homogenised in 225 ml Buffered peptone water (20% m/v) solution (Oxoid, CM1049) for 30 seconds using a Seward stomacher 400 (Seward Ltd) at room temperature. Buffered peptone water was prepared according to the manufacturer's instructions, which instructed the use of 20 g of buffered peptone powder in 1 litre of distilled water.

5.2.6.2 Aerobic Plate Counts (APC)

For samples, serial dilutions were prepared from the suspension made above using peptone buffered water. Plate count agar was used for the plate count method (C6.500, Merck, Modderfontein, South Africa). Pour plates were also done in duplicate. PCA plates were left to solidify at room temperature. Finally PCA plates were incubated at 30°C for 72 hours. After the incubation, colonies were counted using a Suntex colony-counter 570 (Lasec, South Africa). Plates were examined under a light to avoid mistaking particles of matter for colonies (Hoffman *et al.*, 2010).

5.2.7 Statistical analysis

Bacterial counts were converted to their logarithmic form for statistical analysis. Mean bacterial counts were determined for raw previously frozen meat, processed meat and environmental samples. Mean bacterial counts were used to generate bar graphs, to visually show bacterial load from work surfaces, hands and equipment. Whiskers on graphs indicate standard error (S.E) of the means. A Shapiro-Wilks test for normality was done to determine the distribution of the data against a null hypothesis that the data is normally distributed. All parameters tested had normally distributed data, which warranted the use of parametric testing. An ANOVA was done and where significant differences were found, a Bonferroni test was used to determine variation for the 1st and 2nd visit (Figures 4.2, 4.3 and 4.4). The statistical program, Statistica version 12 was used.

5.3 Results

5.3.1 Meat samples

Average microbial counts for APC from previously frozen game meat samples intended for the production of retail products obtained during the 1st and 2nd sampling visits were 5.37 log cfu/g ± 0.11 and 2.43 ± 0.06 log cfu/g, respectively. Average microbial counts for APC from retail products for the 1st and 2nd sampling visits were 6.16 ± 0.163 log cfu/g and 5.96 ± 0.061 log cfu/g, respectively.

5.3.2 Environmental samples

For this abattoir, water comes from a water reticulation system that is treated with chlorine dioxide. Tap water samples collected on the 1st and 2nd sampling visits yielded no bacterial counts (0 cfu/ml). No colonies were enumerated from the air samples during production of game products.

5.3.3 Swab samples

Swab samples from hands, surfaces and equipment were first taken before production, however no bacterial growth was seen, therefore counts before processing were at 0 cfu/cm^2. Therefore, Figure 5.1, 5.2 and 5.3 represent the aerobic bacterial load enumerated from surfaces, equipment and hands that came into contact with meat during the production of retail game products, during the two visits to the game abattoir.

Bacterial counts from the 1st visit were noticeably higher than counts from the 2nd visit. A reduction in aerobic counts after cleaning and disinfecting was seen for all swab samples, irrespective of the visit, however surfaces had the lowest reduction. Furthermore, statistical analysis showed that reduction of bacterial counts was significant for equipment (for the 1st and 2nd visit) and hands (1st visit). However, surfaces did reduction but significant differences were detected (for 1st and 2nd visit).

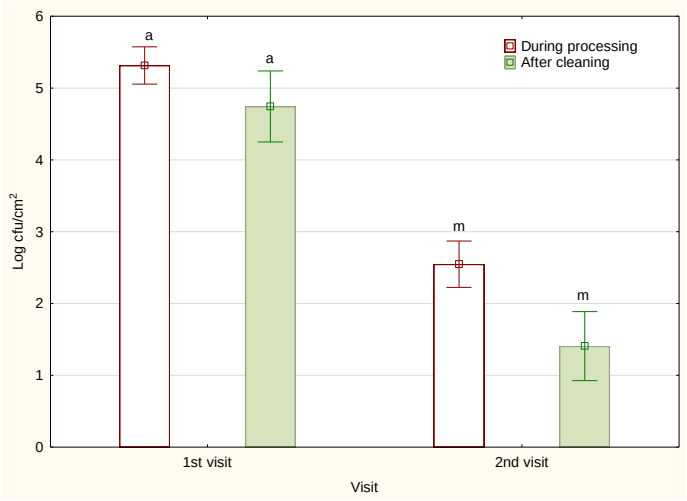

Figure 5.1 Aerobic plate counts from swab samples from surfaces (n= 23) in contact with meat (whiskers indicate S.E). Different letter notations are significantly different at p<0.05.

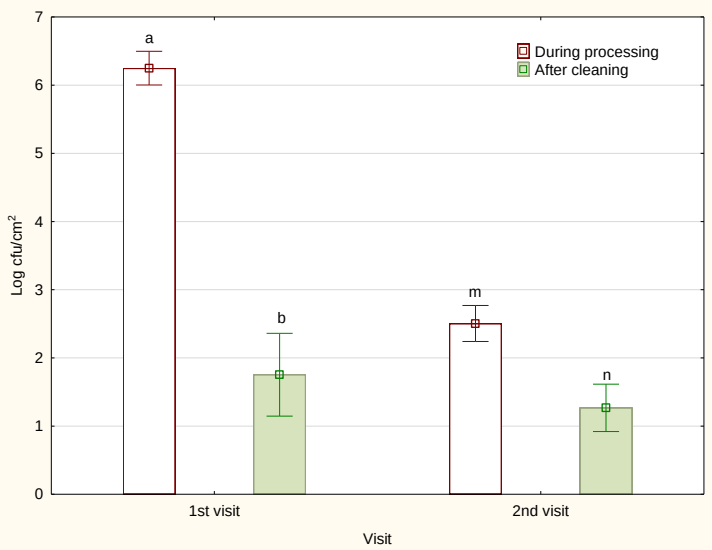

Figure 5.2 Aerobic plate counts from swab samples from equipment (knives etc.) (n = 21) used by meat handlers (whiskers indicate S.E). Different letter notations are significantly different at p<0.05.

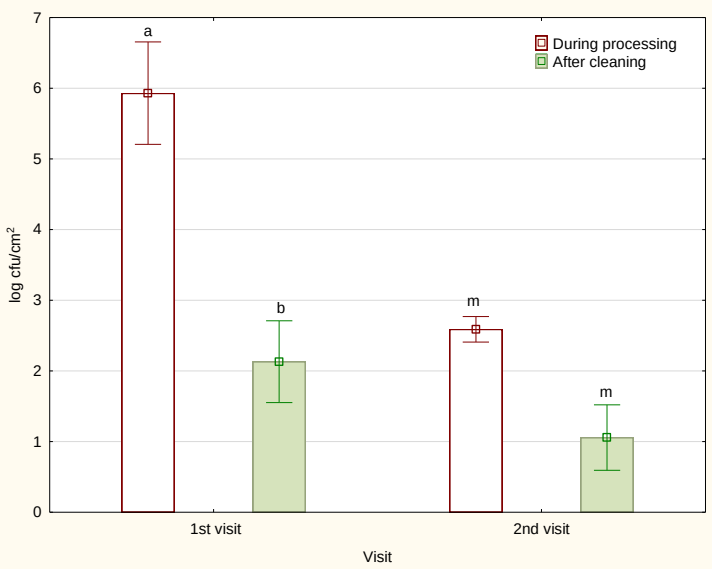

Figure 5.3 Aerobic plate counts from swab samples from hands of meat handlers (n = 22) (whiskers indicate S.E). Different letter notations are significantly different at $p<0.05$.

5.4 Discussion

Swab Samples obtained before processing (at the beginning of the day) yielded no aerobic bacterial counts. These results suggest that cleaning of surfaces and equipment at the end of the day is much more effective. Typically work surfaces and equipment are adequately cleaned at the end of the day due to the absence of production pressure (Green & Selman, 2005). Furthermore the presence of aerobic bacteria on samples gathered during production from hands, knives and surfaces could imply that the meat was contaminated and caused contamination of the hands, equipment and surfaces during processing as has been reported to be the case elsewhere (Nel *et al.,* 2004b, Berends *et al.,* 1998).

