# The relationship between hygiene assessment system audit scores and the bacteriological status of single species red meat abattoirs in the Free State province

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## Declaration

I, **ITUMELENG MATLE,** the undersigned, declare that the dissertation hereby submitted to the **Central University of Technology, Free State** for the degree **Magister Technologiae Environmental Health: Food safety** and the work contained therein is my own original work and has not previously, in its entirety or in part, been submitted to any university for a degree.

Signed .....this.....day of.....2016

## Dedication

This dissertation is dedicated to my wife, Ntsiki.

## Acknowledgements


- The completion of this dissertation would not have been possible without the assistance of a number of people. Many individuals provided inputs in various aspects of this study. I would like to start by thanking our Heavenly Father for giving me the ability to study. For the privilege to study I would like to thank my wife Ntsiki: none of this would have been possible without your continual support dear.

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## List of abbreviations

| | |
|---|---|
| AIDS | Acquired Immunodeficiency Syndrome |
| APC | Aerobic Plate Count |
| API | Analytical Profile Index |
| ARC | Agriculture Research Council |
| $A_w$ | Water Activity |
| BPA | Baird Parker agar |
| BPW | Buffered Peptone Water |
| BRC | British Retail Consortium |
| $^0C$ | Degrees Celsius |
| CAC | Codex Alimentarius Commission |
| CAR | Corrective Action Report |
| CDC | Centre for Disease Control |
| Cfu | Colony forming units |
| $cm^2$ | Centimetre squared |
| DAEC | Diffuse-adhering *E. coli* |
| DAFF | Department of Agriculture, Fisheries and Forestry |
| DoA | Department of Agriculture |
| DoH | Department of Health |
| DNA | Deoxyribonucleic acid |
| EAEC | Enteroaggregative *E. coli* |
| EHEC | Enterohaemorrhagic *E. coli* |
| EIEC | Enteroinvasive *E .coli* |
| EPEC | Enteropathogenic *E. coli* |
| ETEC | Enterotoxigenic *E. coli* |
| EU | European Union |
| FAO | Food and Agriculture Organisation |
| FSMS | Food Safety Management System |
| GHP | Good Hygiene Practice |
| HACCP | Hazards Analysis Critical Control Points |
| HAS | Hygiene Assessment System |
| HMPs | Hygiene Management Programmes |
| HMS | Hygiene Management System |
| H | Flagellar antigen |

| | |
|---|---|
| IFS | International Food Standard |
| IMQAS | International Meat Quality Assurance Services |
| ISO | International Standard Organisation |
| K | Capsule antigen |
| KJ | Kilojoule |
| LT | Heat labile toxin |
| Min | Minute |
| Mg | Milligram |
| ml | Millilitre |
| MRS | De Man Rogosa Sharpe |
| MSA | Mannitol Salt Agar |
| NaCl | Sodium chloride |
| NARS | National Abattoir Rating Scheme |
| NC | Non-conformance |
| O | Somatic antigen |
| OIE | International Alimentarius Commission |
| QA | Quality Assurance |
| RMAA | Red Meat Abattoir Association |
| RSA | Republic of South Africa |
| SABS | South Africa Bureau of Standards |
| SAMAIC | South Africa Meat Industry Corporation |
| SANS | South Africa National Standards |
| SOP | Standard Operating Procedure |
| SRK | Swab rinse kits |
| SS | *Salmonella Shigella* |
| *ST* | Heat stable toxin |
| *STEC* | Shiga toxin-producing *E. coli* |
| *Stx* | Shiga toxin |
| UN | United Nations |
| USA | United States of America |
| USDA | United States Department of Agriculture |
| WHO | World Health Organisation |
| WTO | World Trade Organisation |

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**Summary**

*The Hygiene Assessment System (HAS) is an audit checklist that is used to measure the hygiene status of the abattoir. The final HAS score for individual abattoirs is graded to a sum of 100, and is interpreted as a measurement of the potential risk to public health. Theoretically, the final HAS score reflects the likelihood of safe meat being produced in that specific abattoir on the day of audit. The aim of the study was to test the association between the HAS scores and the bacteriological contamination in six single species high throughput abattoirs in the Free State province. This was done to validate the efficiency of the HAS score as a measure for meat safety and to determine the extent to which HAS audit score and bacteriological tests mirror each other. Each abattoir was visited once and the audit was performed according to official HAS: four carcasses were sampled at four different carcass sites at three processing stations; and ten direct air samples were collected from the slaughter floors. All the abattoirs showed compliance with the meat safety legislation since the total HAS scores ranged from 68 to 94. However, it was found that the effectiveness of HAS audits as a measure of food safety was questionable, since it does not demonstrate the risk/impact of non-compliance. The microbiological analysis for both carcass and air samples included the test for aerobic plate count (APC), Escherichia coli, Salmonella species and Staphylococcus aureus. The APC for the abattoirs ranged from undetectable to 9.9 x $10^4$ CFU.$m^{-2}$ for carcass surfaces and 0 to 2.4 x $10^2$ CFU.$m^{-3}$ for bioaerosols. The total count for E. coli, S. aureus and Salmonella species exceeded the national maximum acceptable limits. These results highlight the possibility of the occurrence of foodborne diseases in the human population. In addition the relationship between E. coli, S. aureus, Salmonella spp, APC, and total HAS score, revealed no significant relationship. These findings further justify the fact that HAS*

*audits should not be used as a measure of meat safety. The results also suggest the importance of the inclusion of bacterial tests in meat safety audits because a high HAS score does not signify that meat is entirely safe for human consumption.*

**Key words**: HAS audits, *E. coli, Salmonella species* and *S. aureus*

# CHAPTER ONE

## General Introduction

## 1.1 Introduction

The slaughtering of animals for human consumption is inevitable in most nations of the world and dates back to ancient times (Jode *et al.,* 1906). In regard to abattoirs, reports indicate that the concept of abattoirs was introduced early in the 19th century, under Napoleon (DAFF, 2012). The term "slaughter poles" was previously used to designate an abattoir (Shale *et al*., 2006): these "slaughter poles" generally consisted of two upright poles with a horizontal crossbar onto which the animal could be hoisted (Nel, 2003). As a result of growing populations and increased awareness of hygiene and foodborne diseases, meat producers became more and more aware of the demand for better and more hygienic practices in their production processes (Van Zyl, 1995). This subsequently rendered the "slaughter poles" inadequate for slaughtering, which led to the construction of a facility known as an abattoir (Nel, 2003).

An abattoir, also known as a slaughter house, is a place where animals are butchered to provide food for humans (Panisella *et al.,* 2000). According to the Abattoir Hygiene Act, Act 121 of 1992 (RSA, 1992), an abattoir is a place where animals are slaughtered or are intended to be slaughtered, and includes all facilities which normally appertain or are attached to such a place, whether or not such facilities are situated at the same place as the actual abattoir. Bello and Oyedem (2009) further define an abattoir as a premise approved and registered by the controlling authority for hygienic slaughtering and inspection of animals, processing and effective preservation and storage of meat products for human consumption.

Today, over 200 years since the origin of the abattoir, there are about 500 red meat abattoirs in South Africa, which are classified as high throughput, low throughput or rural abattoirs, according to the number of animals slaughtered per day (RSA, 2000). These abattoirs slaughter approximately 2,3 million cattle, 2,4 million pigs and 5,5 million sheep on an annual basis, which together contributes 34.1% to the total domestic agricultural production and provides 36% of population protein needs. These abattoirs also account for 21.4% of the total meat produced on the continent of Africa and 1% of global meat production (RMAA, 2014). By virtue of being a major industry, there is a need for careful inspection, monitoring and training of personnel to ensure compliance with health, safety and other relevant regulations, hence the importance of veterinary involvement in the production of meat that is safe for human consumption.

The safety of meat starts at the farm and ends on the dinner plate of the consumer (farm to fork concept). Before the final retail product reaches the hands of a consumer, several hygiene management practices have already taken place (Gillespie & McLanchin, 2007). The first step in a meat hygiene system is the monitoring of all aspects of animal husbandry practices on the farm, for the production of clean and healthy livestock. Farmers and veterinary practitioners are responsible for ensuring that only animals fit for slaughter, loading, travelling and subsequent unloading are transported to the abattoirs (Nørrung & Buncic, 2008). Animals must be sent to the registered abattoirs for slaughter where hygienic processing and meat inspection are compulsory and also regulated by legislation (Mohammed, 2011). The carcasses are then dispatched to wholesalers, retailers and butcheries inside cold trucks, where they are offloaded and kept at a required temperature of ≤ 7°C, then processed, packaged and labelled (RSA, 2000). Consumers however play a significant role in meat safety

as the vast majority of meat-borne bacterial illnesses occur at consumer level due to inadequate hygiene and cross-contamination of raw meat products. Some of these practices include inadequate cooking methods and poor storage conditions, often related to temperature (Nyenje *et al*., 2012).

Farm animals are the original source of meat-borne pathogens causing foodborne diseases in humans and the most significant of these pathogens are zoonotic bacteria (Bolder, 2007). The deep muscle tissues of healthy slaughtered livestock contain few if any bacteria, and contamination of meat occurs mainly during processing at abattoirs, particularly during removal of hides and evisceration (Bouttier *et al.,* 1994). Other possible contamination sources include the design of abattoirs, meat contact surfaces, equipment, utensils, aerosols or condensation forming on ceilings, water supply and personnel (Tshabalala, 2010; Mohammed, 2011). The influence of these factors on the safety of meat cannot be determined by visual observation and necessitates monitoring by bacteriological (or general microbiological) surveillance in order to determine the influence of these factors on meat safety (Derbyshire, 2013). Managing and assuring the safety of meat, given all of these risk variables, requires systematic process control at abattoirs (Govender *et al.,* 2013). Most governments worldwide have adopted the Hygiene Management System (HMS) applicable to the meat industry and regulate the mandatory implementation of such system (Brashears *et al.,* 2001).

The Hygiene Management System (HMS) is a set of pre-requisite food safety programmes that are designed for macro-monitoring of operating processes at abattoirs (Mutsinze, 2013). The HMS is based on the principles of Good Hygienic

Practices (GHPs) and the Hazard Analysis Critical Control Point (HACCP) system. It is also based on the twelve steps that have been recognised by the International Organisation for Standardisation (ISO) as an important tool for assessing and managing health risks posed by foodborne pathogens (ISO, 10330:2007). This system is a food safety system that has been widely tested and established as an effective means of preventing foodborne diseases if correctly implemented (Ehiri *et al*., 1997).

In South Africa the safety of meat is governed by the Meat Safety Act, Act 40 of 2000 (RSA, 2000). The purpose of this legislation is to promote meat safety and the safety of animal products, to regulate the importation and exportation of meat and to establish meat safety within South Africa and across its borders (RSA, 2000). Section 11 of the Meat Safety Act, called “essential national standards”, requires all abattoirs to be managed in accordance with an approved hygiene management and evaluation system, whilst the Red Meat Regulations (RSA, 2004) promulgated in terms of the Meat Safety Act require that the hygiene status of an abattoir be determined by means of the Hygiene Assessment System (HAS).

The Hygiene Assessment System (HAS) is an audit compliance checklist used in abattoirs to conduct audits on adherence to the requirement set by the Red Meat Regulation (RSA, 2004). “It does not only consider the hygiene and hygiene practices within a facility, but takes a holistic view of all aspects that can have an influence or impact on the safety of the products. All hazards as well as quality and managerial aspects are considered in such evaluations” (Derbyshire, 2011). The HAS is the only nationally accepted ‘scientific’ measure of the effectiveness of an abattoir’s HMS (Derbyshire, 2011). The HAS scores are allocated based on the hygiene status of an

abattoir on the day of the audit. The final HAS scores are interpreted as a measurement of the potential risk to public health of products derived from a specific abattoir. It is generally accepted that the HAS score reflects the likelihood of safe meat being produced in that specific abattoir on the day of audit. Therefore the higher the HAS score out of 100, the lower the risk. Hence, together, HMS and HAS are central in the management and demonstration of meat safety during processing at South African abattoirs (Govender *et al.,* 2013). The government and/or local authorities are monitoring and evaluating the compliance of the abattoirs to the set requirements.

The Meat Safety Act (RSA, 2000) enforces the implementation of the HMS and HAS in South African abattoirs. However specific food safety parameters such as bacteriological testing for zoonotic pathogens are not covered under this act, and this gap in the legislation makes the safety of the meat produced in South African abattoirs questionable. Bacteriological tests are used to determine the safety as well as the shelf life of the meat (Brown, 2006). These tests also help the abattoirs towards understanding how the bacteria enter or spread through the food chain and therefore find approaches on how to prevent or minimise exposure to the consumers to such microbial agents (Kiiyukia, 2003). Accordingly, bacteriological testing is essential if the meat safety is to be assured, rather than assumed from non-bacteriological qualities such as HAS which may or may not reflect the bacteriological conditions of the meat.

## 1.2 Rationale

The lack of bacteriological testing of meat and meat products could result in foodborne disease outbreaks if the pathogens are present at levels above the recommended limit.

The potential risk of foodborne diseases is particularly high in groups such as children, the elderly, pregnant women and those with a compromised immune system (Tshabalala, 2010). This places a large percentage of the South African population at risk, considering that the old, the young and those who are immuno-compromised contribute significantly to the total population (Statistics South Africa, 2012). Human infection by foodborne bacterial pathogens can lead to a wide range of non-specific clinical illnesses, from diarrhoea, fever, stomach and abdominal cramps, headache, muscle cramps, nausea, vomiting, bloody diarrhoea to neurological symptoms and death (Mead & Griffith, 1998). Foodborne disease outbreaks may also cause major food safety scares that go beyond monetary losses and can put consumers off a product permanently (DAFF, 2012).

The challenges mentioned above show the significance of the inclusion of bacteriological tests in meat safety assessment in South Africa. If meat safety can indeed be assured by systems of control (HMS) that are based on the subjective assessment of the hygienic condition of an abattoir (HAS), then bacteriological testing may only be peripheral in the system for assuring the safety of meat. This is not acceptable and the HAS on its own is possibly not adequate in determining the safety level of meat, which prompted this study in generating the following questions; (1) can the HAS audit scores of abattoirs be regarded as an indication of the safety of the meat derived from abattoirs and; (2) To what extent do HAS audit scores and bacteriological tests mirror each other?

The main aim of this study was therefore to determine whether there is an association between HAS audit scores and the bacteriological status of single species red meat abattoirs in the Free State province.

The objectives below were established to address the main aim:

- To conduct HAS audits at single species abattoirs in the Free State province.
- To assess microbial levels on carcass surfaces on randomly selected carcasses.
- To assess the prevalence of bioaerosols within selected areas.
- To further assess possible relationships from the HAS audits, microbial levels and airborne bacterial counts.

## 1.3 Study area

This study was conducted in the Free State province, South Africa. The Free State province is situated on an almost flat, boundless plain in the centre of South Africa and is divided into five municipal districts (Figure 1.1). It is the country's third largest province, making up 10.6% of South Africa's land with an area of around 129 825 square kilometres. The Free State province has the largest number of red meat abattoirs in South Africa, accounting for 16% of the total population of red meat abattoirs in the country. The province also commands the second greatest share of red meat production in South Africa (RMAA, 2014).


**Figure 1. 1:** Map reflecting municipalities of Free State province, inclusive of the study area (Google, 2013)

## 1.4 Study protocol

Hygiene Assessment Systems (HAS) audits as well as carcass swabs and air samples were collected at six single species high throughput red meat abattoirs spread over three districts (Motheo, Lejweleputswa and Fezile Dabi) of the Free State province. The abattoirs, designated as A, B, C, D, E and F, are registered to slaughter cattle, sheep and pigs (Table 1.1). The criterion used for selecting these abattoirs to participate in the study was the mean scores of HAS audits of the last three official HAS audits conducted by provincial inspectors at single species abattoirs in the province (Free State Veterinary Service, 2012). Two abattoirs were selected for each species, according to the highest and lowest scores, to participate in the study.

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**Table 1.1:** Details of HAS scores for the abattoirs included in the study (Free State Veterinary Service, 2012)

| Abattoirs | Species | District | HAS Score [Mean] |
|---|---|---|---|
| A | Cattle | Lejweleputswa | 91 |
| B | Cattle | Fezile Dabi | 78 |
| C | Sheep | Motheo | 74 |
| D | Sheep | Motheo | 52 |
| E | Pig | Fezile Dabi | 86 |
| F | Pig | Lejweleputswa | 76 |

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# CHAPTER TWO

## Literature Review

## 2.1 Historical review of laws governing meat safety in South Africa

Concern for the safety of meat intended for public consumption can be traced back to biblical times and in fact long before the time of Jesus Christ. Moses, for example, commanded the Israelites (Leviticus 11:39) not to eat meat from diseased animals (Nel, 2003). In terms of abattoirs, meat safety can be traced back to the early nineteenth century in Europe, with the earliest records indicating that the concept of abattoirs was first introduced in Paris during the 18$^{th}$ century under Napoleon (DAFF, 2012). In South Africa, meat safety was first controlled by the Public Hygiene Act, Act 36 of 1919 (RSA, 1919). According to the Public Hygiene Act, the responsibility of meat safety at abattoirs resided with the Department of Health (DoH) (Derbyshire, 2013). However with the introduction of the Animal Slaughter, Meat and Animal Product Hygiene Act, Act 87 of 1967, the Department of Agriculture (DoA) became the responsible authority for regulating meat safety in South African abattoirs (RSA, 1967). This Act presented requirements to be met by municipal managers of abattoirs. During this time, abattoirs were owned by government and only government abattoirs were legally allowed to slaughter animals for re-sale in South Africa (Govender *et al.,* 2013). The DoA and local municipalities became the sole providers of meat safety services in the country (DAFF, 2012). Nonetheless, the mandate of meat safety beyond the abattoir remained the responsibility of the DoH.

During the 1980s, government abattoirs started to close down. The reason for such closures was the high cost of running the abattoirs, which were generally running at a loss (Govender, 2009). This led to the government encouraging the privatisation of abattoirs, but still providing meat inspection services. The Animal Slaughter, Meat and

Animal Product Hygiene Act, Act 87 of 1967 was repealed and replaced by the Abattoir Hygiene Act, Act 121 of 1992 (RSA, 1992). After the promulgation of the Abattoir Hygiene Act, the meat inspection function at abattoirs was also privatised. This led the public to believe that government has completely given up control and resulted in smaller abattoirs emerging all over the country (Derbyshire, 2013). As meat inspection services were now privatised, abattoir owners and private meat inspection companies concentrated only on primary meat inspection. This led to hygiene control at abattoirs becoming a matter of concern for the government, whose only method of ensuring hygiene at abattoirs was through routine inspections (Govender, 2009). The only way of assuring meat safety was to ensure that slaughtering took place in approved and registered abattoirs, with the meat being inspected and passed by registered meat inspectors (ARC, 2000). Meat inspection only focused on removing diseased carcasses from the human food chain (primary meat inspection) and not on the assurance of the microbiological or bacteriological safety of meat (Tompkin, 1990).

As previous legislation in South Africa had concentrated mainly on the hygienic production of meat and the removal of gross or visible zoonotic pathogens from the food chain through primary and secondary meat inspection, the current legislation, called the Meat Safety Act, Act 40 of 2000, was promulgated. This Act was promulgated in an attempt to improve hygiene conditions at abattoirs. The Meat Safety Act mandates the state veterinary services, as the controlling authority, to provide maintenance of proper standards of hygiene in the slaughtering of animals and in handling of meat and animal products (RSA, 2004). It also compels abattoir owners to protect public health in the safe processing and dispatching of meat through

compulsory HMS. However, safety parameters such as microbiological or bacteriological testing for pathogens are not mandatory under this Act.