Bacterial counts obtained from frozen game meat can be due to contamination that occurred from point of harvest to the deboning step (Chapter 3 and 4). Furthermore, contamination can be attributed to an abattoir's hygiene state during the slaughter of game carcasses (Newton *et al*., 1978). In this study, high counts, especially from the 1st visit can indicate low compliance to hygiene practises during the initial stages of processing which can lead to high microbial contamination (Nel *et al.,* 2004a, Gill & Penney, 1976, Gill, 2007; Paulsen, 2004). This could be due to the game animals being harvested at night where poor working conditions (lack of adequate light, water, etc.) make the adherence to general hygiene practices even more difficult (Van Schalkwyk *et al.,* 2011). Counts obtained from the first round of sampling were higher than those reported on the 2nd visit. However, aerobic bacterial counts were still within acceptable retail limits (<5.6 log cfu/g) (Van Schalkwyk *et al.,* 2011). Counts from the unprocessed raw (previously frozen) game meat were similar and lower to the total bacterial count of 5.2 log cfu/g reported by van Schalkwyk *et al.* (2011) on fresh raw game meat.

When contamination occurs, standard operating procedures for cleaning and disinfecting hands, equipment and surfaces should be adhered to in order to eliminate or reduce further contamination. Hygiene procedures requires the effective cleaning of equipment and surfaces as well as hands, this can decrease further contamination. However, during sampling, observation of the workers showed a flaw in the following of protocol which required washing and disinfecting of surfaces, hands and equipment every time the alarm rang, as sometimes the alarm was ignored by workers. Green & Selman (2005) noted that personnel expressed that they see cleaning of equipment and washing of hands as a waste of time.

After cleaning of hands, equipment and surfaces a reduction of bacterial counts was noted (Figure 5.1, 5.2 and 5.3) however, surfaces had the lowest reduction in APC. Low reduction (indicative of an ineffective cleaning procedure) in bacterial counts can also indicate a worn out state of cutting boards and equipment (such as the appearance of deep grooves on cutting boards), which can occur overtime. Furthermore, a low reduction in bacterial count can possibly indicate the ineffectiveness of detergent and disinfectant used by the value adding section. The ability to yield counts after cleaning and disinfecting, contradicted results obtained by Eisel *et al.* (1997), where sampling after cleaning seldom yielded enumeration of bacteria. The low reduction of APC (especially for surfaces) can be a cause of concern, as harmful bacteria can proliferate on equipment and work surfaces (Berends *et al.,* 1998). However, results corroborated Gill & McGinnin's (2000) notion that bacteria originating from meat can possibly be found on equipment and surfaces, as daily cleaning procedures can prove to be ineffective.

This low reduction in bacterial counts can also show the lack of training of personnel involved in the value adding process of game meat, for this specific abattoir. The efficacy of any cleaning procedures put in place relies on the routine inspection of the cleanliness of equipment used during the production process as well as firm adherence to operating procedures for cleaning and disinfecting (Nel *et al.,* 2004a). A similar notion was expressed by Martinez-Tome & Murcia (2000) where great importance was given to the adequate education for food handlers about hygiene procedures. Adequate knowledge of hygiene practices can possibly go a long way in minimising cross contamination. Low reduction in bacterial counts can also indicate the ineffectiveness of cleaning methods, put in place.

Finished products showed that bacterial counts of processed products were much higher than the counts obtained from the raw meat (previously frozen) samples. A similar increase in bacterial counts was seen by van Schalkwyk *et al.* (2011) where aerobic bacterial counts of processed game products (salami) showed an increase in aerobic bacteria, when compared to the raw unprocessed game meat. The high aerobic bacterial counts of processed products, is expected as processed products such as sausages and mince, are made with other raw materials such as sheep fat, which carries it's on bacterial load. Secondly, bacterial counts from finished products are the combination of the initial bacterial counts from various batches of raw game meat (before the processing of retail products). Lastly, for this abattoir, meat would be cut and sorted in area 1,

weighed into plastic bags in area 2 and finally would be transferred to vacuum packaging in area 3; this alone would increase contact with hands, surfaces and knives (Eisel *et al.,* 1997, Berends *et al.,* 1998).

As mentioned, air and water samples collected from the three areas yielded no bacterial counts. This could be due to the low room temperature (<10ºC) of the value adding working area, low relative humidity and lower air movement (one door and at least one fan per working area). It can be concluded that contamination due to air was not a significant factor (Eisel *et al.,* 1997) whereas Hoffman *et al.* (2010) found that air played an important role in bacterial contamination in an ostrich abattoir along the slaughter line. Similarly, the water used in the processing plant was not a source of bacterial contamination. Eisel *et al.* (1997) also noted that environmental sources of contamination are not always significant sources of the overall microbial contamination.

5.5 Conclusion

In this meat processing plant, incoming contaminated raw meat was a source of contamination and subsequently influenced the bacterial load of the work surfaces, equipment and hands of the personnel involved in the production of game meat products. Cleaning and disinfection if adequately performed should results in substantial reduction in the bacterial counts, however even though a reduction in bacterial counts was reported, it proved to be ineffective for the work surfaces. Therefore, work surfaces can be an important factor in the spread of contamination.

It is also important that application of SOP's further down the value chain (during harvesting field dressing and transport) be monitored to ensure that meat with the minimum contamination enters the abattoir.

The understanding of hygiene practices (and ultimately the importance of food safety) amongst the meat handlers should reduce contamination, therefore the knowledge of game meat handlers have about hygiene practices should be determined in future studies so as to identify areas that require further training.

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CHAPTER 6

pH, colour and microbiological changes of black wildebeest (*connochaetes gnou*) *longissimus thoracis et lumborum* (LTL) muscle with normal or high muscle pH

Abstract

This study aimed to determine if muscle pH influences colour stability and microbial spoilage when meat is categorised as normal and dark, firm and dry (DFD). For the investigation, 12 black Wildebeest *Longissimus thoracis et lumborum* (LTL) muscle were used, of which 6 were classified as DFD samples (pH ≥ 6.06) and 6 were classified as normal samples. The study revealed that pH significantly affected colour, as initial (day 0) L*, a*, b*, C* and H_{ab} values from normal pH samples were significantly higher than values reported for DFD samples. Initial APC and *Enterobacteriaceae* counts from samples with normal pH were not significantly different from counts reported for DFD samples; therefore initial contamination can be due to harvesting and slaughter process. LTL muscle were further refrigerated (5±1ºC) for 12 days in an aerobic environment, to be sampled further every 3 days. The second part of this study revealed that pH did not affect lightness and brownness as L* and b* values for DFD samples did not significantly differ overtime; the same trend was seen for samples with normal pH. Samples with normal pH showed a significant increase in a* and C* values until day 12, whilst H_{ab} values decreased until the 12th day. The same trend was seen for a* and C* values for DFD samples until the 9th day as on the 12th day values increased. Similarly, H_{ab} values for DFD samples decreased until the 9th day, and then increased on the 12th day. Using the microbial spoilage limit of 6 log cfu/g, it was see that DFD meat reached this limit earlier than samples with normal pH (day 9 and 13 for APC and *Enterobacteriaceae*, respectively). Furthermore regression analysis showed more rapid growth of bacteria for DFD meat than normal pH meat.

6.1 Introduction

Prolonged stress or high activity before slaughter can result in the depletion of glycogen stores in the muscle. This can cause a limitation of post-mortem glycolysis which can result in low lactic acid production, maintaining a higher pH than normal in the muscle/meat of an animal *post*-mortem (Kritzinger *et al.,* 2003; Hoffman, 2004). As a consequence, high ultimate muscle pH can lead to the formation of dark, firm and dry (DFD) meat. The defect has been reported in beef and mutton, and to a more limited extent, in pork (Newton & Gill, 1981). Additionally, DFD meat has frequently been reported in ostriches (Hoffman *et al.,* 2010) and in game meat from South Africa; the latter mainly due to the highly stressful manner in which game animals are hunted (Hoffman, 2000). Ultimately, muscle pH will change the quality and safety of meat, as DFD meat has been reported to have a short shelf life and a poor appearance (Lawrie, 1998; Wiklund & Smulders, 2011).

The colour of meat is one of the most important indicators of meat quality (Mancini & Hunt, 2006). It has been reported that whilst trying to determine the quality of meat, consumers pay great attention to colour. To consumers, colour can indicate flavour, tenderness, safety and freshness

(Hoffman *et al.,* 2005). Consumers use colour to visually detect defects in food which ultimately influences the consumer's decision in buying the product or not. In saying that, South African game meat has been perceived to have a dark unappealing colour to consumers, the dark colour is mainly due to the high myoglobin content (Wiklund & Smulders, 2011). DFD meat allows for further deterrent to consumers, as the meat appears to be even darker (Swatland, 1990). In terms of discolouration, it is important to note that even though consumers use colour as an indicator of freshness (Young & West, 2001) discoloration in meat actually occurs prior to microbiological spoilage (Faustman & Cassens, 1990).

Previous researchers have accredited the shortened shelf life of DFD meat to the high ultimate pH, however most bacterial growth is not affected by a variation of pH from 5.5 to 7 (Gill & Newton, 1979). DFD meat's ability to spoil at a faster rate is thought to be due to the absence of glycogen and not the elevation of pH (Gill, 1976). The presence of glycogen, which can be found in meat with normal pH, acts as the primary nutrient source for bacteria, once the glycogen has been depleted; bacteria start the utilisation of free amino acids. This utilisation immediately releases by-products such as ammonia and hydrogen sulphide which are responsible for the development of off odours and flavours; typical symptoms of spoilage (Gill & Newton, 1979). The microorganisms in question have been found to be *Enterobacteriaceae*, Lactic acid bacteria such as *Lactobacillus* and *Leuconostoc. Pseudomonas* spp. has been found to be dominant during the spoilage of refrigerated raw meat in an aerobic environment (Ercolini *et al.,* 2006). However, DFD meat can be used to make salted, smoked or heat treated meat products such as smoke sausages (Korneliussen, 2012).

Previous work has showed the influence pH can have on meat quality. However the bulk of research in this field falls heavily on studies conducted on beef and lamb, very little exists about the spoilage of game meat that is dark, firm and dry. Therefore, the aim of this investigation is to determine the effect pH has on colour and microbial quality of game meat, and to also understand the spoilage process of game meat (originating from South Africa) with high pH (DFD) compared to game meat samples with normal pH (pH of <6), by observing pH, microbial (aerobic plate counts and *Enterobacteriaceae*) and colour changes in the game meat over a 12 day period at low temperature (5 ± 1ºC) storage.

6.2 Material and Methods

Black Wildebeest *Longissimus thoracis et lumborum* (LTL) muscles (12 left and 12 right from the same animals) were delivered frozen and vacuum packed to the laboratory. On arrival samples were given a lab number. To study pH changes, colour changes and microbial growth in an aerobic environment, meat samples were kept at 5 ± 1ºC in a GDC refrigerator (sample temperature was monitored with an infrared thermometer, model number TFC-42, Cape Instrument services Cc) for 12 days and sampled on day 0, 3, 6, 9 and 12.

LTL muscles were left to defrost overnight in their vacuum packed state. The following day packaging was first sprayed with 70% ethanol in order to minimise cross contamination from packaging to the LTL muscle. Each LTL muscle was cut into five steaks which were randomly allocated to a specific day of sampling. On each sampling day each of these steaks were further divided into three subsamples under a laminar flow cabinet: one for measuring the pH, one for colour determination and the third for microbial analyses. Steaks were kept in pre-labelled polystyrene trays, trays with steaks were over-wrapped with a low density polyethylene wrap (LDPE) (moisture vapour transfer rate of 585 gm^{-2} 24 h^{-1} 1 atm^{-1}, oxygen permeability of 25 000 cm^{-3} m^{-2} 24 h^{-1} 1 atm^{-1} and carbon dioxide permeability of 180 000 cm^{-3} m^{-2} 24 h^{-1} 1 atm^{-1}).

6.2.1 pH measurements

A 1 g taken from each subsample (intended for pH determination) was homogenised in a blender with 10 ml of a Sodium-Iodoacetate (Na-Iodoacetate) (5 mM) Potassium Chloride (KCl) solution (150 mM) (pH of Na-iodoacetate/KCl was 7) that was prepared prior to analysis. pH of the homogenate was measured using a calibrated Jenway 3510 pH meter (Lasec, South Africa). pH measurements were done in duplicate. Furthermore, to reduce temperature fluctuations, the homogenate was placed in ice. The pH meter was calibrated using standard buffers of pH 7 and 4.

6.2.2 Colour measurements

The colour of the LTL muscles was evaluated from aseptically cut steaks (~2 cm thick) using a colour guide 45°/0° colorimeter (aperture size 11 mm) (Catalogue number 6801; BYK-Gardner, Geretsried, Germany). The measurements were taken using an illuminant/observer of D65/10° (Neethling, *et al.,* 2015). The colorimeter was calibrated using the standards provided (BYK-Gardner). The CIELab colour variables were measured where L* represents brightness, a* represents the red-green range and b* represents the blue-yellow range. Colour measurements were repeated five times, for each sample and the mean readings used in further statistical analyses. Chroma (C*) and Hue (H_{ab}) values were calculated from individual a* and b* values according to the formula: $C^* = \sqrt{(a^*)^2+(b^*)^2}$ and $H_{ab} = \tan^{-1}\left(\frac{b^*}{a^*}\right)$

6.2.3 Detection of microorganisms

6.2.3.1 Sampling procedure

Samples (~25 g) were homogenised in ~225 ml Buffered peptone (CM1049, Oxoid, Hemisphere, England) water (20% m/v) solution (Oxoid, CM1049) for 30 seconds using a Seward stomacher 400 (Seward Medical UAC House, United kingdom) at room temperature. Buffered peptone water was prepared according to the manufacturer's instructions.

6.2.3.2 Aerobic Plate Counts (APC) (ISO 4833:1991)

Serial 10-fold dilutions were prepared from the above made suspension using peptone buffered water. The pour plates method with plate count agar (PCA) was used (C6.500, Merck, Modderfontein, South Africa). Pour plates were duplicated and left to solidify at room temperature. Thereafter the plates were incubated at 30°C for 72 hours. After the incubation, colonies were counted, using a Suntex colony-counter 570 (Lasec, South Africa). Plates were examined under a light to avoid mistaking particles of matter for colonies.

6.2.3.3 Enterobacteriaceae (ISO 21528-2:2004)

Serial 10-fold dilution was prepared and poured into duplicate plates, using Violet red bile agar (VRBG) (Oxoid, CM0485). Plates were left to solidify at room temperature before a second layer of VRBG was poured and left to solidify. Pour plates were then incubated at 37°C for 24 hours. All dark pink to red colonies were counted using a Suntex colony-counter 570 (Lasec, South Africa). Accurate counting was assisted with a bright light.

6.2.4 Signs of spoilage

After colour measurements, steak samples would then be used for visual inspections. Visual inspections of the meat samples were conducted to see whether there were any detectable signs of spoilage such as slime formation which can be felt on the surface of the sample and whether any off-odours could be smelt.

6.2.5 Statistical analysis

A Receiver Operating Characteristic (ROC) curve was constructed in order to determine a cut-off point; a pH value that will ultimately be able to split samples into two pH groups. In one group, the samples will be considered to be normal in pH and the other, to be high in pH (DFD). Where applicable an ANOVA was performed for all variables to determine significant differences over day 0, 3, 6, 9 and 12 and between pH categories. Where significant differences were found, a post-hoc Fisher's LSD test was done to determine the sources of variation. Where applicable, graphs, tables were generated from the means data (± S.E).

6.3 Results

6.3.1 The effect of pH on game meat samples

6.3.1.1 Receiver Operating Characteristic (ROC) curve to determine cut-off pH value

The ROC curve plots sensitivity (the probability of determining a case as having a pH defect) vs 1-specificity (the probability of determining a sample as having a pH defect), are seen in Figure 6.1. A pH cut-off point of 6.06 was calculated, sensitivity and 1-specificity values were 0.00 and 1.0,

respectively. Therefore, pH values above 6.06 are considered to have a pH defect (DFD), whilst pH values lower than 6.06 will not have a pH defect, and will therefore be considered to be samples with normal pH for this study.