## 2.2 The need for food control systems

Food control systems have been defined by the Food and Agriculture Organisation (FAO) as mandatory regulatory activities of enforcement by controlling authorities to provide consumer protection (Chanda *et al.,* 2010). These activities have to ensure that all foods during production, handling, storage, processing and distribution are safe, wholesome and fit for human consumption, conform to safety and quality requirements, and are honestly and accurately labelled as prescribed by law (FDA, 2012). However, this study is limited to the production and processing of meat in the abattoir.

Food control systems vary in structure and the FAO/WHO has provided three categories of these systems where the first category is referred to as a multiple agency food control system. This type of system is characterised by the structure and functions of the food control system being segregated, usually under different government departments or levels of government (FDA, 2012). However, the segregation can be both horizontal and vertical where food law drafting, inspection and testing services are physically and functionally separated into different departments of the same government (Chanda *et al.,* 2010). The second category of food control systems have their functions consolidated under a single authority, not necessarily within a single government department, but that could include a parastatal body that answers to one government department (FDA, 2012). In this type of system, categorised as a single

agency food control system, food law drafting, inspection and testing services are integrated under one body with the same objective and mandate (Garcia & Jukes, 2004). The third category of food control system is characterised by coordination of various activities of the system by different agencies or departments, for example, food law drafting, inspection and analysing are coordinated independently. The monitoring of the system could also be coordinated by a separate section (FDA, 2012). The South African food control system combines all three food control systems mentioned above.

Due to lifestyle changes, pressure of growing human populations, industrialisation, the globalisation of the food trade and advances in food technology, more opportunities for food contamination result from more diverse sources, rendering individual attempts in assuring food safety ineffective (Garcia & Jukes, 2004). The end point testing of foods at processing facilities, focusing primarily on good hygiene practices to assure safety, has been replaced by food safety management systems (Govender, 2009), and the need to reduce the risk of foodborne diseases through formal control systems is no longer seen as optional but as mandatory. The necessity for an efficient national food control system thus arises not only from public health considerations, but also because of trade and economic implications (Govender *et al.,* 2013). Therefore, the South African food control system as far as meat safety is concerned, can be considered ineffective in assuring food safety for nationally supplied meat, because there are no standards in place to ensure the microbiological safety of meat.

In the past, countries could regulate foreign goods coming into their markets through mechanisms such as subsidies or quotas, and could exclude products from countries posing risks to public health. In an initiative to improve global trade, new World Trade

Organisation (WTO) guidelines now call for quotas and subsidies to be generally disallowed or lowered (Chanda *et al.,* 2010). However, lower tariffs are exposing other access restrictions, such as technical requirements. Technical requirements are a major means by which countries can control access to their markets. The elimination of technical trade barriers can only be achieved if trading countries have confidence in the quality systems of their trading partners to ensure that public health is appropriately protected, quality standards are maintained, and fraudulent practices are prevented (Hugas & Tsigarida, 2008). South Africa should therefore have a food control system that is internationally recognised and accepted as it is member of the WTO, the Codex Alimentarius Commission (CAC) and the World Organisation for Animal Health, subscribing to the International Code for Animal Health (OIE), and it must comply with the requirements of these bodies.

## 2.3 South African food safety control

South Africa has the fundamentals of a food control system in place, although legislation and functions are not confined to a single government department. Rather, laws, regulations, standards, enforcement and testing services are fragmented and control and/or administration of these are a shared responsibility by two main national departments namely the Department of Agriculture, Forestry & Fisheries (DAFF) and the Department of Health (DoH) (Chanda *et al.,* 2010). In addition, provincial and local authorities are also involved in food control for enforcement of legislation drafted at national level (Brückner *et al.,* 1998; DoA, 2003).

The DAFF exercises partial authority over farms, feedlots and abattoirs and is mandated to administer the Animal Diseases Act, Act 35 of 1984 (RSA, 1984) and the Meat Safety Act, Act 40 of 2000 (RSA, 2000). The application of the Animal Diseases Act, which makes provision for the management of diseases in order to safeguard livestock and is applied at producer level, also identifies animal diseases, especially zoonotic diseases, in order to ensure that they do not end up in the food chain (Govender *et al.,* 2013). The powers to legally enforce this act and its related regulations are delegated by the DAFF to each of the nine provincial veterinary services of South Africa (Chanda *et al.,* 2010).

The Meat Safety Act, Act 40 of 2000 (RSA, 2000), governs meat safety in South Africa at the abattoir level. The aim of the regulations, promulgated in accordance with the Act, is to promote meat safety and the safety of animal products, to regulate the importation and exportation of meat and to establish meat safety schemes (RSA, 2000; 2004). Section 7 of the Meat Safety Act requires that all animals be slaughtered in registered abattoirs with the exception of slaughtering for cultural or religious purposes or for personal use (RSA, 2000). Furthermore section (11) (1) (e) of the Act, referred to as “essential national standards”, requires all abattoirs to be managed in accordance with approved HMS. The owner of an abattoir is responsible for the development, implementation and management of HMS. The government (DAFF) is responsible for the evaluation of HMS at abattoirs (RSA, 2004).

When the carcasses and/or meat are removed from the premises of registered abattoirs, the DoH is legally mandated to ensure the safety of this meat. This includes transportation between the abattoir and cutting plants or other retail recipients

(Govender, *et al.,* 2013). The national DoH is entrusted with the Foodstuffs, Cosmetics and Disinfectants Act, Act 54 of 1972 (as amended by Act 39 of 2007) (RSA, 2007a) and is responsible for meat safety at cutting plants and retail outlets for national consumption. District and metro municipalities enforce the Act for food that is manufactured and sold locally, while the provincial level deals with imported foodstuffs (Govender *et al.,* 2013). The public and private role players involved in ensuring production and provision of safe meat in South Africa are represented diagrammatically in Figure 2. 1.

Figure 2.1 also shows that meat-based product processors can source meat or animals directly from the feedlots. This is because previously, under the marketing regime, meat-based product processors mostly bought carcasses through the auction system (DAFF, 2012). Currently, many source live slaughter animals (not weaners) directly from farmers or feedlots on a bid and offer basis, i.e. they take ownership of the animal before the animal is slaughtered. The animal is then slaughtered at an abattoir of the meat-based product processor's choice, after which the carcass is distributed to the retailers. In some instances, the public can also buy carcasses directly from abattoirs.

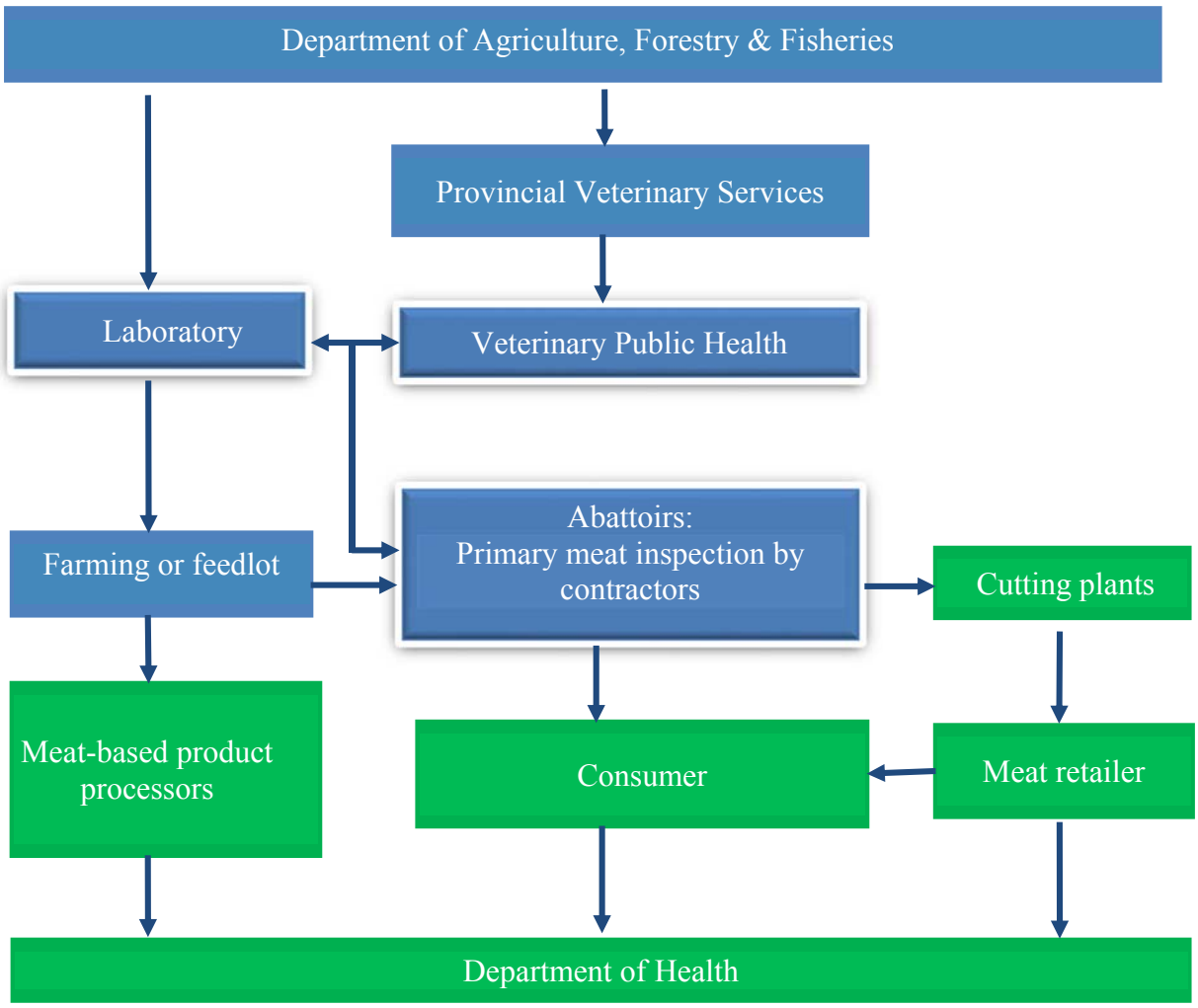


**Figure 2. 1:** Meat safety regulatory control in the meat supply chain (*adapted from Govender et al., 2013*). Blue blocks indicate the areas controlled by DAFF, while green blocks indicate the areas controlled by DoH. White shaded blocks indicate study areas.

## 2.4 Hygiene Management System

Hygiene Management System (HMS) is a set of prerequisite food safety programmes that is designed for the macro-monitoring of operating processes at abattoirs and it is designed to enable abattoirs to become self-governing entities for the production of safe meat (Mutsinze, 2013). “The HMS presents process standards and norms that relate to infrastructure and operating practices. It also provides for quality control measures through the monitoring of specific activities and processes at abattoirs. The HMS requires hazard analysis of all abattoir processing and operations in abattoirs. The HMS also requires mitigation measures to be planned and documented as part of hygiene programmes within the HMS” (Govender *et al.,* 2013). Although this is an improvement on previous legislation, specific food safety parameters such as microbiological or bacteriological testing for pathogens are still not mandatory under the Meat Safety Act, Act No. 40 of 2000. The premise of this type of management system is that if all standards are complied with, the likelihood of unsafe meat being processed at abattoirs is significantly reduced (Govender, 2009).

The HMS requirements may be summarised as follows: (1) assessment of the hygiene status of the abattoir by means of the Hygiene Assessment System (HAS) by Veterinary Public Health (VPH) officers; (2) provision of results to the provincial executive officer for verification as frequently as he or she may require; (3) a documented management system; traceability; (4) a tested product recall procedure; (5) schematic plan of the abattoir; documentation of flow diagrams of the slaughter process; (6) risk assessment of abattoir processes to identify potential hazards; (7) prevention of identified hazards and sampling programmes for laboratory analyses; (8)

written accounts of decisions relating to when corrective actions are taken must be provided; and (9) record keeping (RSA, 2004).

The HMS comprises a minimum of 14 Hygiene Management Programmes (HMPs). The HMP's requirements are fairly comprehensive and cover the following areas: (1) ante-mortem inspection; (2) slaughter and dressing; (3) meat inspection; (4) personal hygiene of workers; (5) medical fitness of workers; (6) temperature of water in sterilizers; (7) availability of liquid soap and soap dispensers, toilet paper and disposable towels; (8) sanitation and continuous cleaning; (9) availability and quality of water; (10) vermin control; (11) waste disposal, including condemned material; (12) contact wrapping and packing materials; (13) maintenance of all equipment and structures and (14) thermo control. These programmes are currently applicable to red meat, poultry and ostrich abattoirs as set out in the various regulations promulgated under the Meat Safety Act (RSA, 2004; 2006; 2007b). Legislation requires the HMS to be audited by means of an HAS.

## 2.5 Hygiene Assessment System

The South African HAS was benchmarked according to the system used in the United Kingdom and was released in 1999 (Govender *et al.,* 2013). Although it was not a regulatory requirement, it was used by state veterinary sections countrywide to assess abattoirs (Govender, 2009). The internal assessment of abattoirs using the HAS was regulated in 2004 and made applicable to red meat abattoirs (RSA, 2004). According to Van Zyl (1998), the HAS is envisioned by the government as a national system implemented in abattoirs, that would promote and facilitate quality and hygiene in

South African abattoirs. Van Zyl (1998) further states that the mission of the HAS is to serve as a general guide to long term hygiene performance at abattoirs; to provide uniformity by establishing norms and standards; to serve partially as an umbrella system for quality in South Africa supported by government and acknowledged by the private and public sectors; and to be used as an auditing tool for VPH officers to compare essential national standards in the provinces effectively.

The Hygiene Assessment System (HAS) has been defined as a quantitative assessment of an abattoir's hygiene status through the use of a compliance audit checklist in order to ascertain the extent to which an abattoir complies with the requirements set by the Red Meat Regulations, Regulations No. 1072 of 2004 (RSA, 2004). An audit of an abattoir using the HAS would yield a conclusion as to the probability of the abattoir producing safe meat or not. The HAS audit checklist (Annexure C) focuses on ten specific categories within an abattoir on which the hygiene status of an abattoir is assessed or compliance to the Meat Safety Act is assessed. Table 2.1 shows these categories as well as their maximum weighted scores. These ten categories are subdivided into specific topics within each division, each of which has an allocated score. All subdivisions add up to a total category score of 100 points while each of the 10 categories in turn has a weighted score. The rationale for weightings assigned to each category is based on the risk that the particular category poses to the contamination of the product (Van Zyl *et al.,* 2008; Derbyshire, 2011). Categories such as slaughtering and dressing, meat inspection, chilling and dispatch therefore have the highest weighted scores, whilst structure and maintenance and personnel carry less weight because of their lesser influence on the safety of the final product (Derbyshire, 2011).

**Table 2. 1:** The main categories and weightings of the HAS (RSA, 2004)

| Category | Description of aspect being assessed | Category Score | Weighting of category | % Total |
|---|---|---|---|---|
| A | Ante Mortem | 100 | 0.07 | 7 |
| B | Slaughter and Dressing | 100 | 0.15 | 15 |
| C | Meat Inspection and Marking | 100 | 0.15 | 15 |
| D | Chilling and Dispatch | 100 | 0.15 | 15 |
| E | Offal Processing | 100 | 0.03 | 3 |
| F | Sanitation and Pest Control | 100 | 0.10 | 10 |
| G | Personnel | 100 | 0.08 | 8 |
| H | General Condition | 100 | 0.07 | 7 |
| I | Structure and Maintenance | 100 | 0.10 | 10 |
| J | Hygiene Management System | 100 | 0.10 | 10 |
| Totals | | | 1.0 | 100 |

In order for HAS audits to be 'scientifically' reliable and also to limit bias, they are performed by trained Veterinary Public Health (VPH) officials who have received training in the allocation of scores. Furthermore, HAS audits should be performed in accordance with internationally accepted auditing principles defined by the International Standards Organisation (ISO) as a systematic, independent and documented process for obtaining audit evidence and evaluating it objectively to determine the extent to which the audit criteria (ISO 19011:2002) are fulfilled (Derbyshire, 2011). In addition to the HAS, a standard operating procedure (SOP) was developed by the South African National Abattoir Rating Scheme for the uniform application of the HAS system throughout all abattoirs in South Africa (RSA, 2004).

Any non-conformances observed during the audit are noted on the HAS document, and the reason/s for not allocating a perfect score are explained in the comments section provided in the HAS checklist. The severity of non-conformances should not be considered when scoring, since the HAS checklist already compensates for this via the weighting scores. On completion of all 10 categories of the HAS document, any non-conformances found are carried over to the Non-Conformance, Corrective Action and Clearance Report and the final scores of each section are transferred to a HAS score sheet (Derbyshire, 2011).

On the Non-Conformance, Corrective Action and Clearance Report, mention is made of each category/division of the HAS to which reference is made, and the non-conformances found (findings) are listed in a specific division, together with references to the relevant section in the Act or regulation that was transgressed. All mentioned non-conformances must consequently be prioritised as critical, major or minor. For the

sake of prioritisation, a “critical” non-conformance is defined as one that will directly influence the safety of the product and which therefore poses an imminent risk to public health. Immediate action must be taken and production may be stopped until the problem is rectified (Derbyshire, 2011). A “major” non-conformance is defined as one with a high potential to directly influence the safety of the product and where the potential impact is likely to compromise food safety if no remedial action is taken. Examples of major non-conformances are the lack of soap/hand sanitizer in the toilet (as observed by the auditor during an audit), and failure to implement effective corrective action for a previously identified minor non-compliance (RSA, 2004). A “minor” non-conformance is noted when the potential impact of the non-conformance is not likely to pose a serious or imminent risk to the product, therefore the overall food safety programme is still effective in controlling the food safety hazards. An example would be that some signatures are missing on a record over a short time period. However, if a number of minor non-conformances are considered collectively and are likely to compromise food safety, the non-conformances are reclassified as “major” or “critical” (RSA, 2004). The time allocated to correcting a major non-conformance is shorter than that given to a minor non-conformance. All non-conformances prioritised as critical or major are listed as non-conformance on the prescribed Non-Conformance, Corrective Action and Clearance Report. This report must be presented to the owner/hygiene manager of the facility where the corrective actions to be taken to prevent recurrence are listed, and a proposed date of completion must be agreed on.

The HAS audit is only completed once the corrective actions have been addressed and the form is signed off by the registered inspector. The scores out of 100 for each

division are carried over to the HAS score sheet and multiplied by the weights of each subdivision. The weighted scores are then added up and totalled as an overall score out of 100, which is the total score for the abattoir. In the event of progressive non-compliance, a veterinary instruction may be issued for the problem to be corrected within a stipulated period of time. Provisions made by section 10 of the Meat Safety Act provide for legal sanctions in the event of failure to comply with the requirements. The abattoir's registration certificate could also be withdrawn (rescinded) or government could refuse to renew the certificate once expired in the event of non-compliance (RSA, 2000).

## 2.6 Foodborne diseases

Foodborne diseases in human beings caused by bacterial pathogens and their toxins are a known reality that has been documented worldwide for centuries (Lotte *et al.,* 2002). Such diseases continue to form part of the major public health threats. They also impose a substantial economic and quality of life burden on society by way of acute morbidity and chronic squeal (Duffy *et al.,* 2003). Each year, contaminated food makes at least two billion people ill worldwide, resulting in more than three million deaths (Govender, 2009). However, these reported incidences of foodborne diseases may represent less than 10% of the real incidence, due to the absence of foodborne disease surveillance systems in many countries and weakness in existing programmes (UN, 2011).

According to a United Nations report (UN, 2011) it is estimated that 800 000 children die each year from foodborne-related illnesses, and especially from diarrhoea and

dehydration in Africa. This is because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment, and lack of education for food handlers. Even though data regarding foodborne diseases in the African region is extremely scarce, studies from available data have shown that the following bacteria are prevalent: *Campylobacter*, *Salmonella*, *Shigella*, *Brucella*, *Staphylococcus aureus, Bacillus cereus,* and *Escherichia coli* (Nel, 2003; Dunkley *et al.,* 2009; Jacob *et al*., 2010).

In South Africa foodborne diseases have been notifiable since 1989, but the statistics on foodborne diseases remain poor and there is currently insufficient data to establish trends of foodborne diseases (Jacob *et al.,* 2010). This is because food-related and other diarrhoeal illnesses are conditions that are clinically mild and resolve within 24 to 48 hours without any medical attention, thus are unlikely to be reported as people do not seek medical attention (Mutsinze, 2013). As a result, many food-related illnesses are not officially diagnosed or treated, and associated foodborne disease outbreaks are often not recognised. Also, when people do seek medical attention, health workers are less likely to report these less severe conditions (DoH, 2009). This poses a challenge to the health care system to maintain the knowledge and resources to identify and respond to these outbreaks.

Foodborne diseases are defined as diseases of an infectious or toxic nature caused by the consumption of contaminated food or water (Nel, 2003). Types of foodborne disease are subdivided into poisoning and infection, where the term food poisoning embraces a group of acute illnesses caused by the ingestion of foods that contain

substances or agents injurious to humans. These substances may be chemical or biological (e.g. cysticercosis) in origin (Quinn & Markey, 2004). A bacterial food infection refers to foodborne illness caused by the entrance of bacteria or their toxins into the body through ingestion of contaminated food and reaction of the body to their presence or metabolites (Nel, 2003).

There are more than 250 known foodborne diseases of which bacteria are the main cause followed by viruses and parasites (Mohammed, 2011). The most common foodborne bacteria, i.e. *Campylobacter*, *Staphylococcus aureus, Salmonella* and *Escherichia coli,* are estimated to have caused over 26 million illnesses and 8000 deaths in the United States of America in 2012 (CDC, 2012). These organisms are found in the intestinal tract of animals and birds and may therefore contaminate poultry and red meat. This contamination may occur during the slaughter and/or processing of animals at abattoirs, and/or may be due to poor hygiene or sanitation practices of food handlers (Hilton, 2002). The two important stages that have the greatest impact on carcass contamination of red meat are the evisceration process and the flaying process (Jacob *et al*., 2010), explaining the reason for the higher category scores in the HAS checklist. Pathogens that are present in raw meat may survive insufficient cooking and may cause human food poisoning as mentioned by Hilton (2002), which explains why the correlation between meat consumption and foodborne disease outbreaks is significant in many countries (Holt & Henson, 2000). This highlights the importance of performing bacteriological testing of meat intended for human consumption.

## 2.7 Selected indicator bacteria significant to red meat abattoirs

An indicator bacterium is a bacterium or larger groups of bacteria, which are relatively easy to measure as a group and whose presence is likely to indicate the presence of pathogenic bacteria (Lues & Van Tonder, 2007). Indicator bacteria have the advantage of being enumerated inexpensively (simple to detect) and easily for quantifying the performance of a production process, when particular pathogens or spoilage organisms might be difficult to detect (Brown *et al.,* 2000). Assessment of the concentration of indicator bacteria on carcasses and in meat products is a key element for evaluation of safety and quality of raw meat and to assess, validate or verify the efficacy of microbial control measure and sanitation programmes (Moore & Griffith, 2002; Lues & Van Tonder, 2007). The absence of indicator bacteria on the carcass and in the meat provides a degree of assurance that the hygiene and meat processing process has been carried out appropriately or meat has not been exposed to conditions that would permit contamination by bacteria or present the opportunity for its growth (Van Tonder, 2004). The presence of indicator bacteria on the carcass usually indicates that a potential problem or failure in the process has occurred. A high number of indicator bacteria in meat signify the reduction of meat palatability, shelf life or quality, increase the chance of meat-related illness, and subsequently influence consumer acceptability of a product (Brown *et al.,* 2000).

Indicator bacteria generally associated with red meat include Aerobic Plate Counts (APC), psychrotrophic counts, mesophyllic counts, Coliform counts, Enterococci counts, *Enterobacteriaceae* counts, *E. coli, Salmonella* spp., and *S. aureus, Pseudomonas spp, Bacillus cereus amongst* others (DoH, 2000). However, for the

purpose of this study three indicator bacteria, discussed below, were selected based on their ability to indicate fecal contamination, poor slaughtering technique, poor sanitation (*Salmonella spp, E. coli* and Aerobic Plate Counts) and poor personnel hygiene (*S. aureus* and Aerobic Plate Counts) (Nel, 2003). In addition, the selected indicator bacteria were also chosen based on Veterinary Procedural Notification (VPN)-15 and EU standard for exporting abattoirs (DAFF, 2010).

### 2.7.1 *Salmonella* species

*Salmonella* is a bacterium that causes one of the commonest and most widely disseminated illnesses called salmonellosis, which is a global public health problem (Hugas & Tsigarida, 2008). Problems related to salmonellosis in recent years have increased significantly both in terms of incidence and severity (Narapati, 2007). It remains the major cause of foodborne hospitalisations worldwide, with more than 16 million cases reported every year to the World Health Organisation (Pui *et al*., 2011). This shows the magnitude of the problem, especially since many cases of salmonellosis are not reported (Narapati, 2007). Although there is no surveillance data for salmonellosis in South Africa, it is estimated that 37% of foodborne diseases are caused by *Salmonella* spp. (DoH, 2009).

This bacterium grows at temperatures between about 5 and 46°C, their optimum temperature for growth is 35 to 43°C, while the optimum pH is 6.6 to 8.2 with a water activity above 0.94 (Clements *et al.,* 2001). These microorganisms utilise simple carbon compounds as sources of carbon and energy. To satisfy their nitrogen requirements they utilise nitrogenous compounds (Dunkley *et al.,* 2009).

2.7.1.1 Ecology/Origin

As a zoonotic foodborne bacterium, *Salmonella* has reservoirs in various animals. The most common hosts of this bacterium are domesticated animals, particularly poultry, pigs and cattle (Mohammed, 2011). Nonetheless many other domestic animals as well as a wide range of wild animals can also harbour this organism. In the abattoir during slaughter, *Salmonella* is passed from the intestinal tract of the host through faecal contamination onto meat products. The *Salmonella* infection is acquired from ingestion of contaminated food of animal origin (Warriner & Namvar, 2009) particularly when undercooked, and this seems to be the predominant situation in most developed countries (CDC, 2012). Other additional routes of human infection by *Salmonella* have been postulated in developing countries and these include hospital-acquired infection, direct and indirect animal contact, and transmission between humans.

2.7.1.2 Importance in food

*Salmonella* illnesses in humans are most commonly associated with food products, especially foods of animal origin such as poultry, eggs, meat and dairy products (Tshabalala, 2010). Clinical illness resulting in salmonellosis disease occurs when 100-1000 cells are ingested by healthy individuals. The symptoms of *Salmonella* infections usually appear between 6 and 48 hours after ingestion of the contaminated food and include diarrhoea, fever, abdominal cramps, nausea and sometimes vomiting, although asymptomatic infections may also occur (Warriner & Namvar, 2009). The illness usually lasts from 4 to 7 days in healthy individuals but is in most cases self-limiting. Complications associated with *Salmonella* infections include

aortitis, cholecystitis, colitis, endocarditis, orchitis, meningitis, myocarditis, osteomyelitis, pancreatitis, Reiter's syndrome, rheumatoid syndromes, septicaemia, splenic abscess and thyroiditis (Adams & Motarjemi, 1999).

2.7.1.3 General characteristics and classifications

The genus *Salmonella* was initially discovered in 1886 by Theobald Smith and Daniel Elmer Salmon. The discovery of the genus originated from the work on swine fever (hog cholera) by Theobald Smith and he named the genus after his supervisor at the US Department of Agriculture (USDA), Daniel E. Salmon (Pui *et al*., 2011). *Salmonella* species belongs to the family *Enterobacteriaceae* which is characterised by medium-sized Gram-negative rods, which are non-spore forming, motile and facultative anaerobic (Narapati, 2007). The genus *Salmonella* comprises a single species that has been divided into over 2600 serotypes in the Kauffmann White Schema, based on the O (somatic), H (flagellar) and occasionally capsular (vi) antigens (Narapati, 2007). More recently each *Salmonella* serovar has been placed into one of two species: *S. enterica* or *S. bongori,* where *S. enterica* is further divided into six subspecies which are designated as *enterica, salamea, arizonae*, *diarizona*, *houtenae* and *indica* (Pui *et al*., 2011).

### 2.7.2 *Escherichia coli*

Infections caused by *Escherichia coli* remain one of the leading causes of foodborne bacterial illness throughout the world (Mohammed, 2011). This bacterium is well recognised as a faecal indicator that provides a fair estimation of the level of faecal contamination. *Escherichia coli* was first discovered in 1885 by a German paediatrician, Theodor Escherich, during his work on bacteria in infant stools (Tshabalala, 2010). *Escherichia coli* is a Gram-negative, facultative anaerobic and non-sporulating, rod-shaped bacterium that belong to the family *Enterobacteriaceae* (Adams & Moss, 1997). *Escherichia coli* isolates are serologically distinct from each other and those serological differentiations are based on three major surface antigens O (somatic), H (flagella) and K (capsule). The K antigen descriptor has been dropped and only the H and O are commonly employed as descriptors of serotypes (Bell, 2002; Mohammed, 2011).

The general growth parameters for all *E. coli* include a minimum temperature of 7°C to 8°C and an optimum temperature of 35°C to 40°C, a minimum pH of 4.4 and an optimum pH of 6 to 7; a minimum water activity of 0.95 with 0.995 as the optimum (Quinn & Markey, 2004). *Escherichia coli* are more resistant to sodium chloride and sodium nitrate than are salmonellae, and growth can occur in 0 to 4% sodium chloride and 0 to 400 µg of sodium nitrite per millilitre (Bello & Oyedem, 2009). Changes in environmental signals such as temperature, ion concentration, osmolarity, carbon source, iron ions, pH and oxygen can be sensed by enterotoxigenic *E. coli (*ETEC) bacteria, resulting in the expression of virulence factors (Quinn & Markey, 2004).

2.7.2.1 Ecology/Origin

*Escherichia coli* is a ubiquitous inhabitant of the gut in both humans and animals (Quinn & Markey, 2004), where it helps to maintain the physiological milieu of the gut and support digestion while also defending against enteric pathogens (Bello & Oyedem, 2009). *Escherichia coli* is excreted in faeces and can survive in faecal particles, dust and water for weeks or months. The presence of *E. coli* in meat samples is taken as evidence of faecal contamination during processing. The faecal contamination can be directly from the animal itself and/or indirectly from the hands of food handlers (Quinn & Markey, 2004).

2.7.2.2 Importance in food

*Escherichia coli* was considered to be harmless or non-pathogenic. This was because, according to the Koch's postulates, a bacterial species was either pathogen or not (Bello & Oyedem, 2009). However a Danish veterinarian proposed that the *E. coli* species comprises of different strains, some being pathogenic, others not (Quinn & Markey, 2004). Not only did his assertion prove to be true, but today *E. coli* is subdivided into several pathogenic strains causing different intestinal, urinary tract or internal infections and pathologies in humans (Nataro & Kaper, 1998).

The pathogenic strains of *E. coli* have been categorised into six groups, which are mostly regarded as Shiga toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC) and diffuse-adhering

*E. coli* (DAEC) (Bell, 2002). The virulence mechanisms that characterise these categories of *E. coli* are genetically encoded by chromosomal, plasmid and bacteriophage DNA and are represented by several virulent genes. These genes include *eae* (attaching and effacing lesions), *bfpA* (localised adherence), the genes encoding enteroaggregative adherence, *ipaH* (enteroinvasive mechanism), the genes encoding heat-labile toxin (*LT*) and heat-stable toxin (*ST*), and *stx1* and *stx2* (Shiga toxins) (Nataro & Kaper, 1998).

*E. coli* are major inhabitants of farm animals and it has been understood that such organisms in the faeces of the animals are spread to meat during slaughter and processing (Scheutz *et al*., 2012). Farm animals are therefore also implicated in direct transmission of *E. coli* to humans. Apart from meat and meat products, other foods have also been implicated as vehicles for the transmission of *E. coli*. These include raw milk, sandwiches, unpasteurised apple juice and vegetables (Talan *et al.,* 2011). These bacterial strains can cause acute gastroenteritis with the following symptoms: abdominal pains, fever, vomiting and diarrhoea that may be prolonged, and blood with mucus in the stools. Symptoms develop 5 to 48 hours after food consumption, but the incubation period can be as short as 1 day or as long as 10 days. Some strains, particularly EPEC and EHEC, cause haemorrhagic colitis and renal disorder (Scheutz *et al.,* 2012).

### 2.7.3 *Staphylococcus aureus*

*Staphylococcus aureus* is an important foodborne pathogen and a major public health concern throughout the world (Kechrid *et al.,* 2011). In the last few decades *S. aureus*

has been reported to be the third-ranked cause of foodborne illnesses in the world (Tshabalala, 2010). In the United States, it is estimated that *S. aureus* accounts for 2.6% of foodborne illness caused by 31 major pathogens (Scallan *et al.,* 2011; CDC, 2012). However, while South Africa does not have systems to keep proper statistics of staphylococcal food poisoning, different researchers have shown that there is a significant increase in the number of *S. aureus* isolates from meat and meat products in South Africa (Tshabalala, 2010).

*Staphylococcus aureus* are able to grow in a wide range of temperatures ranging between 6.5 and 48.5ºC, but grow optimally at 37ºC (Quinn & Markey, 2004). The microorganism grows well in the presence of oxygen and is capable of growing anaerobically. It can grow within a pH range of 4.2 to 9.3, with optimum growth occurring at a pH of 7.0 to 7.2. *Staphylococcus aureus* are resistant to drying due to toleration of low water activity (0.85) and optimal growth at $a_w$ 0.99. High salt concentrations up to 15% sodium chloride are also tolerable by *S. aureus* strains (Talan *et al.,* 2011). These characteristics explain the growth of *S. aureus* strains in a wide variety of foods and give them a competitive advantage over other organisms in foods with low $a_w$ and high salt concentrations (Mohammed, 2011).

2.7.3.1 Ecology/Origin

*Staphylococcus aureus* is a common commensal of the skin and mucosal membranes of humans and animals, with estimates of 20–30% for persistent and 60% for intermittent colonisation (Shale *et al.,* 2006). Food handlers carry *S. aureus* in their noses or on their hands, and these are regarded as the main source of food

contamination, i.e. via manual contact or through respiratory secretions (Quinn & Markey, 2004). Foods that have been vehicles for *S. aureus* in food poisoning incidents include meat and meat products, poultry and egg products, and milk and milk products (Gill & Jones, 2000). Air, dust and food contact surfaces can also serve as vehicles in the transfer of *S. aureus* to foods (Quinn & Markey, 2004). *Staphylococcus aureus* does not compete well with indigenous microbiota in raw foods; contamination is mainly associated with improper handling of cooked or processed foods, followed by storage under conditions which allow growth of *S. aureus* and production of the enterotoxin(s).

2.7.3.2 Importance on food

*Staphylococcus aureus* is capable of hiding in pores and hair follicles and is difficult to remove from the skin. Once hands become damp, pathogens can be drawn to the surface and transferred to foods (Adams & Motarjeni, 1999). Staphylococcal food poisoning is caused by the ingestion of highly heat-stable proteins and is generally mild and self-limiting (Aberle, 2001). *Staphylococcus aureus* strains are capable of producing toxins called enterotoxins when high cell density, estimated at $10^5$ CFU/g, is reached. Ingestion of food contaminated by the bacteria or its toxin leads to nausea, vomiting, cramps and diarrhoea. Symptoms may appear within 30 minutes of, or up to 8 hours after, ingestion and may last between 1 to 2 days (Gill & Jones, 2000).

2.7.3.3 General characteristics and classifications

*Staphylococcus aureus* is a facultative anaerobic Gram-positive coccal bacterium, with an average diameter of 0.8 to 1 µm that tends to occur singly, in pairs, tetrads, short chains or irregular grapelike clusters. This organism is non-motile, catalase positive, oxidase negative and non-spore forming with fermentative metabolism (Quinn & Markey, 2004). It produces staphylococcal enterotoxins, which cause staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms (Montville & Matthews, 2008).

## 2.8 Intrinsic and extrinsic factors affecting microbial growth

The characteristic development of a microbiological population in meat is the result of the surrounding environmental conditions on the type of microorganisms which are present on the raw meat or which are introduced by cross-contamination or processing (Gill & Jones, 2000; Nel, 2003). The factors affecting microbial growth in meat can be either intrinsic or extrinsic. Intrinsic factors are predominantly chemical including the concentration or availability of nutrients, pH, redox potential, water activity, competitive microflora, antimicrobial substances and biological structures, thus all factors or parameters that are an inherent part of the tissues (Ahmad & Sarangi, 2013). Extrinsic factors are concerned mainly with the storage and processing conditions. Extrinsic factors also include storage temperature, handling, composition, processing steps and relative humidity of the gasses in the atmosphere surrounding the meat (Jay, 1996). Some intrinsic factors are however interlinked with some extrinsic factors; for example, water activity rises with increasing temperature, and there is an increase in water activity of 0.03 with every 10°C rise in temperature (Quinn & Markey, 2004).

## 2.9 Meat as a medium for microbial growth

The term “meat” was originally used to describe any solid food (Adams & Moss, 1997), but is now applied almost only to all parts of an animal carcass that are intended for, or have been judged as safe and suitable for, human consumption. According to Aberle *et al.* (2001) meat can be subdivided into several categories: beef, pork, lamb and mutton which are commonly referred to as red meat; poultry meat, or the flesh of chickens, turkeys, ducks and geese, is commonly referred to as white meat, as is seafood, including the flesh of aquatic creatures such as fish, clams lobster, crabs, mussels and other shellfish. The third category is game meat such as venison (deer). In principle this category consists of the flesh of any non-domesticated animal (Nel, 2003).

Meat is considered to be an excellent source of high quality animal protein, vitamins and certain minerals especially iron (Gracey *et al*., 1981). Meat has a high moisture content, high percentages of nitrogenous compounds, plentiful supply of minerals and some fermentable carbohydrates (glycogen) of a favourable pH for the growth of most of the enteric microorganisms (Mohammed, 2011). The composition of meat renders it the most perishable of all important foods (Jay, 1996). Fresh meat, with a pH between 5.3 and 6.4, is within the growth range of most pathogenic bacteria. The oxidation-reduction potential on the surface of processed meat tends to be higher than that of whole meat, which results in suitable growth conditions for strict aerobes and facultative anaerobes, whereas antimicrobial constituents are not known to occur in meat or meat products. Added to this is the fact that meat has water activity ($a_w$) values

of 0.97-0.99 that are close to the optimum growth level of most bacteria, thus rendering it a perfect growth medium for microorganisms (Nel *et al.,* 2004).