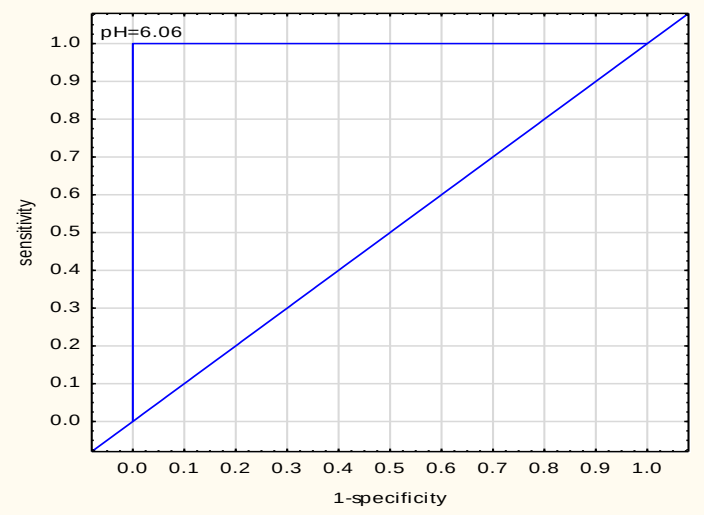

Figure 6.1 ROC curve based on pH values (determined on day 0) for separating samples with Normal pH from samples with high pH (DFD)

Table 6.1 is a summary of samples with their initial (day 0) corresponding pH values which can be classified as normal (n=6) and high (n=6) in pH (DFD) as determined by the cut-off point determined by the ROC curve. The pH values for samples with normal pH values, ranged from 5.55 to 5.86 and samples with high pH ranged from 6.06 to 6.45 at day 0.

Colour co-ordinates (L*, a*, b*, C* and H_{ab}) revealed that they were significantly affected by muscle pH category (Table 6.1). Normal pH meat had higher L* values than samples with high pH (indicating a dark appearance in the latter). Samples with normal pH, also had higher a* (redder colour) and b* values (more yellow in colour) when compared to samples with high pH. Furthermore Hue (H_{ab}) and Chroma (C*) values for muscle steaks with normal pH were higher than those reported for samples with high pH.

Aerobic bacterial counts from samples with normal pH were not significantly different from counts reported for samples with high pH. However, *Enterobacteriaceae* counts obtained from samples with normal pH were significantly lower by ~1 log cfu/g (Table 6.1).

Table 6. 1 The effect of pH on meat colour and initial microbial load in black wildebeest samples which have normal pH and are DFD

Parameter	**Normal pH (pH < 6.06) (n=6)**	**High pH (pH > 6.06) (n=6)**
	Mean (±S.E)	
pH	5.65^{a} ± 0.043	6.29^{b} ± 0.059
L*	33.08^{a} ±1.018	27.21^{b}±0.22
a*	13.60^{a}±0.313	11.10^{b} ±0.26

b*	10.29^{a} ±0.582	6.97^{b} ±0.32
C*	17.10^{a} ±0.503	13.12^{b} ±0.35
H_{ab}	36.85^{a} ±1.549	32.08^{b} ±0.96
APC	3.56^{a} ± 0.215	3.53^{a} ± 0.137
Enterobacteriaceae counts	2.24^{a} ± 0.360	3.02^{a}± 0.317

^{a,b} Means in rows with different superscript differ significantly ($p < 0.05$)

6.3.2 Spoilage of game meat with high pH and normal pH under chilled storage

6.3.2.1 Change in muscle mean pH overtime

Figure 6.2 represents the mean pH values (categorised as either Normal or DFD) obtained during 12 days of analysis of black wildebeest LTL muscles that were kept at refrigerator temperatures (5 ± 1ºC), under aerobic conditions.

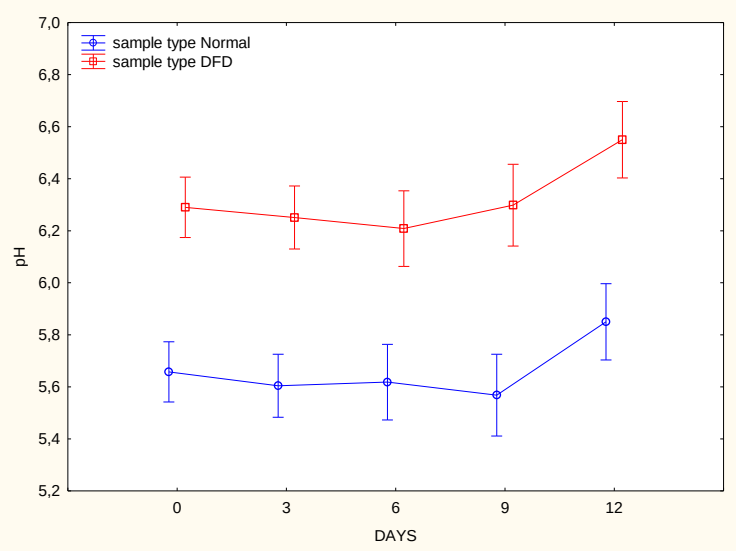

Figure 6.2 pH changes (mean ± S.E.) of black wildebeest LTL muscle muscles categorised as normal pH (n=6) and DFD (n=6) at day 0.

For samples with normal pH (Figure 6.2), a decrease in pH occurred until day 9, however this was not so for samples with high pH (DFD), where a pH decrease only occurred until day 6. Thereafter, an increase in muscle pH values was observed; from day 9 for samples with normal pH from day 6 for DFD samples.

6.3.2.2 Colour measurements overtime

L*, a*, b*, hue angle (H_{ab}) and chroma (C*) values of black wildebeest LTL muscle stored at refrigerator temperature (5 ± 1ºC), under aerobic conditions for 12 days are depicted in Figure 6.3 to 6.7.

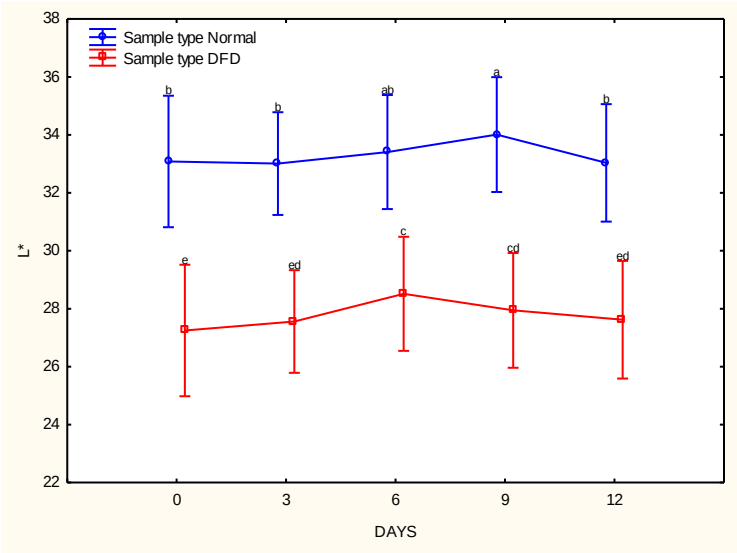

Figure 6.3 L* values of black wildebeest LTL muscle that have a normal pH or are DFD