After slaughtering, slaughter stock undergoes many processes before the carcasses hang dressed in the abattoir chiller (see Figure 2.2). Skilled workers perform these processes in some cases at great speed because their remuneration is linked to the number of animals slaughtered per day: workers therefore tend to increase the slaughter line speed (Tshabalala, 2010).

The deep muscle tissues of healthy slaughtered livestock contain few, if any bacteria; however, due to post mortem handling, contamination by microorganisms occurs on both the surface and interior tissues of meat (Nel *et al.,* 2004). Together with its natural surface microorganisms, the exterior of the animal harbours large numbers of many kinds of microorganisms originating from soil, water, feed and manure; furthermore the animal's intestinal contents contains various intestinal microorganisms (Mohammed, 2011). During bleeding, skinning (flaying) and cutting, the main sources of contamination originate from the exterior of the animal (hide, hooves, skin and hair) as well as the intestinal tract. Utensils, equipment, water supply, air, hands and clothing of meat handlers also serve as intermediate sources of contamination (Quinn & Markey, 2004). Knowledge of sources of infection and the spreading and control of microbiological growth is therefore of utmost importance for the effective implementation of hygiene measures to contain possible microbiological contamination (Aberle *et al.,* 2001). Table 2.2 indicates the sources of different foodborne pathogens associated with abattoirs.

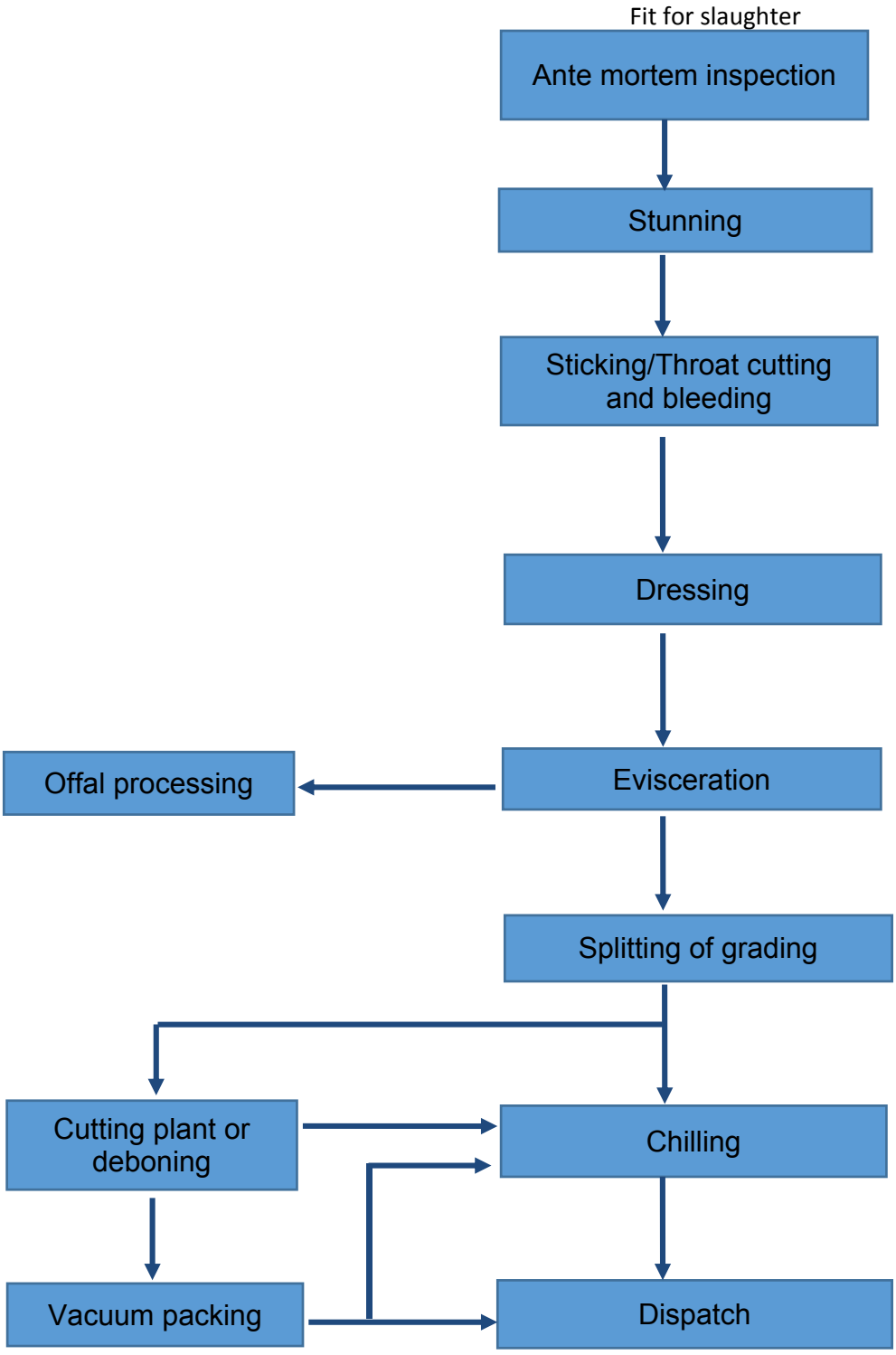


**Figure 2. 2:** Schematic representation of the slaughtering process

**Table 2.2:** Different source of pathogenic bacterial species in the abattoir

| Source | Organism | Characteristics | | References |
|---|---|---|---|---|
| | | Pathogen | Spoilage | |
| Equipment & Utensils | *Escherichia coli* | X | | Rivera-Betancourt *et al.,* 2004 |
| | *Salmonella spp* | X | | |
| | *Staphylococcus aureus* | | X | |
| | *Pseudomonas aeruginosa* | X | | |
| | *Listeria monocytogenes* | X | | |
| | *Campylobacter* spp | X | | |
| Hides & Skins | *Escherichia coli* | X | | Downes & Ito 2001 |
| | *Salmonella* spp | X | | |
| | *Bacillus cereus* | X | | AK *et al.,* 1994 |
| | *Bacillus subtilis* | X | | |
| | *Bacillus anthracis* | X | | |
| | *Staphylococcus aureus* | X | | RSA, 2000 |
| | *Staphylococcus epidermidis* | X | | |
| | *Pseudomonas* spp | | X | |
| | *Lactobacillus* spp | X | | Quinn & Markey 2004 |
| | *Listeria monocytogenes* | X | | |
| | *Listeria ivanovii* | X | | |
| | *Neisseria* spp | X | | |
| Environment & Air | *Escherichia coli* | X | | Nel, 2003 |
| | *Salmonella* spp | X | | |
| | *Shigella* spp | X | | Warriner et al., 2002 |
| | *Yersinia enterocolitica* | X | | |
| | *Yersinia pestis* | X | | |
| | *Camplyobacter* spp | X | | Shale & Van Tonder 2007 |
| | *Bacillus* spp | X | | |
| | *Listeria* spp | X | | |
| | *Pseudomonas spp* | | X | |
| | *Staphylococcus aureus* | X | | |
| Personnel | *Escherichia coli* | X | | Aberle *et al.,* 2001 |
| | *Staphylococcus aureus* | X | | |
| | *Staphylococcus epidermidis* | X | | |
| | *Mycobacterium tuberculosis* | X | | |
| | *Salmonella spp* | X | | |

## 2.10 Bioaerosols

In recent years concerns have emerged in relation to the potential for aerial bacterial contamination of meat carcasses during the slaughter process (Sutton, 2004), as the presence of such bacteria in meat can increase the possibility of foodborne illness and reduce the meat's palatability and shelf life (Okraszewska-Lasica *et al.,* 2012). Air is an important vehicle for the distribution of bacterial contamination within various food processing environments including those that process meat (Sutton 2004). These airborne contaminants of biological origin are known as bioaerosols. The role of bioaerosols in various industrial settings has been well studied in developed countries. Their role in the South African food industry and particularly in the meat industry, however, has only been investigated to a very limited extent, "mainly because of lack of proper equipment, lack of expertise to perform bioaerosols survey, fear of how the outcome of such studies will affect various companies or some combination of those factors" (Shale *et al.*, 2006).

Bioaerosols may attach to dust particles or may survive as free floating particles surrounded by a coating of dried organic or inorganic material, but those microorganisms cannot multiply in the air due to a lack of nutrients (Sutton, 2004). However due to their size, bioaerosols can remain airborne for a long time and are capable of migrating through buildings; this consequently increases the likelihood of meat contamination occurring (Cox & Wathes, 1995). Depending on their type and origin, the size of bioaerosol particles can range between 0.01 and 100 mm in aerodynamic diameter (Hirst, 1995). Airborne microorganisms can settle and contaminate the meat itself, as well as the working surfaces, equipment and hands of

employees, which could possibly lead to cross-contamination of meat (Cox & Wathes, 1995; Ellerbroek, 1997). Due to its nutritional quality, meat is prone to microbial contamination and some of the contaminants are aerosols (Cox & Wathes, 1995). Many innocuous and ordinary activities such as cutting, grinding, washing, spraying, talking, coughing and sneezing, as well as open doors, the air conditioning system and cleaning of equipment, can create and spread bioaerosols in food processing environments (Ellerbroek, 1997). The removal of cattle hides is also a recognised source of bioaerosols (Shale *et al.,* 2006). Furthermore, airborne microorganisms may be of human origin from purulent discharge of an infected finger or eye, from abscesses, facial eruptions or nasophryngeal secretions, or from normal skin (Ellerbroek, 1997). Other sources that are indirectly linked to bioaerosols are contamination from waste handling and disposal, fungal or microbial growth niches in the building and unhygienic practices including improper maintenance and poor operations and sanitation (Shale *et al.,* 2006).

Geographical location and environmental conditions such as humidity, density, oxygen concentrations, temperature, exposure to sunlight and air flow (direction and speed) are known to influence bioaerosols within food processing environments such as abattoirs (Maier *et al.*, 2000). These parameters affect the generation and distribution of airborne contaminants (Ellerbroek, 1997). For example, a small decrease in humidity, from 82 to 75%, has been known to result in a 26% reduction in *E. coli* survival rates (Okraszewska-Lasica *et al.,* 2012). A strong relationship between humidity, density, oxygen concentrations, temperature, air flow and the presence of airborne contaminants has raised concern regarding the contribution of ventilation systems to the distribution of airborne contaminants. In the food production

environment, a strong correlation exists between the efficiency of ventilation systems and the concentration of bioaerosols as the ventilation system can significantly influence temperature changes in the indoor environment and also impact on the dispersal, dilution and removal of air pollutants (Shale & Lues, 2007).

Generally, exposure to bioaerosols in an indoor environment could be associated with a range of health effects (Shale & Lues, 2007), as bioaerosols contribute roughly about 5-34% of indoor air pollution (Srikanth *et al*. 2008). Bioaerosols cause bacterial spoilage of meat and reduce shelf life of the end product or, in the worst case scenario, cause foodborne infection or poisoning of the consumers, as well as affecting the health and well-being of workers (Brown *et al.* 2006). For quantification and characterisation, bacteria in the air may be recovered using various methods, which include sedimentation or impaction on agar surfaces, impingement in liquids, filtration, centrifugation and electrostatic or thermal precipitation (Sutton, 2004). The method most commonly used in meat abattoirs is impaction on agar (Burfoot *et al.,* 2006; Pearce *et al.,* 2006).

Impaction methods use the inertia of particles to separate them from the air currents (Bitton, 2002). Impactors collect airborne microorganisms onto an agar surface or an adhesive coated surface with the aid of a vacuum. An impactor consists of an air jet that is directed over the impaction surface causing the particle to collide and stick to the surface. There are two types of impactors: slit or sieve samplers. A slit sampler is cylindrical in shape and has a tapered slit tube that creates a jet stream when an air samples are pulled by a vacuum. The air sample is collected onto an agar plate which rotates on a turntable to create an even distribution of particles. A slit sampler requires

a vacuum to draw a constant flow rate usually of 28.3 litres per minute (Sutton, 2004). Sieve samplers function by drawing air (i.e. 28.3 l/min) through a metal plate with many small holes. Air particles impact on the agar surface which is a few millimetres below the metal sieve. Sieve samplers like the Andersen sampler may consist of a single stage, or two, six or eight stages (Pearce *et al.,* 2006).

## 2.11 Conclusion

The HAS is the only nationally accepted “scientific” measure of compliance used by the government and as such, the HAS score is interpreted as a measurement of the potential risk to public health of products derived from a specific abattoir. Moreover, pathogenic bacteria of great concern in the meat industry have been isolated in a number of studies conducted in South African abattoirs. The opportunity for contamination of the meat therefore exists, and may occur from the slaughter floor, for instance, through environmental air, from contact with surfaces or through handling.

It is important that the government should develop and facilitate the implementation of proper guidelines, standards and limits in terms of bacterial levels on carcasses and in relation to air contaminants for the abattoir industry. In order to achieve these goals an investigation into the role of the Hygiene Assessment System (HAS) audits in guaranteeing microbiological meat safety needs to be conducted. The possible relationship between HAS audits and microbial contaminants that could affect the quality of meat in the abattoirs should also be assessed.

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# CHAPTER THREE

## An evaluation of hygiene assessment system audits at selected high throughput single species red meat abattoirs in central South Africa

# An evaluation of Hygiene Assessment System audits at selected high throughput single species red meat abattoirs in central South Africa

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## Abstract

*Typically, the establishment and the functioning of abattoirs are governed by legislation, the Hygiene Management System (HMS), compliance and Quality Assurance (QA) systems and audits. The emerging challenges requiring immediate attention are the translation and implementation of those legislative requirements and quality assurance standards into the abattoir hygiene management system and the assessment of the effectiveness of the HMS in producing safe meat. As a result of this, various audit tools have been developed to determine the compliance of the abattoirs to the set requirements. The present study was carried out to evaluate the compliance levels at six selected single species high throughput red meat abattoirs in the Free State province of South Africa. The selected abattoirs were registered to slaughter cattle, sheep or pigs. The Hygiene Assessment System (HAS) checklist was used for this purpose. The HAS scores of individual abattoirs ranged from 68 to 94. The findings of this study showed inconsistencies of the HAS score in relation to guaranteed meat safety, thus its effectiveness is questionable. This is demonstrated firstly by the fact that HAS audits do not measure the impact of non-compliance or the risk imposed by the non-compliance to meat safety, nor do they show the risk to meat safety. Secondly, there are many inconsistencies of the final HAS score in relation to meat safety. Those inconsistencies include the fact that many abattoirs obtained a total HAS score rated as good yet several categories with varying effects on meat safety had alarmingly low scores and critical NCs which posed a serious risk to public health. Thirdly the weighted scores in individual categories do not entirely measure risks posed by those categories to the actual meat safety. In addressing the identified challenges regarding the HAS audits the category score, type of non-conformance and the impact/risk posed by the non-conformance need to be investigated to derive a formula or factor that reflects the actual impact of the final HAS audit score on the meat safety. It is therefore recommended that government should initialize further study into what audit or monitoring systems are needed in the abattoir industry as a measurement for meat safety which would ensure the production of safe meat based on the real risks to public health.*

**Keywords:** HAS audit, Final HAS score, meat safety, abattoirs.

## 3.1 Introduction

It is internationally accepted that the establishment and functioning of food facilities such as abattoirs are governed by legislation, Hygiene Management Systems (HMS), compliance and Quality Assurance (QA) systems and audits (Masanganise *et al*., 2013). The reason for such governance is to prevent and/or minimise the occurrence of foodborne diseases in the human population (Haileselassie *et al.,* 2013). The legislation governing the abattoir industry varies from country to country, but it is usually enforced by public sector institutions (Mutsinze, 2014). According to the Codex Alimentarius Commission (CAC) each country must ensure that the legislation underpinning its food control system is scientifically based and must work to establish equivalency and transparency among national food control systems (Domenech *et al.,* 2008). The CAC is a set of food standards, guidelines and codes of practice produced with the aim of protecting consumer health and facilitating international trade (Luning & Marcelis, 2007). The CAC is open to the governments or associate members of the Food and Agricultural Organisation/World Health Organisation (FAO/WHO), which currently has over 185 members (FAO/WHO, 2015). Compliance to the CAC recommendations is voluntary but many governments and non-government institutions use the CAC guidelines as the basis for legislation (FAO/WHO, 2003).

A hygiene management system (HMS) in the South African context can be defined as a minimum standard and its components are guided by, or derived from and audited against, regulations promulgated under the Meat Safety Act, Act 40 of 2000 (Mutsinze, 2014). Hygiene Management Systems are designed to enable abattoirs to become self-governing entities for the production of safe meat (Mutsinze, 2013). This is

achieved through identification of normal, reaction to deviation from normal, and record keeping, and implements both short and long term preventive actions of such demonstrated deviation. It also includes internal audits, which are used to ascertain the extent to which an abattoir complies with the requirements, and serves as continual improvement initiatives designed to meet public health authority requirements (Hepner *et al.,* 2004). Therefore, an ideal HMS implemented in an abattoir should be based on Good Hygienic Practices (GHPs) and Hazard Analysis Critical Control Point (HACCP) principles, and should address both food safety control and assurance activities in order to guarantee meat safety (Jacxsens *et al.,* 2009), and should then be audited using various tools. Food safety control activities in food processing facilities aim to keep product and process conditions within acceptable parameters in order to ensure meat safety. Assurance activities on the other hand are concerned with the evaluation of system performance and organising necessary changes (Luning & Marcelis, 2007).

Specific food safety and quality standards that were previously developed are currently being used in food processing industries. These include International Standard Organisation (ISO) 9001:2000, ISO 22000:2005 (Holt & Henson, 2000), British Retail Consortium (BRC 2008), Global Standard for Food Safety (Pinillos & Jukes, 2008) and the International Food Standard (IFS 2007). The emerging challenges regarding food safety and requiring immediate attention particularly in developing countries, are as follows: how to translate and implement legislative requirements and quality assurance standards into the current abattoir HMS, and how and when to assess the performance of the existing HMS in producing safe meat (Jacxsens *et al.,* 2009). These challenges have resulted in the development of various audit tools to determine compliance of abattoirs to set requirements (Cormier *et al.,* 2007; Domenech *et al.,* 2008), such as

Hygiene Assessment System (HAS) audits (which are mostly compliance-orientated) in South Africa and food safety audits in Australia.

In the food industry, audits are defined as activities used to determine compliance with specific food safety guidelines, quality assurance standards and legislation (FOA, 2015). They involve the systematic, independent examination of quality activities (this does not always apply for internal audits), safety activities, records and processes to verify their compliance to set requirements and whether these requirements are implemented effectively (Sampers *et al.,* 2012) or are adequate for their intention. There are several types of audits, and the FAO/WHO has provided two categories of these audits. The first category is referred to as internal audits (1st party audits) which are performed by a food establishment itself and may have good potential for reducing risk if the methods followed are those outlined in widely accepted codes and risk assessment guidelines (FAO/WHO, 2015). The second category is referred to as external audits (3rd party audits which are performed by external bodies that usually verify the parameters of product quality in relation to product compliance on specifications, legislation, QA standards and customer specification (Hepner *et al.,* 2004). External bodies normally issue an audit certificate which gives confidence regarding compliance to the requirements of certain QA standards and legislation (Van Gerven *et al.,* 2007; Albersmeier *et al.,* 2009). Through auditing, food facilities can verify their operational practices and improve the uniform application of standards to their products and services. This is also particularly valuable for trade as some retailers require similar or such certificates before they can purchase from abattoirs.

In South Africa, meat safety is governed by the Meat Safety Act, Act 40 of 2000. The Act requires that all abattoirs be managed in accordance with approved hygiene management and evaluation systems (RSA, 2000). The Red Meat Regulations (Regulations No. 1072 of 2004) promulgated in term of regulation 49 (e) of the Meat Safety Act requires an HMS to be audited by means of the Hygiene Assessment System (HAS). The HAS audit is an assessment of an abattoir's hygiene status through the use of a compliance audit checklist in order to determine the extent to which an abattoir complies with the requirements. The government uses the HAS as a national system that is implemented at abattoirs, to promote and facilitate quality and hygiene in South African abattoirs. Its mission is to serve as a general guide to long term hygiene performance at abattoirs; to provide uniformity by establishing norms and standards; to serve partially as an umbrella system for quality in South Africa; and to be used as an auditing tool for VPH officers to effectively compare essential national standards in the provinces (Van Zyl, 2008). Therefore the HAS audit system assigns "poor" HAS scores to those premises with "poor" hygiene standards and higher HAS scores to better premises.

The HAS is the only nationally accepted "scientific" measure of compliance used by the public sector and as such, the HAS score is interpreted as a measurement of the potential risk to public health of products derived from a specific abattoir. Questions arise on the effectiveness of HAS audits alone in driving the continual improvement of the abattoir HMS towards improving meat safety in South Africa. Alternatively, there is a question as to whether adjustments can be made to HAS scores and/or scoring system so that the information collected during compliance audits may be translated into output predictions on safety of the products. Thus the aim of this study was to

conduct HAS audits to determine compliance levels of single species high throughput red meat abattoirs in order to evaluate the effectiveness of the final HAS score as an indicator of the safety of products produced.

## 3.2 Methods and materials

Hygiene assessment system (HAS) audits were performed at six single species high throughput red meat abattoirs that are spread over three districts (Motheo, Lejweleputswa and Fezile Dabi) of the Free State province. The study was performed during a period of four months between April and July 2013. The abattoirs designated as A, B, C, D, E and F are registered to slaughter cattle, sheep and pigs respectively. The criterion used for selecting these abattoirs to participate in the study was the mean scores of the last three official HAS audits conducted by provincial inspectors at single species abattoirs in the province (Free State Veterinary Service, 2012). Two abattoirs of each species with the highest score and lowest score were than selected to participate in the study.

Each abattoir was visited once and a single HAS audit was performed. The audits were conducted using the legislated HAS audit checklist (Annexure C). The audits were performed by two trained provincial VPH officers. Auditors have received training in auditing according to the ISO 19011 standard and in the application of HAS according to standard operational procedures of the DAFF to ensure objectivity in their scoring methods and to standardise the application of the HAS audit checklist. The scope of the audits was from the point of receiving livestock to the dispatch of carcasses. In order to ensure objectivity, the same two auditors were utilised for all audits, which

they performed together and thus submitted a single combined audit report of their results for each abattoir.

Abattoir owners were notified in advance of the date of the intended audit, the scope, expected duration and purpose of the audit. During the opening meeting the abattoir owner or their representative and the hygiene manager were invited to join the audit process as prescribed. Upon completion of the audit the owner was notified of critical (urgent), major and minor non-conformances in the closing meeting. Dates were agreed upon for the closure of non-conformances captured during the audit by means of corrective action reports. A digital thermometer (Snookums Digital Beer, Gauteng), with a range of at least –50°C to 150°C was used to measure the temperature of the carcasses, environmental, sterilizer (≥82°C) and chiller. A light meter (Lutron, Gauteng), was also used to measure light intensity of up to 2000 lux, to audit the slaughter and dressing categories of the HAS checklist. The instruments had valid calibrated certificates (valid till 2014) issued by a South African National Accreditation System (SANAS) accredited facility at the time of the audits. Once the audits were completed, the findings (critical non-conformance, the category score and final HAS score) were recorded and analysed. Statistical analysis to determine whether the significance of the results between the various species was performed by Biometric Section or the Agricultural Research Council (ARC

## 3.3 Results and discussion

The mean audit scores per species and category, as well as the total HAS scores and the number of critical non-conformances identified are shown in Table 3.1.

**Table 3. 1:** Mean scores of audits performed at high throughput abattoirs in the Free State

| *Categories | A | B | C | D | E | F | G | H | I | J | Total Scored | Total Critical NC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Abattoir** | | | | | | | | | | | | |
| Cattle A | 97 | 97 | 96 | 98 | 94 | 95 | 97 | 90 | 75 | 100 | 94 | |
| *C/NC per category | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | | 2 |
| Cattle B | 76 | 78 | 76 | 80 | 85 | 60 | 45 | 54 | 67 | 60 | 68 | |
| C/NC per category | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | | 3 |
| **Mean(Cattle)** | **86.5** | **87.5** | **86** | **89** | **89.5** | **77.5** | **71** | **72** | **71** | **80** | **81** | |
| Sheep C | 80 | 80 | 85 | 90 | 79 | 87 | 66 | 68 | 67 | 84 | 79 | |
| C/NC per category | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 1 |
| Sheep D | 76 | 66 | 52 | 76 | 95 | 72 | 58 | 78 | 75 | 51 | 70 | |
| C/NC per category | 0 | 3 | 2 | 0 | 0 | 0 | 1 | | 0 | 0 | | 6 |
| **Mean(Sheep)** | **78** | **73** | **68.5** | **83** | **87** | **79.5** | **62** | **73** | **71** | **67.5** | **74.5** | |
| Pig E | 92 | 90 | 74 | 86 | 85 | 81 | 74 | 74 | 57 | 86 | 80 | |
| C/NC per category | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 |
| Pig F | 76 | 60 | 73 | 78 | 83 | 78 | 76 | 64 | 69 | 78 | 74 | |
| C/NC per category | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | | 1 |
| **Mean score(Pigs)** | **84** | **75** | **73.5** | **82** | **84** | **79.5** | **75** | **69** | **63** | **82** | **77** | |

*The various categories audited as indicated as A: Ante-mortem, B: Slaughtering and dressing, C: Meat inspection and marking, D: Chilling and dispatch, E: Offal processing, F: Sanitation and pest control, G: Personnel, H: General conditions, I: Structural requirements, and J Hygiene management systems, whilst the alphabets in the first column reflect various abattoirs evaluated. Red colour indicates the poor category score. * C/NC is number of critical non-conformance per category. Blue colour indicates categories with critical non-conformances

## 3.1 General observations

Abattoir A had the highest total HAS score of 94 (Table 3.1), which according to the HAS is rated as “excellent”. This score can be attributed to the fact that abattoir A exports meat and meat products to other countries and continents, and therefore is compelled to meet international trading standards by implementing other food safety systems and quality systems over and above the existing HMS system. Those systems include ISO 9001:2000, ISO 22000:2005 and the International Food Standard. These quality systems have the advantage that they improve the hygiene condition in the abattoir, as they enforce certain hygiene practices which are not covered under the HAS audit checklist (Tshabalala, 2010). The findings of this study are in agreement with those of Tshabalala (2010), who found that an abattoir that uses HAS alone has a lower score compared to those abattoirs that use both HAS and other quality systems. The study by Tshabalala (2010) analysed the effect of hygiene and safety management systems on the microbiological quality of fresh beef in South Africa.

Despite the fact that abattoir A had a high total HAS score, two critical non-conformances were identified during the audit. Both originated from category H (General condition) and both were related to compromised security during transportation of condemned material to secondary processors. Critical non-conformances are recorded when the non-conformance has a direct influence on the safety of the product and therefore poses an imminent risk to public health (Derbyshire, 2011. These non-conformances poses a risk to public health as the condemned material could be accessed by the public during transportation and has the possibility

to re-enter the food chain and subsequently cause food related disease to the public (Shale & Lues, 2007). Furthermore the abattoir failed to comply with waste management regulations as covered in HAS audit checklist under category H (General Conditions), which states that it is the responsibility of the abattoir to ensure that the waste leaving its premises is the same waste received at the secondary waste site.

Abattoir B recorded the lowest total HAS score of 68 which is rated as “fair” according to the HAS. This abattoir also attained low scores in two categories, namely G (Personnel practices) (45) and H (General conditions) (54), which can be regarded as “poor”. The low score obtained in category G (Personnel practices) is due to poor personal hygiene, workers wearing their protective clothing outside the abattoir premises, lying on the ground with their protective clothing on during lunch time, workers from dirty areas found in clean areas, and germicidal soap not being available in the toilets during production. Three critical non-conformances were raised in abattoir B which originated from this category and were due to some of the above reasons. This further justifies the low total HAS score obtained and the need to measure the impact of non-compliance. Personnel practices represents 8% of the total HAS score which is indicative of its greater importance in meat safety. Given that workers are of the utmost importance when an effort is made to deliver a safe product of high quality to the consumer, a low category score and critical non-conformances can be indicative of a potential microbiological contamination of carcasses due to personnel practices.

Category H (General conditions) only represents 7% of the total HAS score which is indicative of its lesser influence on the safety of the final product. General conditions covers aspects such as compliance of structure with approved design drawings,

premises, water supply and quality, effectiveness of drainage system/effluent disposal, disposal of waste material and disposal of condemned material. This category does not have a direct influence on the safety of meat, although individual factors included in this category are linked to other categories where they may have a direct impact on meat safety. For example water quality used at final wash is covered under slaughter and dressing (Category B), which weighs higher in HAS. The low score in this category was due to inadequate temperature of the sterilizer (40°C), and improper disposal of condemned material, amongst other things.

Abattoirs C and F achieved a score above 60 in all categories which is reflected in the total HAS score of 79 and 74 respectively, and is rated as good. However, each of these abattoirs had a single critical non-conformance. The critical non-conformances originated from category B for abattoir C and category H for abattoir F. The reason for the critical non-conformance raised in category B was due to improper slaughtering technique (all opening lines were made from outside to inside), this led to the puncturing of stomach and intestines and subsequent contamination of the carcasses with microorganisms. The critical non-conformance in category H of abattoir F was due to the improper storage of condemned material that could be a problem as condemned material was not securely stored and therefore could be accessible to the community around the abattoir (including the workers themselves), since there was not proper fencing and security.

Table 3.1 indicates that abattoir D had a total HAS score of 70, which is rated as good, but this abattoir achieved a poor score in three relatively highly-rated categories, namely category C (52), G (58) and J (51). The reasons for the low score in category

C (Meat Inspection) is that the Meat Inspector was found not competent enough according the competency checklist that is part of the HAS. The purpose of meat inspection is to identify abnormalities (parasites, aesthetics, colour and consistency size) and disease conditions on carcasses and offal (Govender, 2009). The production of visually clean meat, monitored by meat inspection, is an important starting point for meat safety (Tompkin, 1990), hence it has high weighted score on HAS checklist.

Meat inspection represents 15% of the total HAS score which is indicative of its greater importance in meat safety. Therefore the low score for meat inspection can potentially expose consumers to the risk of food poisoning. The low score for Category G was due to poor personal hygiene (workers wearing their protective clothing outside the abattoir premises and workers from dirty areas being found in clean areas). The HMS (Category J) represents 10% of the total HAS score which is indicative of its greater importance in meat safety. The poor score in this category was due to corrective action reports which are not signed by the owner of abattoir, no record for training of work on slaughter procedure, the HMS was not approved by the Provincial Executive Officer and there were no results of laboratory tests for the efficacy of the sanitation process and water tests (microbiological and chemical).

Abattoir D had the highest number (6) of critical non-conformances as well as the highest number of categories with a poor score. The majority of critical non-conformances originated from categories B (3) and C (2), which have a direct influence on the safety of the meat, and only one originated from category H. The nature of those critical non-conformances were that each animal was not bled with a clean and sterilized knife; animal opening lines were made from outside to the inside, puncturing

the intestine and causing contamination of the carcass by intestinal contents; the heads and feet were not available for meat inspection; and lack of secondary meat inspection. These critical NC's can be attributed to lack of supervision during slaughtering and/or training of the slaughters. Therefore this highlights the need for re-training of personnel.

Table 3.1 shows that abattoir E achieved the second highest total HAS score of 80, which is rated as good. Although no critical non-conformances were raised, this abattoir achieved a poor score of 57 in category I (Structural requirements and Maintenance). This category represents 10% of the total HAS score which is indicative of its greater influence on the safety of the final product. This category covers areas such as pens, lairages, crushes, races, sanitizing facilities for trucks, slaughtering and dressing area, detention facilities, condemn room, freezer, chiller, dispatch area, offal room, offal facilities, change room and dining facilities. Given the fact that the abattoir structure is a known source of carcasses contamination (Sutton, 2004), measures must be taken to prevent cross-contamination.

The above findings reveal inconsistencies of the HAS score in relation to guaranteed meat safety. For example, despite the fact that abattoir A has obtained a total HAS score of 94 (Excellent score), two critical non-conformances were raised which have a direct impact on the safety of the product. In comparison, abattoir E had a poor score in a relatively high-rated category (Structural requirements), but still got a total HAS score rated as good (80) without any critical non-conformances. Therefore this raises the question: which abattoir is better: the abattoir with an "excellent" HAS score of 94 and 2 critical non-conformances, or the abattoir with a "good" HAS score of 80 and no

critical non-conformances but poor structure? Similar results were observed when comparing abattoir A with abattoirs C and F. Given that critical non-conformances have a direct impact on the safety of meat, it should be assumed that an abattoir with no critical non-conformances has a better chance of producing safe meat than the abattoir with critical non-conformances. Thus from these observations it is clear that more needs to be taken into consideration when interpreting the HAS score, such as types of non-conformances raised, impact of such non-conformances, individual category scores and importance (rating) of individual categories in relation to the scores. Therefore a formula or factor needs to be developed to reflect the actual impact of the final HAS audits score on meat safety.

### 3.3.2 Species

An investigation into the overall performance of individual species revealed that cattle (A & B) abattoirs achieved the highest total HAS score with the mean of 81, followed by pig (E & F) and sheep (C & D) abattoirs with means of 77 and 74.5 respectively. There was no significant difference ($p < 0.05$) in the scores found between different species abattoirs, indicating that the hygiene management of an abattoir is not necessarily related to the species being handled.

### 3.3.3 Categories

Further investigation into the mean score of different categories of individual species shows that categories G, H and I obtained relatively low scores with the exception of H in sheep abattoirs (Table 3.1). Category G (Personal hygiene) was the main

contributing factor to the low score for this category. This highlights the need for training of personnel, as well as reviewing of training manuals and methods being used. The benefits of training have been proven in many companies (Mutsinze, 2014): a non-trained or poorly trained worker can serve as a potential source of carcass or food contamination in the abattoir (Goveden, 2009). There should be no compromises when it comes to the importance of training (Goveden, 2009). SABS 10049 clearly states that adequate and continued training in personal hygiene and the preferred practices of hygienic handling of food are a must (SANAS, 2005). Category H (General conditions) had the highest number of critical non-conformances across the three different species abattoirs. This may explain the poor performance of this category: it indicates the need to pay more attention to training with regards to management of the abattoir.

Category I (Structural requirements and Maintenance) achieved the lowest mean score compared to other categories. This can firstly be attributed to the fact that the majority of abattoirs were built over 25 year ago and require renovations. It was observed that the ceilings and windows were broken, paint was flaking and some walls were cracked (indicating a poor maintenance programme). According to Galland (1997), contaminants may originate from poor structural maintenance such as broken windows, floors, walls, ceilings, doors and processing equipment. Good abattoir sanitation practices and prevention of carcass contact with any surfaces reduce the risk of contamination. Sanitation of older abattoirs may be harder to manage and may serve as a source of contamination (Govender & Genis, 2009). Secondly, there is ineffective separation between neighbouring areas in which there are incompatible activities, such as having a dirty area (Stunning and bleeding) right next to a clean

area (Meat inspection area) with no demarcation. For example there was a big hatches between the slaughter hall and the rough offal and hides and skins room. Therefore given that the layout of the abattoir contributes to the transmission of bacteria, the low scores in category I may potentially serve as a source of contamination.

Table 3.1 also shows that categories A, D and E have achieved the highest scores across three different species abattoirs. Category A (Ante-Mortem) represents 7% of the total HAS score which is indicative of its perceived lesser importance in meat safety. The ante-mortem, which is performed by at least a registered meat inspector (RSA, 2004), serves as the first line of defence at production level to protect consumers from potential foodborne illnesses originating from meat and meat products (Demarchelier *et al.,* 2007). Therefore only animals that are fit for slaughter (not too dirty and visibly healthy) and capable of being converted into wholesome products for human consumption are accepted and allowed to pass ante-mortem inspection before slaughter (Tshabalala, 2010).

Category D (Chilling and dispatch) account for 15% of the total HAS score which shows its larger impact on meat safety. The objective of chilling is to control the proliferation of bacteria and other microbes such as yeast and fungi so that they cannot negatively influence the safety and quality of meat. By slowing down the multiplication of organisms that cause meat to spoil, and of microbes which cause food poisoning (Gracey, 1990), the shelf life of meat is lengthened. According to Bailly and Guere (2009), temperature plays a significant role in meat safety as a vast majority of meat-borne bacterial illnesses occur due to poor storage conditions related to temperatures which may support the growth of bacteria. The fact that this category obtained

relatively high rankings throughout the three species abattoirs indicates that bacterial load as a result of contamination during production can be reduced significantly before the carcass leaves the abattoir, as found by the study performed by Cohen *et al.* (2006). However, chilling should not be used as a measure to compensate for poor hygiene management, as the changing of the temperature at retail or consumer points can cause the proliferation of bacteria to undesirable levels (Goveden, 2009).

Category E (Offal processing) represents 3% of the total HAS score which is indicative of its lesser importance in meat safety. Basic requirements for this category include the need for adequate clean running water and availability of facilities for storing products off the floor. A system should also be put in place to handle full production effectively to prevent congestion. Red offal must be separated from rough offal to prevent cross-contamination. Red offal should be chilled to <7°C within 16 hours if not removed continuously, edible washed rough offal must be stored in a chiller at an air temperature not exceeding -2°C (RSA, 2004). All abattoirs in this study complied with the temperatures mentioned above for red offal. The influence of this category to the total HAS score is very low however, given that 57-67% of the population in South Africa use offal as a staple food especially in the winter season (Derbyshire, 2013). The allocated score for this category needs to be reviewed to match the risk this product poses to the public health. Several researchers have proven that offal has been incriminated in a number of foodborne poisoning outbreaks worldwide (Cohen *et al*., 2006, Magwedere *et al*., 2013; Edris *et al*., 2013). The pathogenic bacteria associated with red offal include *Salmonella* spp., *E. coli*, *Campylobactor* spp., *Listeria monocytogenes*, *Clostridium perfringens* and *Shigella* spp. amongst others (Cohen *et al*., 2006).