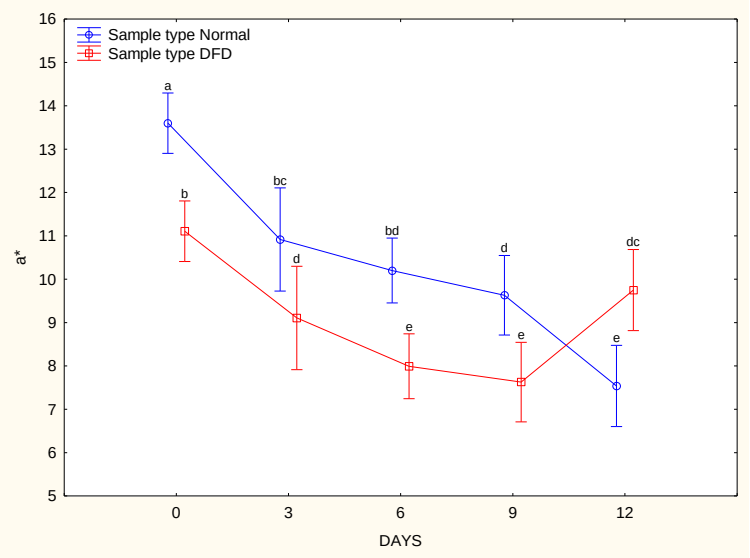

Figure 6.4 a* values of black wildebeest LTL muscle that have a normal pH or are DFD

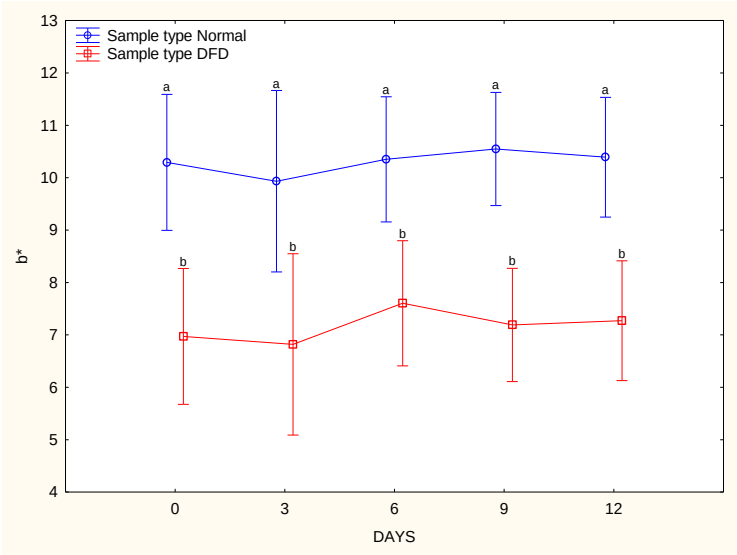

Figure 6.5 b* values of black wildebeest LTL muscle that have a normal pH or are DFD

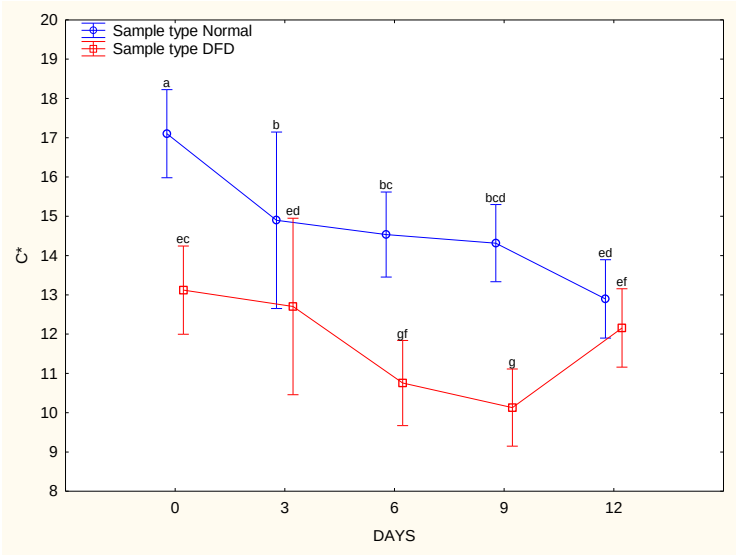

Figure 6.6 Chroma (C*) values of black wildebeest LTL muscle that have a normal pH or are DFD

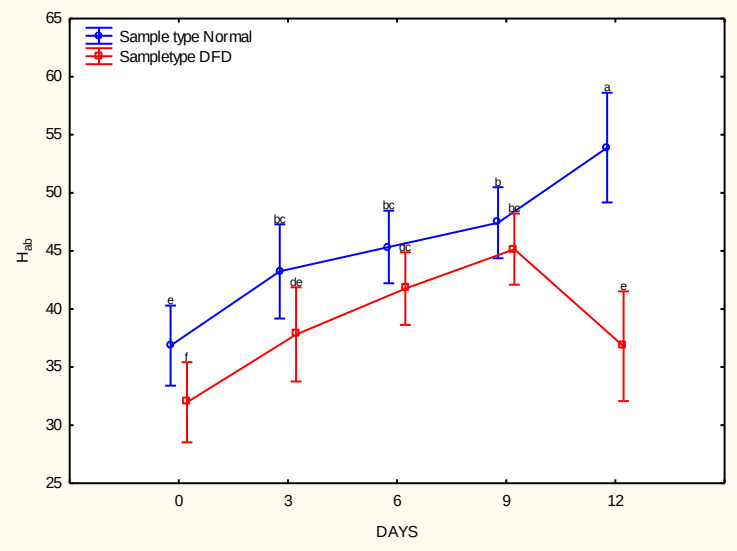

Figure 6.7 Hue values of black wildebeest LTL muscle that have a normal pH or are DFD

L* and b* values for samples with normal pH showed no fixed trend over the refrigeration period. This was also seen for DFD samples as L* and b* values remained consistent over the refrigeration period (Figure 6.3 and 6.5). When mean L* and b* values from normal pH samples were compared to L* and b* values from DFD samples, It could be noted that, the L* and b* values for normal pH samples remained significantly higher than the L* and b* values reported for DFD samples overtime.

For samples with normal pH, a* and C* values decreased from day 0 to 12. For DFD samples, a* and C* values decreased until the 9th day. On the 12th day, C* values reported for DFD samples significantly increased. Coordinates, a* and C* from samples with normal pH were also significantly higher than a* and C* values reported DFD samples overtime.

The H_{ab} values continuously increased over the refrigeration period for samples with normal pH. However, for the DFD samples, H_{ab} values increased until the 9th day, and significantly

decreased on the 12th day; this change could be attributed to the significant increase in the a* values on the 12th day (Figure 6.4). Over the refrigeration period it was also noted that, H_{ab} values from samples with normal pH were significantly higher than H_{ab} values reported for DFD samples.

6.3.2.3 Microbial counts overtime

Figures 6.8 and 6.9 represents the mean viable counts (log mean ± SE) of aerobic and *Enterobacteriaceae* of normal and DFD categorised black wildebeest LTL muscles that were kept at refrigerator temperature (5 ± 1ºC) under aerobic conditions measured over a 12 day period.

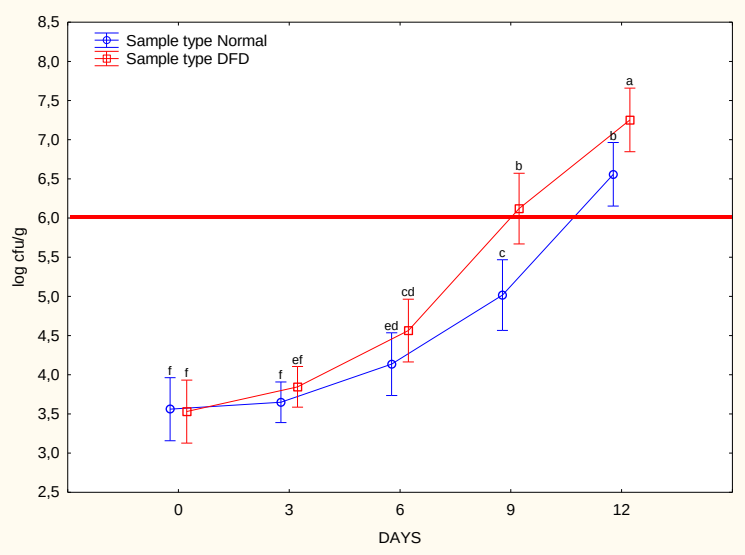

Figure 6.8 Viable counts (log mean ± S.E.) of aerobic bacteria detected on black wildebeest LTL muscles (n=6) with normal pH and DFD at day 0. Letters on whiskers indicate significance difference ($p < 0.05$)