### 3.3.4 Hygiene Assessment System document

From the study, it was found that the effectiveness of the HAS checklist audit is questionable. This is because the results of this study demonstrate that even though the various categories are weighted in order to demonstrate their importance to meat safety, the total HAS score of the various abattoirs does not demonstrate food safety risks. The HAS is said to be a compliance measure to regulatory requirements, as set out in the Meat Safety Act. The HAS is also seen as a tool to measure the level of compliance of an abattoir's HMS, which is said to be based on HACCP principles as it contains a level of hazard identification (RSA, 2004). However HAS control still does not demonstrate to the consumer the level of confidence in the safety of the meat they intend to purchase as scoring is solely based on the presence of a non-compliance and not the impact of that non-compliance. This is demonstrated by the fact that all abattoir final scores are seen as good, yet there are several categories with varying effects on meat safety that have alarming low scores. As an example, abattoir D obtained an overall HAS score of 70, which is regarded as good, whilst obtaining only 52 for meat inspection and marking (marking relates to the approval stamp containing the abattoir number, which indicates that the meat has been inspected and passed for human consumption and the abattoir number enables traceability) (Category C) which is of critical importance to meat safety, 58 for personnel practices and only 51 for hygiene management systems. These scores are regarded as poor for categories that are of utmost importance to meat safety, yet the abattoir obtained an overall HAS score of good. If this is compared to abattoirs C and E, where all the category scores were well managed, yet their overall HAS scores were also regarded as good, again demonstrates that there is reason to question the effectiveness of the HAS audit. In

addition there is an element of subjectivity in the HAS audits. The scores are allocated by auditors, and therefore the experience level and history of audits they have performed at abattoirs may indirectly influence the scores given to each abattoir.

## 3.4 Conclusion

In conclusion, this study has revealed that HAS audits with the use of the current tool are ineffective as a tool to determine the hygiene status of the abattoir and to guarantee safe meat to the public. This is demonstrated firstly by the fact that HAS audits do not measure the impact of non-compliance or the risk imposed by the non-compliance to meat safety, nor do they show the risk to meat safety. Secondly, there are many inconsistencies of the final HAS score in relation to meat safety. Those inconsistencies include the fact that many abattoirs obtained a total HAS score rated as good yet several categories with varying effect on meat safety had alarmingly low scores and critical NCs that pose a serious risk to the public health. Thirdly the weighted scores in individual categories do not entirely measure risks posed by those categories to the actual meat safety. Therefore, there is an urgent need to review allocated category score contributions to final HAS scores in order to equalise the risk posed by those categories to the safety of meat. Finally the fact that abattoirs that are managed as companies and that have implemented additional safety and quality systems, achieved higher HAS scores than those managed by a single owner using the HMS as the only system in their abattoirs, further proves that HAS audits alone are ineffective in assuring meat safety. It also shows that the measure of hazard evaluation as part of the HMS is not effective.

Given all of the above factors, it can be concluded that an abattoir's total HAS score cannot be used as a measure of the abattoir's ability or guarantee to produce safe meat, and that HAS audits are merely a compliance audit and not a meat safety audit. Therefore adjustments need to be made to the scoring system used, in order to determine the HAS score so that the information collected during compliance audits may be translated into output predictions on the safety of the products.

## 3.5 Recommendation

In addressing the identified challenges regarding the HAS audits, it is therefore recommended that controlling authorities should initialize further study into what audit or monitoring systems are needed in the abattoir industry as a measurement for meat safety. These audits or monitoring system should ensure that production of safe meat based on the actual risks to public health. It is proposed that the Meat Safety Act, Act 40 of 2000 should refer and/or include meat safety aspects such as bacteriological testing of meat, bacterial limits on meats (acceptable levels of bacteria on meat) and measuring the actual risk imposed by the non-compliance to meat safety as there are current not covered under this Act.

It is also recommended that the government should legally mandate the incorporation or the combination of HMS and quality assurance systems into the abattoir HMS to guarantee better hygiene condition. This could then be combined into one adapted HAS audit checklist that would result in a score that would truly reflects the safety of the meat being produced which would guide consumers to make informed choices regarding meat safety in South Africa.

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# CHAPTER FOUR

## The occurrence of indicator bacteria on carcasses and in the air environment of single species high throughput red meat abattoirs in the Free State province

# The occurrence of indicator bacteria on carcasses and in the air environment of single species high throughput red meat abattoirs in the Free State province

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## Abstract

*Meat safety is an emerging public health hazard requiring immediate attention due to the high incidences of food poisoning outbreaks worldwide. Meat is a perishable product containing plenty of nutrients that can support the growth of many microorganisms. The threat of foodborne poisoning as a result of such microorganisms is particularly great. In this study a total of 288 carcasses and 96 air samples were collected at six single species high throughput red meat abattoirs in the Free State province. Samples were collected from the carcasses at four sites at three different processing stations using swab rinse kits. The samples were then tested for indicator bacteria. Air samples were collected using the SAS Super 90 air sampler by impaction on agar. Ninety-nine percent of the carcass samples were positive for aerobic plate count (APC) bacteria (≥ 1.0x $10^6$ CFU.$cm^{-2}$). Staphylococcus aureus were isolated from 26.0% of the samples and were recovered at a level of ≥ 1.0 x$10^3$ for 75.0% of the positive samples. Escherichia coli were isolated from 21.0% of the samples and were recovered at a level of ≥ 3.16 x$10^2$ for 25.4% of the positive samples. Seventeen (5.9%) of the samples analysed were found to be positive for Salmonella spp. The presence of these pathogens is of concern due to their ability to cause foodborne diseases in humans especially to those with suppressed host immunity defences. The APC for bioaerosol concentration varied considerably among the abattoirs investigated, ranging between undetectable levels and 2.4 x $10^2$ CFU.$m^{-3}$. Staphylococcus aureus counts were ≤ 94 CFU.$m^{-3}$. Escherichia coli and Salmonella spp. were not found in any of the air samples. Results also emphasise the importance of bacteriological monitoring of carcasses and air in the abattoir surroundings in order to prevent possible contamination of the meat. It is recommended that special attention be given to slaughter hygiene, sanitation and structural design in order to decrease high occurrences of indicator bacteria. Therefore proper slaughtering techniques as prescribed by Standard Operating Procedures, if applied correctly, appear to be advantageous in controlling the increase of undesirable bacterial contamination. It's recommended that the government to*

*legally mandate the inclusion of bacteriological analysis of meat and air samples into the hygiene and safety evaluation system at a prescribed frequency.*

**Keywords:** *Salmonella*, *Escherichia coli*, *Staphylococcus aureus,* APC, meat, bioaerosols

## 4.1 Introduction

A large proportion of the world's population depends on meat as a source of high quality proteins (Clarence *et al.,* 2009). The protein profile of meat consists of amino acids that have been described as excellent due to the presence of all essential amino acids required by the human body (Bradeeba & Sivakumaar, 2012). It has also been established that the iron, protein and vitamins in meat cannot be replaced by plant sources, further justifying the nutritive importance of meat (Huda *et al.,* 2010). However, this composition of meat also makes it a good medium for the development and spread of a great number of foodborne bacteria, thus rendering meat a most perishable foodstuff (Bradeeba & Sivakumaar, 2012) that if not managed could have devastating effects on the health and economy of populations.

Bacterial foodborne diseases due to consumption of contaminated meat remains a public health and economic problem in most countries in spite of the improvement in hygiene standards and food processing practices, education of handlers and consumer awareness (Lues *&* Van Tonder, 2007). It has been reported that there is a significantly high correlation between meat consumption and bacterial foodborne disease outbreaks (Govender *et al.,* 2013). This observation supports the statement that food management systems managing food hygiene alone cannot ensure that consumers would not be exposed to infectious doses of bacteria in meat and meat products (Adak *et al.,* 2005). Consequently monitoring the bacteriological safety and quality of meat at abattoirs remains essential, although it is currently not mandated by the South African Meat Safety Act, Act 40 of 2000 (RSA, 2004).

There are 250 known genera of bacteria and 25 of these are incriminated in meat-borne diseases (Quinn & Markey, 2004). Testing of each individual bacterium within these genera can be costly and time consuming, making it highly inefficient to test larger batches of samples (Mboto *et al.,* 2012). This challenge was addressed by the introduction of indicator organisms testing. An indicator organism is a bacterium or larger groups of bacteria which are relatively easy to measure as groups and whose presence is likely to indicate the presence of pathogenic bacteria (Lues & Van Tonder, 2007).

Indicator bacteria generally associated with red meat include Aerobic Plate Counts (APC), *E. coli, Salmonella* spp., and *S. aureus* amongst others (DoH, 2000). The detection and enumeration of these indicator organisms are used for evaluation of safety and quality of raw meat and to assess, validate or verify the efficacy of microbial control measure and sanitation programmes (Moore & Griffith, 2002; Lues & Van Tonder, 2007). A high number of indicator bacteria in meat signifies the reduction of meat palatability, shelf life or quality, increases the chance of meat-related illness, and subsequently influences consumer acceptability of a product (Brown *et al.,* 2000). The absence or a low concentration of an indicator bacteria means that meat has not been exposed to conditions that would permit contamination by bacteria or present the opportunity for its growth. Therefore those low numbers present useful information on system contamination and the extent of downstream processing steps (Tshabalala, 2010).

In abattoirs the contamination of meat by indicator organisms occurs mainly during processing at abattoirs, particularly during the removal of hides and evisceration

(Bouttier *et al.,* 1994). Other possible contamination sources include the design of abattoirs, meat contact surfaces, equipment, utensils, aerosols (air), water supply and personnel practices (Tshabalala, 2010). The use of indicator organisms is highly dependent upon microbiological criteria (standards or guidelines) that are in place for the food product. These can be standards or guidelines (limits) recommended and/or enforced by government agencies, or specifications stipulated in commercial contracts (Barza, 2004). There is limited information available in terms of standards or limits associated with the occurrence of indicator organisms and a pathogenic bacteria on raw meat and in the air in the South African abattoir industry. The only national bacterial standard available is the one used for exporting abattoirs, which is set out in the national Veterinary Procedural Notification (VPN)-15 (DAFF, 2010) (Annexure A). As there is no specific standard available for abattoirs supplying the local market, their bacteriological status is unknown. This study was therefore aimed at investigating the occurrence and quantity of indicator bacteria on carcasses and air from single species high throughput red meat abattoirs in the Free State.

## 4.2 Methods and materials

### 4.2.1 Sampling protocol

#### 4.2.1.1 Carcass and site selection

A total of 288 carcass samples were collected from six selected single species high throughput abattoirs in the Free State province of South Africa over a period of four months between April and July 2013. The bacteriological sampling was performed in

a single run. Sampling was carried out on the processing line at three processing stations. The first collection point (P1) was before final wash, but after evisceration; the second collection point (P2) was after final wash, but before chilling; and the third collection point (P3) was after approximately 24 hours of chilling. Sample processing stations were selected based on the DAFF sampling protocol for the national microbiological monitoring programme (DAFF, 2012). Moreover, these processing stations also provide useful information regarding the sources of contamination within the abattoir. For example if high counts of bacteria are present after evisceration (P1), the possible sources of contamination would be linked to slaughtering technique or utensils used, while at P2 the possible sources of contamination would be water and handling, and at P3, the effectiveness of chilling would be called into question.

Four carcasses were randomly selected at each abattoir and each was sampled at P1, P2 and P3. The carcasses were identified by marking them with a number on the leg. Samples were taken from each of the four identified carcasses from pre-determined sites (Figures 4.2, 4.3 and 4.4), with a total of 48 samples per abattoir being collected. The sampling sites were chosen according to ISO 7604:2003 and European Union (EU) Directive 2001/471/EC as stipulated on Veterinary Procedural Notification VPN-15 (DAFF, 2010). These sites have the advantage of accessibility while the carcasses are on the line and they provide critical information regarding slaughtering techniques and personnel hygiene.


**Figure 4. 1:** Sampling sites for bovine carcasses

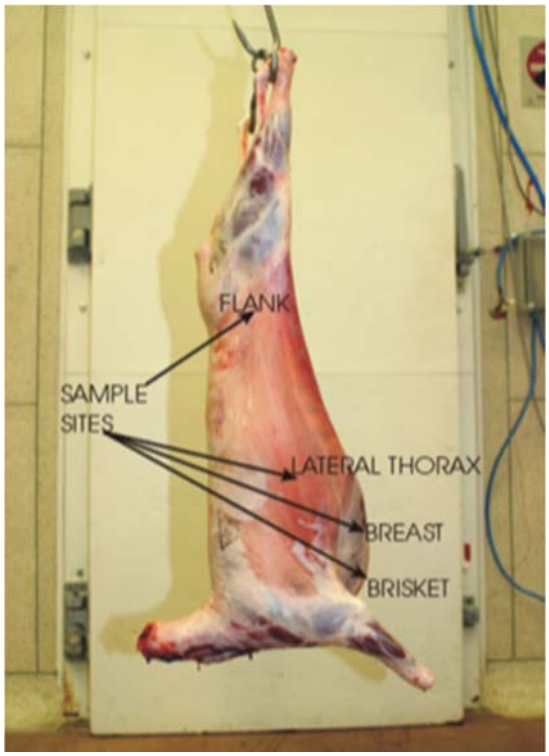


**Figure 4. 2:** Sampling sites for bovine carcasses

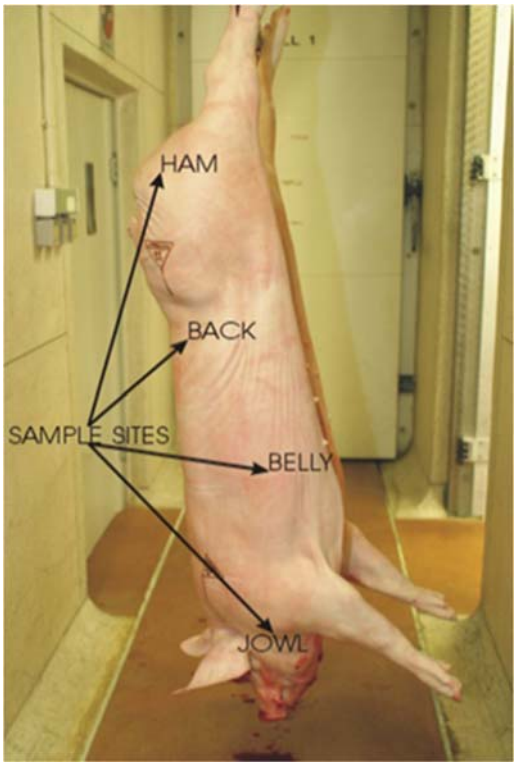


**Figure 4. 3:** Sampling sites for swine carcasses

4.2.1.2 Carcass bacterial sample collection procedure

Swab rinse kit (SRK) swabs (Copon Innovation, Italy) were used to collect samples from selected carcasses. Swab rinse kits are typically comprised of a labelled screw cap tube filled with 10 ml of rinse solution, with a swab stick attached to the cap (Figure 4.5). Prior to swabbing, each SRK swab was moistened by placing it in the 10 ml of sterile rinse solution provided in the tube. The tip of the swab was then pressed against the wall of the tube to remove excess liquid. Sampling was done by swabbing each of the four sampling sites. The area for swabbing on the carcass was created using sterile metal templates (USA) of 100 $cm^2$ for cattle and 25 $cm^2$ for sheep and pigs (Figure 4.6).

**Figure 4. 4:** Swab rinse kits

**Figure 4. 5:** Sampling templates

At each sampling site a moistened swab was rubbed vertically, horizontally and diagonally across the sampling site, with four swabs being used for each of the four sampling sites. Sterile gloves were worn and changed between carcasses. Subsequent to swabbing, each SRK swab was placed back into its original tube of solution. Each tube containing the swab was closed tightly to avoid spillage during chilled transportation. All the swab samples were packed into a cooler box containing ice packs to maintain a cold temperature followed by transportation to Bloemfontein Provincial Veterinary Laboratory for microbial analysis within 12 hours.

4.2.1.3 Air bacterial sample collection procedure

At each of the selected abattoirs, air samples were aseptically collected within the average breathing zone of humans which is 1.5 meter above floor level and within one meter from the carcass during processing (Lues & Van Tonder, 2007). Direct air samples were collected from the slaughter floor in duplicate on the hour, every hour

between 09:00 – 14:00, therefore a total of 10 samples were collected at each abattoir. Sterile 45 mm Petri dishes containing non-selective media and selective media were inserted, without their tops, into the SAS super 90 air sampler (PBI International, Milan, Italy) (Figure 4.7). The air sampler was calibrated at an airflow rate of $0.03m^3.min^{-1}$ and detachable parts were autoclaved before use and sterilised with 70% ethanol between sampling runs (Shale *et al*., 2006). The sampler was turned on for two minutes prior to sampling to allow the alcohol to evaporate and not affect the amount of bacteria recovered. After sampling the Petri dish was removed from the sampler and inverted in its cover. Petri dishes were stored in sterile bags to prevent contamination before further analysis and were then packed into insulated containers with chiller packs for transportation to Bloemfontein Provincial Veterinary laboratory. Temperature levels were obtained from selected abattoirs using a digital thermometer (Lasec, Gauteng) and recorded for possible relationships with microbial distribution.


**Figure 4. 6:** SAS super 90 air sampler

### 4.2.2 Carcass and air microbiological sample analysis

#### 4.2.2.1 Bacterial isolation and identification from carcass samples

In the laboratory the tube containing the swab and solution was vortexed (Lasec, Gauteng, South Africa) to release the sample material from the swab to the solution. A portion of each sample suspension was used to prepare a 10-fold dilution, up to $10^{-5}$. A conventional plate count analysis was performed for the following bacteria: aerobic plate count, *Staphylococcus aureus, Escherichia coli* and *Salmonella* species. Each sample was assayed in duplicate.

##### 4.2.2.1.2 Aerobic Plate Counts (APC)

For the enumeration of APC for carcass samples, Plate Count Agar (PCA) (CM0463, Oxoid, Basingstoke, Hampshire, England), was used. 1 ml of the bacterial suspension of each diluent was pipetted onto sterile Petri dishes and approximately 20 ml of PCA was added. The contents were thoroughly mixed and then the plates were incubated (Labcon, Western Cape, South Africa) at 35$^{o}$C for 48 hours (Quinn & Markey, 2004). Plates with growth in the range of 25 to 250 colonies were selected and the exact counts on both plates were determined; the arithmetic mean of the two counts (plates) was then calculated. The formula below was used to calculate the number of colony-forming units (CFU) per cm$^2$ for each sample.

**Formula 4. 1 …….** $\left(N\ x\frac{F}{A}\right)x\ D$

N= the number of CFU in 1 ml dilution (rinse solution),

F= the amount (ml) of dilution fluid (rinse solution),

A= the surface investigated ($cm^2$) {A= 100 $cm^2$ for cattle carcasses and 25 $cm^2$ for pig and sheep carcasses} and

D= the reciprocal of the dilution used.

4.2.2.1.3 Isolation of *Staphylococcus aureus*

*Staphylococcus aureus* were isolated according to the modified method described by Giaccone *et al.* (2000) for the carcass samples. In short, 1 ml of the sample was added to 9 ml of buffered peptone water (CM0509, Oxoid, Basingstoke, Hampshire, England) for the preparation of serial dilutions in test tubes. The tubes were vortexed and 0.1 ml of each dilution was spread out on Baird Parker (BP) plates containing egg-yolk tellurite emulsion (CM0275, Oxiod, Basingstoke, Hampshire, England) and Mannitol Salt Agar (MSA) plates (CM0085 Oxiod, Basingstoke, Hampshire, England) and incubated aerobically at 36ºC for 48 hours. Black colonies surrounded by an opaque halo on BPA and yellow colonies on MSA were considered presumptive for *S. aureus*. The black colonies surrounded by an opaque halo from BPA were enumerated. Typical *S. aureus* colonies were confirmed by a rapid latex agglutination test (Slidex Staph plus test kits, Biomerieux, Omnimed, South Africa).

The Staph latex agglutination test was performed according to the manufacturer's instructions. A drop of Staph latex test reagent was dispensed into a circle on the test

card. Using a sterile loop, two colonies of the presumptive *S. aureus* isolates were transferred into the circle and mixed with latex test reagent. The test card was gently shaken to allow the mixture to flow slowly over the entire test ring area. The agglutination was observed for up to 20 seconds. The positive control used was *S. aureus* ATCC 25923 and the negative control was *S. epidermis* ATCC 35984.

4.2.2.1.4 Isolation of *Escherichia coli*

The detection of *E. coli* was performed according to the "Most Probable Number Method" (Oblinger & Koburger, 1975). Each sample was transferred into sterile Buffered Peptone Water (BPW), a non-selective liquid medium for per-enrichment at 36°C for 24 hours. A decimal dilution of incubated samples was prepared with sterile Ringer Solution (BR0052, Oxoid). Using 5 consecutive dilutions, 1 ml aliquots from each dilution was transferred into 5 tubes of 2% Brilliant Green Bile Broth (CM0263, Oxoid, Basingstoke, Hampshire, England) followed by incubation at 44°C for 48 hours. The samples were examined for gas production after 48 hours. If gas was observed, an inoculum was streaked onto to MacConkey agar plates (CM0007, Oxoid, Basingstoke, Hampshire, England) and incubated at 36°C for 24 hours. Subsequently, 0.1 ml of Brilliant Green Bile Broth from a tube with gas formation was inoculated into 10 ml of Tryptone water (CM0087, Oxoid, Basingstoke, Hampshire, England) and incubated at 44°C for 48 hours. Five drops of Kovac's reagent (Biolab, Merck, Gauteng, South Africa) were added to the Tryptone water after 48 hours of incubation. Tubes were then allowed to stand for 5 min to permit the reaction to occur. Colour changes in the tubes were examined. A deep pink colour in the top layer of the tube is positive for *E. coli* and absence of red colour is negative for *E. coli.* All presumptive

pathogenic-like colonies of *E. coli* on MacConkey agar were cultured on Blood Tryptose agar and incubated at 36ºC for 24 hours and were then sent to the Onderstepoort Veterinary Institute, Reference Laboratory for serotyping of *E. coli*. The positive control used was *E. coli* ATCC 25922 and the negative control was *Enterobacter cloacae* ATCC 23355.

4.2.2.1.5 Isolation of *Salmonella* species

The detection of *Salmonella* species was performed according to the method described by Poppe *et al*. (2001). A 1 ml aliquot of sample was inoculated onto 9 ml of buffered peptone water (a non-selective pre-enrichment liquid medium) and incubated at 36ºC for 18 hours to 24 hours. A 0.1 ml aliquot of pre-enriched sample was then transferred into 10 ml of Rappaport Vassiliadis (RV) broth (CM0669, Oxoid, Basingstoke, Hampshire, England) and incubated at 42ºC for 24 hours. After incubation, a loop-full of enrichment broth culture was streaked onto Xylose Lysine Deoxycholate (XLD) agar (CM0469, Oxoid, Basingstoke, Hampshire, England) plates and *Salmonella Shigella* (SS) agar plates (CM0099, Oxoid, Basingstoke, Hampshire, England), and incubated at 36ºC for 24 hours. The plates were examined for the presence of typical colonies of *Salmonella*, i.e. transparent colonies with black centre on SS agar and red colonies with a black centre and lightly transparent zone of reddish or pinkish colour on XLD agar.

All presumptive *Salmonella* isolates from carcass and air samples were subjected to a battery of biochemical tests including triple sugar iron (TSI) agar, urea agar, malonate broth, phenol red dulcitol broth, lysine decarboxylase broth, decarboxylase

broth control and thio-gelatinate. Typical *Salmonella* isolates were cultured on BTA and incubated at 36ºC for 24 hours. These were then sent to the Onderstepoort Veterinary Institute, Reference Laboratory for *Salmonella* serotyping.

4.2.2.2 Bacterial isolation and identification from air samples

The air samples were also analysed for the presence of APC, *S. aureus*, *E. coli*, *Salmonella* spp., and other airborne-related bacteria. Since there are no official methods for culturing and isolating bacterial pathogens for air samples, the methods used for identification of bacteria on the carcass samples were adopted for air samples analysis, with the exception of *E. coli*. Air samples collected on Petri dishes filled with selective media (Tryptone Bile X-glucuronide (TBX) medium, Violet Red Bile MUG (VRBM) agar, XLD and BP agar) and non-selective media (PCA and BTA) were incubated for specific times at relevant temperatures.

4.2.2.2.1 Aerobic plate count and isolation of *Escherichia coli*

For enumeration aerobic plate counts, PCA plates were incubated at 36ºC for 24 hours. Tryptone Bile X-glucuronide and VRBM plates were used to isolate *E. coli*. Tryptone Bile X-glucuronide medium was used specifically to target *E. coli* strains that grow at high temperature (44ºC), while VRBM agar was used for those that grow at normal temperature of 36ºC. Inoculated plates were incubated at 44ºC and 36ºC respectively for 24 to 48 hours. The VRBM plates were observed for fluorescence under UV light. *Escherichia coli* colonies were detected as dark red on VRBM plates and as blue on TBX plates. All presumptive pathogenic-like colonies of *E. coli* from

both carcass and air samples were cultured on BTA and incubated at 36ºC for 24 hours and were sent to the Onderstepoort Veterinary Institute, Reference Laboratory for serotyping of *E. coli*.

4.2.2.2.2 Isolation of *Salmonella* species and *Staphylococcus aureus*

Xylose Lysine Deoxycholate (XLD) agar plates were used for culturing and detection of *Salmonella* spp., and incubated at 36ºC for 48 hours, while enumeration of *S. aureus* was done on Baird Parker (BP) agar, after 48 hours of incubation at 36ºC. All presumptive *Salmonella* and *S. aureus* colonies were subjected to the same biochemical tests as those from the carcass samples.

4.2.2.2.3 Isolation of other bacterial species

Blood Tryptone Agar was used for the isolation and identification of bacteria other than those mentioned above. Identification of unknown bacteria on BTA was performed using colony morphology and phenotypic tests, together with a biochemical test (analytical profile index). The phenotypic tests used for identification of bacteria were Gram stain, catalase reaction, oxidase reaction and spot indole as recommended by the Bloemfontein Veterinary Laboratory standard operating procedure. Based on the phenotypic test results the appropriate biochemical tests (Analytical Profile Index) were selected.

4.2.2.2.4 Analytical Profile Index (API)

The analytical profile index (Biomerieux, Omnimed, South Africa) was performed according to the manufacturer's instructions. In short, 10 ml of fresh culture in De Man Rogosa Sharpe (MRS) broth was vortexed and 1.5 ml of the fresh culture was transferred into Eppendorf tubes, centrifuged (Heraeus Sepatech, Biofuge A) for 5 min at 67.2 × g and the supernatant was discarded. 1 ml of sterile distilled water was added into the Eppendorf tubes containing the pellet. The bacterial suspension was added to the API medium and mixed. Following this, sterile saline or API medium was inoculated into the wells according to the manufacturer's instructions. The API wells were covered with sterile mineral oil and incubated at 37ºC for 24 to 48 hours. Colour changes of the API strips were recorded after 24 hours and 48 hours. Results were analysed according to the interpretation sheet provided with the kit and APILAB software (Biomerieux, Omnimed, South Africa).

## 4.3 Statistical analysis

Duplicate plates showing 25-250 CFU were counted and the means determined. The bacterial counts were expressed as $CFU.cm^{-2}$ of carcass and $CFU.m^{-3}$ for air samples. Pearson's correlation was calculated statistically between the microorganisms and summarized by means of a correlation matrix of meat samples. In attempt to address this, raw data was sent to the Biometric Section at the ARC. Based on the response from the Statistician (Eric Mathebula) at Agriculture Research Council, Pearson's correlation could not be used to establish relationship between the actual microbial

results for the respective samples (species) and the procession stations (P1, P2 and P3). This was due to the fact that environmental factors such as water, environmental temperature, chilling temperature, handling and air flow/rate at various processing stations (P1, P2 & P3) were different and not measured. For example carcass samples at P1 were not exposed to water used at final washing P2, which the quality was unknown as it was not part of the study.

## 4.4 Results and discussion

### 4.4.1 Carcass samples results

#### 4.4.1.1 Aerobic plate count (APC)

The APC is a widely accepted measure of the general degree of microbial contamination (Cohen *et al.,* 2006). Raw meat is generally considered of poor quality when the APC on its surface exceeds 1.0 x $10^8$ CFU.$cm^{-2}$ (Lues & Van Tonder, 2007). Low levels of APC are regarded as an indicator of good hygiene practices and the effectiveness of food safety or of the hygiene management system.

Table 4.1 shows the mean and total counts for APC across all tested abattoirs. At processing station P1, P2 and P3 samples were at 4.8 x $10^3$, 2.7 x $10^3$ and 2.7 x $10^2$ CFU.$cm^{-2}$ respectively. The APCs, levels ranged from undetectable to 7.0 x $10^3$ CFU.$m^{-2}$ across all the abattoirs. These counts were considerably lower than the standardised maximum limit of 1.0 x $10^5$ CFU.$m^{-2}$ for raw meat proposed by Veterinary Procedural Notification (VPN)-15 (DAFF, 2010).

**Table 4. 1:** The mean of aerobic plate counts (CFU.cm$^{-2}$) of single species abattoirs in the Free State province

| **Abattoirs and species** | Processing station | | | |
|---|---|---|---|---|
| | **P1** | **P2** | **P3** | **Total mean** |
| A Cattle | 5.0 x $10^3$ | 3.7 x $10^3$ | 2.4 x $10^2$ | 3.0 x $10^3$ |
| B Cattle | 3.8 x $10^3$ | 3.1 x $10^3$ | 9.8 x $10^2$ | 2.6 x $10^3$ |
| C Sheep | 7.0 x $10^3$ | 3.9 x $10^3$ | 2.0 x $10^2$ | 3.7 x $10^3$ |
| D Sheep | 6.2 x $10^3$ | 5.5 x $10^3$ | 1.2 x $10^2$ | 3.9 x $10^3$ |
| E Pig | 2.5 x $10^3$ | 9.3 x 10 | 6.5 x 10 | 9.0 x $10^2$ |
| F Pig | 4.2 x $10^3$ | 1.2 x $10^2$ | 2.2 x 10 | 1.5 x $10^3$ |
| **Total mean** | **4.8 x $10^3$** | **2.7 x $10^3$** | **2.7 x $10^2$** | **2.6 x $10^3$** |

P1: before final wash; P2: after final wash; P3: after chilling

The samples collected after chilling (P3) contained the least APC of the three processing stations, followed by samples collected after final wash (P2). The low counts of APC at P3 could possibly be attributed to chilling which may induce stress on bacterial cells due to its synergistic effect of low water activity (≤ 0.97), pH (≤5.3) and temperature (≤7°C), and subsequently inhibit the proliferation of the bacteria (Derbyshire, 2011). The low levels of APC at this phase do not necessarily imply safe meat at consumption. A number of factors including the meat storage conditions at the market or consumer level may affect the quality of the meat (Mutsinze, 2013). In some instances the meat could be exposed to temperatures above 7°C at the consumer phase, and this would lead to the proliferation of bacteria, which could subsequently increase to levels that may cause diseases (Quinn & Markey, 2004).

The APC varied between collection stations. The highest count was recorded on the samples collected before final wash (P1). This result may be due to the fact that P1 samples were collected after slaughter and dressing where the potential for microbial contamination is highest. These results showed a change of practical significance on tested carcasses at different processing stations during the slaughter process. These findings suggest that the APC found on carcass surfaces may vary, depending on a number of factors affecting the proliferation of bacteria in abattoirs.

#### 4.4.1.2 *Salmonella* species

*Salmonella* species in food industry are used assess the level of contamination on meat arising from gut contents (including faeces), which includes both that originating

directly from the alimentary track of the animal and that arising indirectly via the integument or processing environment (Cohen *et al.,* 2006; Shale & Van Tonder, 2007)

The presence or absence test was used for *Salmonella* detection and results are reported as percentage (%) detection. The maximum limit stipulated by VPN-15 states that *Salmonella* spp. should be undetectable (0 CFU.cm-$^{2}$) on raw meat samples. Table 4.2 shows that 5.9% (n=17/288) of the analysed samples were found to be positive for *Salmonella* spp. 53% (n=9/17) of them were isolated from pig abattoirs and 47% (n=8/17) were isolated from sheep abattoirs. *Salmonella* spp. were not isolated from samples collected at cattle abattoirs. These findings are in contrast to other studies involving *Salmonella* detection in red meat, such as the study performed by Nørrung and Buncic (2007) on microbial safety of meat in the European Union, who reported that *Salmonella* spp. was found most frequently in pigs followed by cattle and then sheep. The finding of this current study was however supported by another study performed by Tshabalala (2010) on the effect of hygiene and safety management system on the microbiological quality of fresh beef in South Africa, that no *Salmonella* spp. was isolated from the samples.

In this study, a possible source of contamination could have originated from the dirty water in the scalding tank as the pigs are de-haired and not skinned prior to evisceration. Poor personal hygiene and sanitation may also lead to contamination of meat with *Salmonella* spp. *Salmonella* spp. can be introduced to the abattoir by the animals being slaughtered on a particular day. The dirtier the animals received, the higher the chance of the carcasses being contaminated (Sutton, 2004).

**Table 4. 2:** Distribution of the presence of *Salmonella* isolated in single species high throughput red meat abattoirs in the Free State province

| Abattoir and species | n (288) | Carcass site | Processing stations | | | |
|---|---|---|---|---|---|---|
| | | | P1 | P2 | P3 | Total |
| | | Neck | 0 | 0 | 0 | |
| A Cattle | | Brisket | 0 | 0 | 0 | |
| B Cattle | 96 | Flank | 0 | 0 | 0 | |
| | | Rump | 0 | 0 | 0 | |
| **Total** | | | **0** | **0** | **0** | **0** |
| | | Brisket | 0 | 0 | 0 | |
| C Sheep | | Breast | 0 | 0 | 0 | |
| D Sheep | 96 | Lateral thorax | 0 | 1 | 1 | |
| | | Flank | 3 | 1 | 2 | |
| **Total** | | | **3** | **2** | **3** | **8** |
| | | Jowl | 0 | 0 | 0 | |
| E Pig | | Belly | 0 | 0 | 0 | |
| F Pig | 96 | Back | 0 | 4 | 2 | |
| | | Ham | 3 | 0 | 0 | |
| **Total** | | | **3** | **4** | **2** | **9** |

n= number of carcasses tested per abattoir species. P1=before final wash P2= after final wash; P3= after 24 hours of chilling.

Table 4.2 also shows that samples collected in sheep abattoirs at processing stations P1 and P3 had the highest levels of *Salmonella* spp., with each recording 37.5% (n=3/8), followed by processing station P2 with 25.0% (n=2/8). At processing station P3 the bacterial count was expected to be lower due the effects of low temperatures. This effect was apparent in pig abattoirs, where the *Salmonella* contamination was 44.4% (n=4/9) after final wash and then reduced to 22.2% (n=2/9) after chilling. The high counts of *Salmonella* spp. at P3 of the sheep abattoirs signify the ability of these *Salmonella* serotypes to survive at lower temperature (≤-2$^{o}$C) during chilling (Derbyshire, 2011). These results still highlight the need for sheep abattoirs to assess the effectiveness of their chillers, as such high counts pose a risk of foodborne illness. Furthermore, this result also shows that chilling cannot always reduce contamination, therefore it should only be used for the purpose of chilling since *Salmonella* spp. including other pathogenic bacteria (*Listeria* spp) can survive at very low temperature (≤-2$^{o}$C). Other factors such as air speed and relative humidity of the chiller environment might also have played a role (i.e. influenced the growth) in the high presence of *Salmonella* spp. at P3.

The flank of the sheep was found to be the most contaminated carcass sampling site with 75.0% (n=6/8), followed by the lateral thorax with 25.0% (n=2/8) positive samples in sheep abattoirs. It is not surprising that the flank was found to be the most contaminated site as this site is most handled by the hands of the workers when the sheep is re-hung after the slaughter process. *Salmonella* spp. were only isolated from ham site samples collected at P1 in pig abattoirs. The backs of the carcasses had the highest levels of *Salmonella* spp. at P2 and P3, which could have been the result of

the spread of contaminants from the ham site to other parts of the carcass, hence the high levels of *Salmonella* spp. in those parts after final wash.

All seventeen strains of *Salmonella* spp. positive samples were typed and classified as six different serovars. The predominant serovar was *Salmonella heidelberg,* which was found in 41.2% (n=7/17) of positive samples and the majority of these serovars were isolated from pig abattoirs (n=6/7) and sheep abattoirs (n=1/7). *Salmonella typhimurium* was found in 23.5% (n=4/17) of the positive samples with the majority being isolated from sheep abattoirs. *Salmonella anatum* was found in 17.6% (n=3/17) of the positive samples while *Salmonella enteritidis, Salmonella schwarzengrund* and *Salmonella muenchen* were isolated in only 11.8% (n=2/17) of positive samples; 5.9% (n=1/17) of the isolates were un-typeable. *Salmonella schwarzengrund* was exclusively detected from sheep carcasses. The presence of these serotypes on meat may result in serious and sometimes fatal diseases in humans, especially in immune-compromised, old and young individuals, and it has been associated with severe gastroenteritis, nausea, vomiting, abdominal cramps, diarrhoea, fever, chills and headache (Kidanemariam *et al.,* 2010). These results also show that there may be a lack of adequate control strategies or failure in the HMS of tested abattoirs. Such failure may include improper slaughtering techniques, poor personal hygiene, poor sanitation and inadequate structural requirements.

In South Africa, a report published by the Agriculture Research Council (Kidanemariam *et al*., 2010) has shown that *Salmonella heidelberg* has increased in the rankings to be among the most frequently isolated *Salmonella* serotypes from animal sources. Furthermore, an international survey of public health conducted by Herikstad *et al*.

(2002), which included 191 countries, reported that *Salmonella heidelberg* was distributed in 37 countries. *Salmonella heidelberg* has been incriminated in many foodborne disease outbreaks throughout the world, with the most recent outbreak reported in Tennessee (CDC, 2014). Most persons infected with *Salmonella heidelberg* develop severe diarrhoea, fever and abdominal cramps and the illness usually lasts from 3 to 7 days (CDC, 2014). *Salmonella typhimurium* and *Salmonella enteritidis* have been reported most frequently as the major causative agent of human salmonellosis, with more than 1.7 million cases reported every year to the World Health Organisation. In South Africa, *Salmonella enteritidis* is a legally notifiable disease (Kidanemariam *et al.,* 2010).

#### 4.4.1.3 *Escherichia coli*

A group of bacteria that live in the intestines and are normally shed in the faeces of man and food producing animals. Presence of *E. coli* on the surface of carcases is an indicator of faecal contamination (poor slaughter technique) and environmental contamination (Cohen *et al.,* 2006).

Twenty one percent (n=59/288) of the samples analyzed in this study were found to be positive for *Escherichia coli* (Table 4.3). The pig abattoirs were found to be the main contributors with 45.8% (n=27/59) of the positive samples. This observation was important as pig abattoirs also recorded the highest presence of *Salmonella* species, due to the sample size this was an observation and cannot be regarded as a trend. The sheep and cattle abattoirs contributed 28.8% (n=17/59) and 25.4% (n=15/59) respectively.