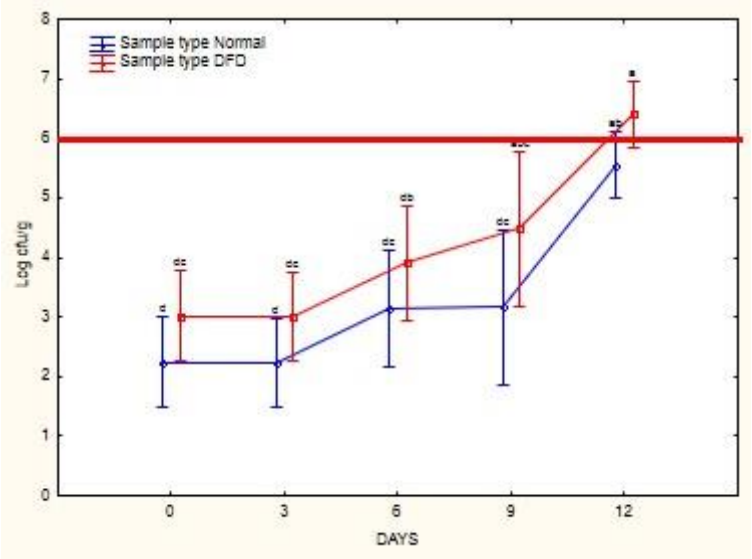

Figure 6.9 Viable *Enterobacteriaceae* counts (log mean ± S.E.) detected on black wildebeest LTL muscles (n=6) with normal and DFD at day 0. Letters on whiskers indicate significance difference ($p < 0.05$)

Aerobic bacterial and *Enterobacteriaceae* counts increased from day 0 to 12 for normal and DFD samples stored in an aerobic environment at refrigerator temperatures (5ºC ± 1). Bacteria grow more rapidly for DFD samples when compared to samples with normal pH. This was more prominent on the 9th and 12th day for DFD samples. On the 9th and 12th day, APC were significantly higher than APC reported for samples with normal pH. The same could be said for *Enterobacteriaceae* counts, however prominent differences were seen at a later stage; day 12. On the 12th day, *Enterobacteriaceae* from DFD samples were significantly higher, than those reported for samples with normal pH.

From regression models below (Figure 6.10 and 6.11), it can be seen that, DFD sample's APC and *Enterobacteriaceae* had a more rapid growth rate when compared to the growth rate seen for APC and *Enterobacteriaceae* from samples with normal pH; The DFD reaching the APC microbiological spoilage of 6 log cfu/g on day 9 and the Normal on day 12 Whilst DFD reached a *Enterobacteriaceae* count of 6 log cfu/g on 13th day and Normal pH samples reached 6 log cfu/g on the 17th day, as calculated using the regression equations displayed above.

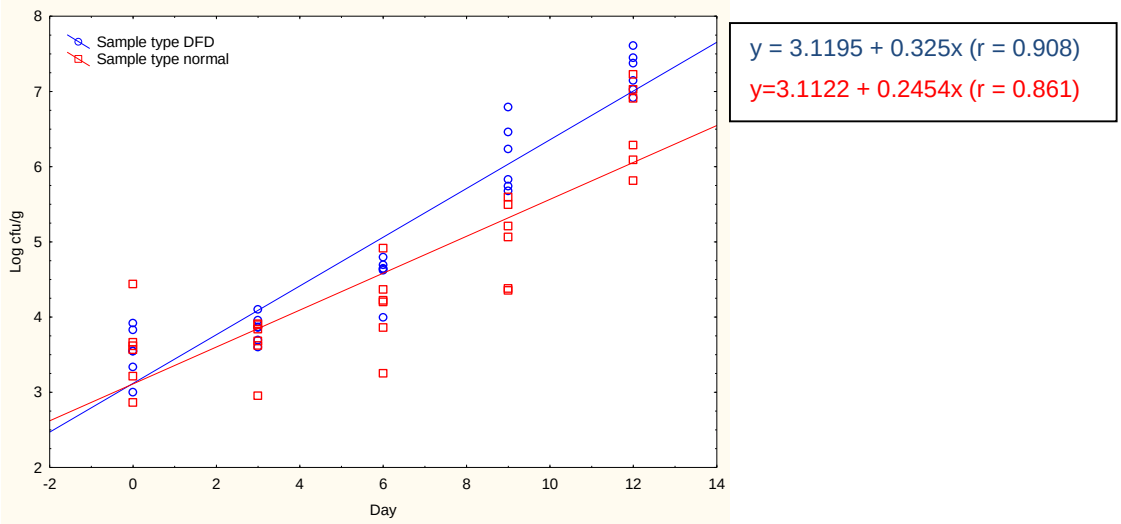

Figure 6.10 APC linear regressions fitted for each pH category

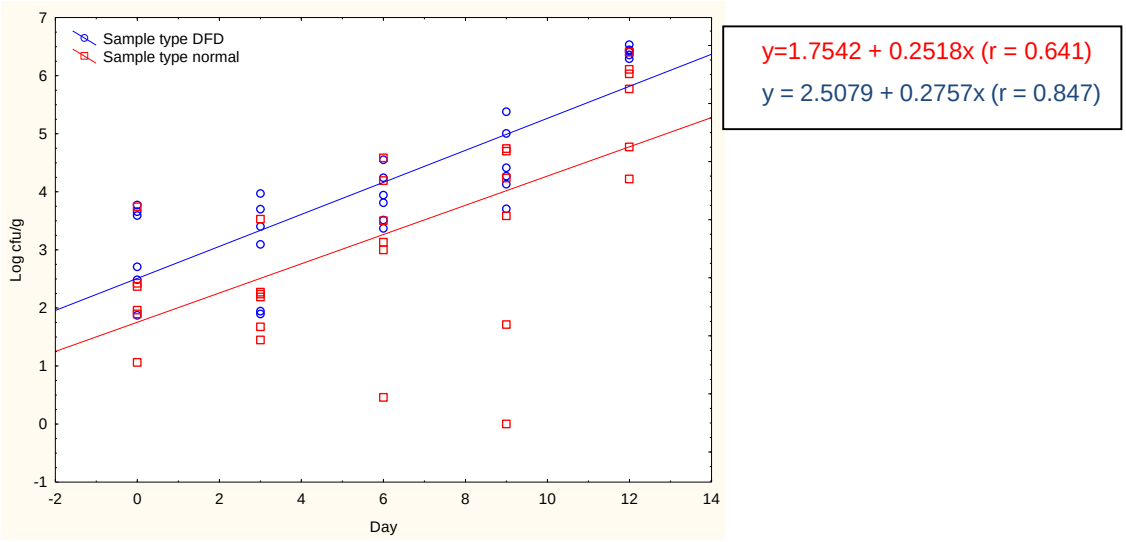

Figure 6.11 *Enterobacteriaceae* counts linear regressions fitted for each pH category

6.3.2.4 Signs of spoilage

Visual inspections for DFD meat samples showed prominent signs of spoilage such as slime and off-odours on the 9th day of the experiment. Samples with Normal pH showed signs of spoilage on the 12th day.

6.4 Discussion

The first aim of this study was to report on the effect of meat pH on muscle colour and initial microbial contamination whilst the second was to see whether meat pH influences the shelf-life stability (colour and spoilage) of game meat when stored under refrigerated conditions. The typical dark brown to red brown colour found in South African game meat has been attributed to the fact that wild angulates tend to be more active than normal livestock. Higher activity correlates to a higher myoglobin content in muscles. Hoffman *et al.* (2005) reported a myoglobin content of 7.3 mg/g for Impala. The higher myoglobin content makes it less attractive when compared to traditional meat types such as beef (Young & West, 2001). Additionally, the low intramuscular fat in game meat makes the meat appear darker to consumers (Hoffman & Wiklund, 2006). Volpelli *et al.* (2003) reported that typical colour coordinates for game meat can be as follows: L* < 40, high a* and low b* values. This typical pattern was followed by both Normal pH samples and DFD samples used in this study. During the death of an animal anaerobic glycolysis in the muscle is responsible for the breakdown of glycogen and formation of lactic acid, which dissociates in the muscle and the H^+ accumulates in the muscle and results in the lowering of muscle pH. However, during stressful situations ante-mortem, glycogen stores can be depleted before death, which can affect the reduction of pH (Hoffman, 2001; Hoffman, 2002). The depletion of glycogen can result in low lactic acid production, which can result in high muscle pH. Meat with high pH is referred to as ‘dark, firm and dry’ (DFD) meat. Therefore, the use of inefficient cropping methods can increase DFD meat incidences in game meat. Colour co-

ordinates corroborated with this theory, as colour results revealed that game meat with high pH was darker, due to the low L* value, more red and less yellow in colour, due to the lower a* and b* values (Hoffman & Laubscher, 2009; Hoffman, 2000). Initial colour L* ordinates were similar to those (28.40-29.45) recorded by Hoffman & Laubscher (2009) for Impala with a high ultimate pH.

DFD samples were found to have slightly higher initial APC and *Enterobacteriaceae* counts from day 0, the difference between the initial days of the study were less than 1 log cfu/g and did not differ statistically, which would indicate that the initial muscle pH did not play a part in the initial contamination as was reported by van der Merwe *et al.* (2013). Initial contamination can possibly be attributed to the harvesting (typically in the field) and/or the slaughter/dressing processes (Humphrey & Jørgensen, 2006). More specifically, Gill (2007) reported that the microorganisms on the skin, gastro intestinal tract and/or the bacteria found on the muscle tissue can contribute to the microbiological condition of game meat. Ultimately, the accurate execution of cropping and slaughtering techniques can determine the extent of contamination. As reported by van der Merwe *et al*. (2013), high bacterial counts can be due to the negative effect night cropping has on the ability to adhere to the hygiene principles put in place. Poor conformance can be due to poor visibility and inadequate lighting during evisceration. Poor working conditions during processing can also allow the transference of bacteria from the hide and gastrointestinal tract to sterile meat surfaces. Furthermore, handling of large game animals such as black wildebeest during the slaughter process, compared to small game animals can prove to be more difficult (Paulsen & Winkelmayer, 2004, Hoffman *et al.,* 2009). Irrespective of these factors, the fact that the initial bacterial load was similar for all the carcasses indicates that similar slaughtering and dressing procedures were followed.

The black wildebeest muscle could be categorised into two distinct pH categories: Normal ($pH < 6.06$) and DFD ($pH \geq 6.06$). A pH of 6.06 is higher than what is normally used for the classification of DFD meat although not all authors agree on the cut-off value, a pH of 6.0 seems to be a preferred cut-off point. The two different pH categories were ideal to answer the question of: does the difference in pH affect the rate of spoilage? Spoilage of game meat was investigated in an aerobic chilled temperature of 5 ± 1ºC. Normally meat is stored at 2ºC to inhibit bacterial growth. The higher temperature can warrant the classification of this study as an accelerated shelf life study, as temperatures above 2ºC can result in a suitable environment for elongation of microbial cells (Gill *et al.,* 1998).

During the study it was observed that pH values significantly increased from the initial value until day 12 for both normal and DFD samples (Figures 6.1). A similar occurrence of a rise in pH values was reported by Rodriguez *et al.* (2005) when aerobic spoilage of rabbit meat was investigated. In the present study, pH values for DFD meat increased earlier, as an increase was seen on day 6, whilst meat samples with a Normal pH, only experienced an increase in pH on the 9th day. The early increase in pH can be attributed to the depletion of glycogen by bacteria during the utilisation of glycogen as a nutrient source forcing the use of amino acids as a secondary nutrient source (Newton & Gill, 1978, Newton & Gill, 1980; Rodriguez *et al.*, 2005). The breakdown of amino

acids releases by-products such as ammonia, amines and other basic compounds, that can increase the environmental pH (increase in alkalinity), as seen from day 6 onwards in this investigation for the DFD classified samples. These by-products are responsible for spoilage symptoms such as off-flavours and off-odours. The delayed increase in the muscle pH seen for the game meat samples with Normal pH can be attributed to the fact that samples with Normal pH have a significant amount of residual glucose available for utilisation by the bacteria. Newton & Gill (1978) reported glucose content of 90-202 µg/g (wet weight) for samples with Normal pH, whilst samples with a high pH were reported to have a lower glucose content of 0 to 33 µg/g (wet weight).

Colour ordinates; L*, a*, b*, C* and H_{ab} were used to evaluate colour changes in game meat overtime. Slight changes in L* and b* values overtime were seen for samples with Normal pH and DFD pH values however, statistical tests detected no significant differences over storage period (within each category). This was similar to the stability found in L* values by Gomez *et al.* (2012) for foal meat stored at an aerobic chilled environment. An increase in the a* values can be an indication of meat samples becoming more red. For this study, a* values significantly decreased from day 0 for meat classified as DFD and Normal; stability in a* values were not observed for this study. Overall, Day 12 a* ordinates were significantly lower than values recorded on day 0 indicating a reduction of the red colour overtime (Pizato *et al.,* 2014). The reduction of the redness was visually more apparent for samples with Normal pH than DFD samples. The chroma values for the Normal pH category decreased overtime and were significantly higher than chroma values reported for DFD samples, which meant that the level of saturation decreased by the 12th day. For DFD samples, chroma values decreased until the 9th day, where after a significant increase in saturation from day 9th to 12th was recorded. Normal muscle pH samples and DFD samples showed an increase in Hue values overtime, this is indicative of the development of unacceptable discolouration. Although the same pattern was seen in DFD samples, a significant decrease in Hue values was recorded on the last sampling date. An increase in Hue values was also reported by Stevenson *et al.,* (1999) in red deer samples that showed Hue values of 43.89 on the 1st day, and on the 5th day values were as high as 51.74. However, the significant increase and decrease of chroma and Hue values respectively can possibly be attributed to the interference of slime produced by the bacteria on the surface of the samples as day 12 was also the peak day of spoilage (as bacterial counts exceeded 6 log cfu/g) for DFD samples. Overall colour changes can be attributed to the oxygen from air reacting with myoglobin, giving a bright red oxymyoglobin. This change in colour in an aerobic environment is short lived, allowing stabilisation of at least 1 to 2 days, before oxidation to metmyoglobin which is responsible for the grey-brown appearance (Wiklund & Smulders, 2011), which is not associated with freshness by consumers.

Bacterial counts ranging from 10^6 cfu/g to 10^9 cfu/g can allow for spoilage symptoms to show in meat (Gill & Gill, 2005). The highest bacterial growth was seen between day 6 and 12 for DFD meat samples. Aerobic and *Enterobacteriaceae* counts were reported to be >6 log cfu/g on the 9th and 12th day, respectively. Furthermore, slime formation; a symptom of spoilage was observed on

the 13th day when microbial counts reached >6 log cfu/g, this result concurs with the observations by Gill & Gill (2005). For game meat samples with a Normal pH, aerobic bacterial and *Enterobacteriaceae* counts reached 6 log cfu/g on the 12th and 17th day, respectively Furthermore, slime was also observed to have formed on the 12th day. Ultimately, microbial spoilage of DFD samples spoilt earlier than samples with a Normal pH.

6.5 Conclusion

This study reiterated that muscle pH can affect colour, as game meat with higher pH (DFD), had lower L*, a*, b*, C* and H_{ab} values, which resulted in darker game meat when compared to samples with Normal pH. However, muscle pH had no effect on the initial bacterial load. Secondly, muscle pH influenced the spoilage of game meat with samples exhibiting a high pH spoiling at a faster rate; the DFD muscles reached the microbial spoilage limit of ≥6 log cfu/g on the 9th day whilst samples with a Normal pH reached >6 log cfu/g on the 12th day. Due to the nature of the game animals harvested, the occurrence of DFD meat seems to be more prevalent and it is clear from this investigation that the initial muscle pH will influence the shelf-life of the meat/steaks when packaged under aerobic refrigerated conditions. However, spoilage of game meat at different pH categories still warrants further investigation particularly as pertaining to the changes of both amino acid and glucose levels, overtime *post mortem*. Another aspect that also warrants further research is the development of alternative products/processes for DFD game meat.

6.6 References

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CHAPTER 7
GENERAL CONCLUSION AND RECOMMENDATIONS

The increase in demand for game meat is increasing both locally and internationally. The demand can be associated with the fact that game meat is low in kilojoules, low in cholesterol and is also protein dense (Hoffman & Wiklund, 2006). Furthermore the rise of the industry can be seen as many cattle and sheep farmers are now shifting into game farming where income is generated from the following components: ecotourism, hunting, breeding for the selling of game animals and meat production (Bekker *et al*., 2011). The fact that meat production is an integral part of the game meat industry does warrant the investigation of microbial safety of game meat.

The safety of game meat can be compromised by inadequate hygiene practices during the harvest and slaughter process (Galland, 1997; Gill, 2007), which can lead to microbiological hazards being present in game meat, which will ultimately pose a threat if consumed by human beings and consequently, decrease quality of game meat.

The first aims of this study were to determine possible steps within the harvest and slaughter processes that could be major sources of contamination of game meat and ultimately determine the microbial quality of commercial game meat. Chapter 3 revealed that the evisceration step (which is the first point that exposes flesh) can contribute to the microbial quality of game (springbok) meat. At this point high aerobic plate counts were reported. Furthermore, *Enterobacteriaceae*, *Clostridia* spp., Lactic Acid Bacteria (LAB) were also found at this point, which led to the conclusion that poor hygienic practices in the field, due to inadequate working conditions and/or insufficient training can lead to the presence of unwanted bacteria (van der Merwe *et al.* 2013). Furthermore, high counts can be attributed to the dusty terrain created, as springbok tend to run in herds. The study also showed a consistent presence of aerobic bacteria, *Enterobacteriaceae*, *Clostridia* spp. and LAB post-evisceration, post-skinning and post-chilling. However, *E. coli* was only detected for samples taken after skinning, which corroborated the notion that the skinning process is indeed a point of contamination.

The revelation that the skinning step can contribute to contamination of game carcasses (Chapter 3) led to the study of the slaughter process in more detail. Firstly, Chapter 4 corroborated that skinning process can indeed contribute to the contamination of carcasses, this corroborated with studies conducted by Gill (2007) and other authors. Bacterial counts from carcass surfaces after skinning, were significantly lower than those found on the skin, however counts were higher than those reported for other studies. Secondly, Chapter 4 also revealed that bacterial counts decreased as the slaughter processed commenced, however bacterial counts were once again higher than counts reported in other studies. As part of Chapter 4, the distribution of bacteria after skinning was investigated. Results showed that during skinning cross contamination due to neighbouring carcasses and hanging hides, can allow for contamination to occur. For the purpose of spoilage, the presence of *Clostridia* spp. was investigated in Chapter 4. Results showed a consistent presence of

Clostridia spp. from post-skinning samples until post-chilling samples. Skinning was an additional step that could lead to contamination. Chapter 4 also revealed that personnel had a major role in the extent of the contamination, as personnel directly handle game carcasses. This led to the conclusion that if hygienic practices have to be adhered to so as to reduce the extent of contamination, an initial step would be proper food safety training aimed at personnel in contact with carcasses/meat.

Chapter 5, focused on the hygiene levels of the value adding section of a game abattoir. Hygiene was assessed during the production of game meat. This section of the investigation investigated the effect that the value adding process has on the microbial quality of game meat products. This chapter revealed that points of contamination found in Chapters 3 and 4 (evisceration and skinning) that were responsible for the initial bacterial load on game carcasses could also be responsible for the hygiene levels (or lack thereof) found during the production of game meat products (Nel *et al.,* 2004, Berends *et al.,* 1998). This was seen from the lack of bacterial counts enumerated from the surface, equipment and personnel samples taken before the day's production started versus bacterial counts found during production. Furthermore, this study revealed that microbial quality of game meat products can be influenced by the inadequate cleaning procedures in place as was also noted by Green & Selmans (2005); cleaning procedures decreased bacterial counts but did not eliminate bacteria from the surfaces, equipment and hands. This Chapter again exposed the need to train personnel on the importance of hygiene practices put in place. With the proper knowledge personnel can then help to reduce further contamination by making sure that hygiene practices are being used to the maximum efficacy.

Process steps that contribute to contamination of game carcasses can be summarised in a hazard analysis as depicted in Table 7.1. Table 7.1 also lists possible control measures that can be implemented to reduce contamination.

Lastly, the effect of the slaughter/culling process on the quality of game meat was conducted in the form of a shelf life study. For this study, the effect of pH on game meat as influenced by *ante-mortem* stress was identified as normal in pH or high in pH. High pH samples were referred to as dark, firm and dry (DFD). Samples from 12 commercially harvested black wildebeest were used and the data was categorised into Normal pH (pH < 6.06) and DFD (pH ≥ 6.06). These results revealed that pH indeed influenced the appearance of game meat, as colour ordinates were lower than those reported for samples with Normal pH, however pH had no influence on the initial *Enterobacteriaceae* and aerobic plate counts. The latter results once more indicating that the slaughter process was the probable cause of this initial contamination. This study also revealed that muscle pH influenced shelf life with the DFD meat experiencing the following: an increase in pH at day 6, a progressive decrease in colour ordinates and reaching the microbial spoilage limit of >6 log cfu/g on day 9. Whilst muscle samples with a Normal pH range had a similar spoilage trend; but microbial spoilage occurred at a later stage.

Table 7.1 Hazard analysis for a game abattoir

Process step	Hazard	Cause	Control measure	Critical limit
Evisceration in the field	*Salmonella* spp. and *E. coli.*	Presence of bacteria is due to possible spillage of intestinal content during evisceration in the field	Tie oesophagus before the removal of offal and ensure that offal is not punctures	*Salmonella* spp. -absent in 25 g *E. coli* - <10cfu/cm^2
Receiving game carcasses		Presence of bacteria is due to insufficient hygiene practices during evisceration in the field	Trim visible contamination, during primary inspection of carcasses	
Cold storage of game carcasses		Presence and growth of bacteria is due to ineffective cooling of carcasses	Air temperature should be 2 °C Internal muscle temperature should be <7 °C for large game carcass	
skinning, trimming and halving of carcasses		Cross contamination from: hide, left over intestinal content, worker's hands and clothing, equipment used (such as knives and electronic saws)	Visible contamination should be removed Equipment (knives, electronic saw) should be washed disinfected	
Deboning and portioning of carcasses		Cross contamination from: Worker's hands and Clothing, equipment used (such as knives), work surfaces and air supply	Equipment, hands and work surfaces should be washed and disinfected regularly	

Overall the microbial quality of game meat is largely dependent on the harvesting process and slaughter process. Therefore, it is important to control the steps listed above in order to decrease the initial bacterial load found on carcasses and consequently that on game meat products within a robust practical food processing system.

Additional research could focus on the use of risk reduction techniques on the carcasses and in the production process such as hot water washes, antimicrobial chemical agents (permitted by the South African legislation). Furthermore, re-evaluation and training of staff involved in the production processes about the overall importance of food safety and the adherence to hygiene practices put in place could help to decrease the spread of contamination. Additionally, future studies should look at investigating more than one abattoir in order to obtain more robust data. A bigger study of this kind would present useful information that would help in the evaluation of the microbial quality of game meat.

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Annexure A

(Logo of Ranch Owner) Record for traceability

(This form must be completed for each days hunting)

A. PARTICULARS OF FARM AND OWNER

Registered Scheme number of farm: _____

Name of farm: _____

Name of owner / manager: _____

B. PARTICULARS OF HUNTED CARCASSES

Carcass Number	Inspection status A-approved, E-partially condemned, C-totally condemned	Comments relating to ante- and post-mortem inspection

C. DECLARATION BY AUTHORISED PERSON

I, _____ hereby declare that:
(name of GME)

The abovementioned game carcasses have been hunted and recorded correctly.

Signed at _____, (date) _____
(place)

_____
Signature