**Table 4. 3:** Distribution of presence of *Escherichia coli* isolated in single species high throughput red meat abattoirs in the Free State province

| Abattoir and species | n (288) | Carcass site | Processing stations | | | |
|---|---|---|---|---|---|---|
| | | | P1 | P2 | P3 | Total |
| A Cattle | 96 | Neck | 2 | 2 | 1 | |
| | | Brisket | 2 | 0 | 1 | |
| B Cattle | | Flank | 0 | 0 | 1 | |
| | | Rump | 4 | 2 | 0 | |
| **Total** | | | **8** | **4** | **3** | **15** |
| C Sheep | 96 | Brisket | 2 | 1 | 0 | |
| | | Breast | 0 | 0 | 0 | |
| | | Lateral thorax | 3 | 3 | 1 | |
| D Sheep | | Flank | 3 | 3 | 1 | |
| **Total** | | | **8** | **7** | **2** | **17** |
| E Pig | 96 | Jowl | 3 | 6 | 1 | |
| | | Belly | 2 | 3 | 0 | |
| F Pig | | Back | 2 | 3 | 0 | |
| | | Ham | 3 | 2 | 2 | |
| **Total** | | | **10** | **14** | **3** | **27** |

n= number of carcasses tested per abattoir species. P1=before final wash P2= after final wash; P3= after 24 hours of chilling.

Table 4.3 shows that samples collected in cattle abattoir at processing station P1 had the highest counts of *Escherichia coli* with 53.3% (n=8/15), followed by P2 with 26.7% (n=4/15) and P3 with 20% (n=3/15). The similar trend was also observed in sheep abattoirs. The high presence of *Escherichia coli* at P1 may be accounted for by the cross contamination between clean and contaminated meat and faecal spillage when intestines are removed.

Table 4.3 also shows that samples collected in cattle abattoir at processing station P1 had the highest presence of *Escherichia coli* with 53.3% (n=8/15), followed by P2 with 26.7% (n=4/15) and P3 with 20% (n=3/15). The similar trend was also observed in sheep abattoirs. The high counts of *Escherichia coli* at P1 may be accounted for by the cross contamination between clean and contaminated meat and faecal spillage when intestines are removed. In pig abattoirs, samples collected at processing station P2 had the highest counts of *Escherichia coli*, with 51.9% (n=14/27), followed by P1 37.0% (n=10/27) and P3 with 11.1% (n=3/27). Similar reasoning behind the *Salmonella* distribution at P2 and P3 of sheep abattoir applies the pattern observed in pigs.

In cattle abattoirs, 40% (n=6/15) of the rump site was found to be the most contaminated carcass site, followed by the neck, brisket and flank with, 33.3% (n=5/15), 20% (n=3/15) and 6.7% (n=1/15) out of a total of 15 positive samples respectively. These results can be attributed to the possibility of water used at final wash to have spread contaminants from anal cavity and/or removal thereof, which are known to harbor bacteria. Of the positive samples in sheep abattoirs (n=17), the lateral thorax and flank had the highest counts of *Escherichia coli* with each recording 41.2%,

followed by the neck with 17.6%. The flank had the highest counts of *Salmonella* species as well. Therefore this may suggest the possibility of the same source of contamination, as these bacteria both inhabit the intestinal tract. Of the 27 positive samples in pig abattoirs the jowl (29.6%) was found to be the most contaminated carcass site, followed by the ham (25.9%). The back and belly site were found to be less contaminated as compared to the other two sites with 18.5% each. This can be attributed to good slaughtering technique and good personnel hygiene.

*Escherichia coli* counts for positive samples ranged from undetectable to 3.6 x $10^7$ CFU.cm-$^{2.}$ Twenty five (n=15/59) of the positive samples had a higher count than the maximum standard (10 CFU.cm $^{-2}$) set out in VPN-15. Of these 25% samples with higher counts 66.7% (n=10/15) originated form sheep abattoirs, followed by cattle abattoirs 20% (n=3/15) and pig abattoirs 13.3% (n=2/15). *Escherichia coli* is used as indicator bacteria that provide a fair estimation of faecal contamination and poor sanitation during processing (Tshabalala, 2010). High counts of *Escherichia coli* on meat are always alarming as most *E. coli* strains are highly pathogenic and have been associated with severe gastroenteritis through internalization, toxin production and interference with physiological functions (Cohen *et al.,* 2006). The counts of *Escherichia coli* found in this study were in agreement with the findings of Cohen *et al.* (2006), in their study they found *Escherichia coli* counts ranging from undetectable to 2.0 x $10^6$ CFU.cm$^{-2}$.

The 59 *Escherichia coli* isolates were subjected to serotyping, of which 9 of these samples were identified as rough biotypes that could not be typed. Enteropathogenic *E. coli* (EPEC), Enterohaemorrhagic *E. coli* (EHEC), Enteroinvasive *E. coli* (EIEC) and Enterotoxigenic *E. coli* (ETEC) were the four virotypes that were isolated from the remaining 50 samples (Table 4.4).

The Enterohaemorrhagic *E. coli* (EHEC) was the dominant virotype at 80% (n=40/50), and serotypes O8, O83, O132 and O149 were detected from this virotype. The public health importance of these EHEC serotypes has been attributed to their enterotoxins, which are implicated in causing gastroenteritis, epidemic and sporadic diarrhoea in children. Moreover, Bell (2002) reports that three principle syndromes caused by EHEC are hemolytic uremic syndrome leading to renal failure in children, haemorrhagic colitis with bloody stools and thermbutic thrombocytopenic purpura syndrome causing brain damage and high mortality.

A total of 12% (n=6/50) of the strains were classified as ETEC with serotypes O9 and O141. Enterotoxigenic *E. coli* are an important cause of diarrhoea in children and in travellers to areas with poor sanitation (Bell, 2002). In developing countries, children under the age of three experience multiple ETEC infections and the primary symptom of ETEC infection is diarrhoea without fever

**Table 4. 4:** Distribution of *Escherichia coli* serotypes isolated from single species high throughput red meat abattoirs

| Serotypes | *E. coli* virotype | | | | |
|---|---|---|---|---|---|
| | **EHEC** | **EPEC** | **EIEC** | **ETEC** | **Total** |
| O8 | 3 | 0 | 0 | 0 | **3** |
| O83 | 5 | 0 | 0 | 0 | **5** |
| O132 | 21 | 0 | 0 | 0 | **21** |
| O149 | 11 | 0 | 0 | 2 | **13** |
| O9 | 0 | 0 | 0 | 3 | **3** |
| O141 | 0 | 0 | 0 | 1 | **1** |
| O18 | 0 | 2 | 0 | 0 | **2** |
| O28 | 0 | 0 | 2 | 0 | **2** |
| **Total** | **40** | **2** | **2** | **6** | **50** |

Serotypes O18 and O28 belonged to the EPEC and EIEC virotypes respectively with each having a prevalence of 4.0% (n=2/50). Enteropathogenic *Escherichia coli* (EPEC) are characterised by adhering to human intestinal epithelial cells, causing watery, persistent diarrhoea (Bell, 2002). Enteroinvasive *Escherichia coli* (EIEC) is known to cause enteritis. Patients often develop the symptoms of bacillary dysentery (Prats & Llovet, 1995).

#### 4.4.1.4 *Staphylococcus aureus*

*Staphylococcus aureus* is the leading species involved in staphylococcal food poisoning outbreaks worldwide, caused by the handling of carcasses during slaughtering and dressing by persons who carry enterotoxigenic staphylococci on their skin. Staphylococci represent or indicates contamination with bacteria from the nasal passages, skin hands, fingers and face lesions of humans (Shale & Lues, 2007).

Table 4.5 indicates that *S. aureus* were isolated from 26.0% (n=75/288) of the tested samples. Of all bacteria isolated in this study *S. aureus* were isolated in the highest number of samples. The occurrence of *S. aureus* was 38.7% (n=29/75) in sheep, 37.3% (n=28/75) in cattle and 24.0% (n=18/75) in pig abattoirs. In studies by Cloete (2009) and Tshabalala, (2010) high levels of *S. aureus* has been found in the South African abattoirs and was seen as emerging as a potential concern for meat handlers and consumers. The species of meat-producing animals that are frequently implicated included pigs, poultry, sheep and cattle. The results of this study indicate that *S. aureus* was predominately isolated in sheep and cattle abattoirs. The source of contamination can be due to frequent handling during slaughtering and dressing.

**Table 4. 5:** Distribution of *Staphylococcus aureus* in single species red meat abattoirs

| Abattoir species | n (288) | Carcass site | Processing stations | | | |
|---|---|---|---|---|---|---|
| | | | P1 | P2 | P3 | Total |
| | | Neck | 1 | 2 | 1 | |
| A Cattle | | Brisket | 2 | 2 | 0 | |
| B Cattle | 96 | Flank | 5 | 5 | 1 | |
| | | Rump | 5 | 4 | 0 | |
| **Total** | | | **13** | **13** | **2** | **28** |
| | | Brisket | 5 | 3 | 1 | |
| C Sheep | | Breast | 2 | 1 | 2 | |
| D Sheep | 96 | Lateral thorax | 4 | 2 | 0 | |
| | | Flank | 5 | 3 | 1 | |
| **Total** | | | **16** | **9** | **4** | **29** |
| | | Jowl | 1 | 3 | 2 | |
| E Pig | | Belly | 2 | 5 | 0 | |
| F Pig | 96 | Back | 2 | 1 | 0 | |
| | | Ham | 0 | 2 | 0 | |
| **Total** | | | **5** | **11** | **2** | **18** |

n= number of carcasses tested per abattoir species. P1=before final wash P2= after final wash; P3= after 24 hours of chilling.

*Staphylococcus aureus* was isolated mainly from the samples collected at processing stations P1 and P2 in cattle abattoirs with counts of 13 (46.4%) in each, while processing station P3 had a low count of 2 (7.4%). In sheep abattoirs, samples collected at processing station P1 had a count of 16 (55.2%), which was the highest count of *S. aureus* followed by P2 with 9 (31.0%) and P3 with 4 (13.8%) respectively. In pig abattoirs the isolation of *S. aureus* was 27.8% for P1, 61.1% for P2 and 11.1% for P3. These results raise the concern that water used, especially under pressure at final wash, could be the vehicle for the spreading of contamination all over the carcass.

Carcass sites with *S. aureus* were the belly in pigs, flank in cattle, and brisket in sheep in descending order. This could be because of slaughter techniques and the fact that these sites are used to move or push sheep and pig carcasses around while on the slaughter line. It could be deduced that food-handlers are mostly implicated as a source of *S. aureus* because of personal hygiene practices.

The *Staphylococcus aureus* counts ranged from undetectable to 9.2 x $10^5$ CFU.$cm^{-2}$. 77% (n=58/75) of the positive samples did not comply with the maximum limit of 1.0 x$10^{-3}$ CFU.$cm^{-2}$ for *S. aureus* proposed by the guidelines to environmental health officers (EHOs) on the interpretation of microbiological analysis of data (DoH, 2000). 43% (n=24/58) of these samples originated from cattle abattoirs, followed by 31% (n=18/58) and 26% (n=15/58) from pig and sheep abattoirs respectively. These high counts of *S. aureus* in the meat indicates the possible of occurrence of staphylococcus foodborne poisoning which is characterised by nausea, vomiting, retching, abdominal cramping, and prostration (Tshabalala, 2010). Other staphylococci species isolated from tested carcasses were *S. arlettae, S. capitis, S. chromogenes, S. cohnii,* and *S.*

*saprophyticus.* These bacteria have little if any significance in causing foodborne diseases (Shale *et al*., 2006). However their presence in the meat indicates possible failure in the hygiene management system.

4.4.1.5 Association between APC and specific microbial count from carcass samples

The correlation between the APC and other microbial types was investigated. Relationship coefficients of 0.901 and 0.723 were found between the APC and *E. coli*, and the APC and *S. aureus* respectively (Table 4.6). This suggests that the APC could be used to estimate the amount of *E. coli* and *S. aureus* present on the meat. *Escherichia coli* is well recognised as a faecal indicator that provides a fair estimate of the level of faecal contamination and the hygienic conditions during handling and processing (Cohen *et al.,* 2006).

Aerobic plate counts also correlated (r = 0.723) with *Staphylococcus aureus*, which is a principal inhabitant of the skin, glands and mucous membranes of animals and humans and therefore expected to occur in high numbers on meat (Kidanemariam *et al.,* 2010). A low (negligible) correlation (r= -0248) was noted between *E. coli* and *S. aureus.* This was not surprising, as these bacteria are not interrelated in terms of their habitat (Nel, 2003). The correlation amongst the bacteria is important in shedding light on possible associations between them during processing. However, considering the relatively short period that bacteria inhabit meat between sample collection points, as well as the low temperature maintained on the slaughtering line and in chillers, it is unlikely that a correlation between microbial groups is a result of contamination during slaughtering.

**Table 4. 6:** The correlation (inter-relationship) between the various bacteriological groups

| | Aerobic plate count | *Staphylococcus aureus* | *Escherichia coli* |
|---|---|---|---|
| Aerobic plate count | | 0.723 | 0.901 |
| *Staphylococcus aureus* | | | -0.248 |
| *Escherichia coli* | | | |

### 4.4.2 Air sample results

#### 4.4.2.1 The aerobic plate count

The aerobic plate counts in abattoir A were fairly the same throughout the sampling period with the exception of high counts (8 CFU.m$^{-3}$) recorded during the second hour of sampling. The lowest APCs (2 CFU.m$^{-3}$) in abattoir A were recorded in the first hour of sampling (Table 4.7).

A strong correlation exists between the efficiency of ventilation systems and the concentration of bioaerosols. The ventilation system can significantly influence the temperature changes in the indoor environment and also impact on the dispersal, dilution and removal of air pollutants (Venter *et al*., 2004; Shale & Lues, 2007). The APCs in abattoir A were significantly below the maximum value (90 CFU.m$^{-3}$) recommended by the American Public Health Association (APHA). The use of a negative pressure air system with air filter and low temperature (12ºC) readings recorded at abattoir A may be the reasons for such low counts. Moreover, the air filter reduces the concentration of airborne bacteria in the abattoir and the low temperature inhibits the proliferation of microorganisms.

**Table 4. 7:** Aerobic plate count results collected in various single species high throughput red meat abattoirs (CFU.m$^{-3}$)

| Abattoir | Species | Sampling hours | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | Mean |
| **A** | **Cattle** | 2 | 8 | 3.5 | 4.5 | 4 | 4.4 |
| **B** | **Cattle** | 86 | 79 | 150 | 54 | 57 | 85.2 |
| **C** | **Sheep** | 239 | 0 | 20 | 1 | 0 | 51.8 |
| **D** | **Sheep** | 0 | 0 | 0 | 0 | 0 | 0 |
| **E** | **Pig** | 0 | 0 | 0 | 0 | 0 | 0 |
| **F** | **Pig** | 10 | 0 | 0 | 0 | 0 | 2.0 |

The APCs in the air were inflated in abattoir B (150 CFU.m$^{-3}$) during the third hour of sampling. The lowest APCs (54 CFU.m$^{-3}$) in abattoir B were recorded in the fourth hour (Table 4.7). The environmental temperature (20ºC) and confined space of the abattoir result in high amounts of condensation and may have influenced these results. According to Sutton (2004), airborne contamination particularly in a confined area can be high, as a confined area can allow aerosols to build up to infectious levels. It was also observed that the layout of abattoir B was affected by the distribution of airborne contamination because the separation of clean and dirty areas of the slaughter line was compromised, with a dirty area (bleeding area) right next to a clean area (carcass dressing area)( Chapter 3, Table 3.1, Category I) with no demarcation between them. As a result, air contaminants from the dirty area can be introduced to the clean area during the slaughter and dressing process.

The APCs were high in abattoirs C and F during the first hour, with counts of 239 CFU.m$^{-3}$ and 10 CFU.m$^{-3}$ respectively. The APC in abattoir C was the highest in this study and this result may have been affected by renovations (removing and/or repairing of floor tiles and changing of the door) occurring during the sampling period. This could have led the introduction of air contaminants from the external environment into the abattoir as the doors and windows were opened. Animals from feedlots and /or pens entering a slaughtering facility have a vast number of bacteria affixed to their hooves and hides (>10$^{9}$ CFU.cm$^{-2}$) (Sutton, 2004). It was observed during the inspection, that animals presented for slaughter in both abattoirs were dirty with mud and faeces. This can be attributed to weather conditions as it was raining on the days before sampling and on the day of sampling. Processes such as hide removal within

the abattoir will cause those bacteria to aerosolise. This might also be the reason for such high counts in abattoir B and abattoir C.

An APC count of 10 CFU.m$^{-3}$ was recorded during the first hour in abattoir F. This low count is attributed to the low environmental temperature (15ºC) and well ventilated systems. No counts were recorded from abattoirs D and E, which may be due to the fact that the air sampler was unable to detect the bacteria from the air as result of low bacterial concentration in the air. The low bacterial concentration can be attributed to low temperature (13ºC) and proper ventilation. The APCs obtained in various abattoirs in the Free State ranged from undetectable levels to 239 CFU.m$^{-3}$. Similar counts were recorded in the study performed by Agabou *et al*. (2013) in Algeria, which found APCs ranging from undetectable levels to 246 CFU.m$^{-3}$ in a variety of airborne bacteria in the municipal abattoirs.

4.4.2.2 *Staphylococcus aureus*

The *S. aureus* counts in the bioaerosols of abattoir A were relatively low; ranging from 0 to 4 CFU.m$^{-3}$ throughout the sampling period (Table 4.8). Abattoir B had the highest *S. aureus* counts, where high counts were recorded during the first hour (48 CFU.m$^{-3}$) and third hour (94 CFU.m$^{-3}$) of sampling. This observation is significant since the total aerobic counts were found to be high in abattoir B as well. The results obtained in abattoir B can be attributed to poor personal hygiene (Chapter 3, Table 3.1, Category H), the lack of good processing practice in the abattoir, the relatively high temperature of 27ºC (*S. aureus* can grow between 25 and 39ºC) and the very poor ventilation system as there was condensation during slaughter.

**Table 4. 8:** *Staphylococcus aureus* results collected in various single species high throughput red meat abattoirs (CFU.m$^{-3}$)

| | | Sampling hours | | | | | |
|---|---|---|---|---|---|---|---|
| | **Abattoir** | **1** | **2** | **3** | **4** | **5** | **Mean** |
| **A** | | 0 | 0 | 0 | 4 | 0 | 0.8 |
| **B** | **Cattle** | 48 | 20 | 94 | 0 | 5 | 33,4 |
| **C** | | 0 | 0 | 0 | 1 | 0 | 0.2 |
| **D** | **Sheep** | 0 | 0 | 0 | 0 | 2 | 0.4 |
| **E** | | 0 | 2 | 0 | 1 | 0 | 0.6 |
| **F** | **Pig** | 0 | 0 | 1 | 0 | 0 | 0.2 |

The remaining abattoirs (C, D, E and F) registered very low *S. aureus* counts, ranging from 1.0 CFU.m$^{-3}$ to 2.0 CFU.m$^{-3}$. The relatively low counts recorded in these abattoirs do not necessarily indicate a clean or contaminant free environment, as counts of less than 10 CFU.m$^{-3}$ have been found to contaminate meat and meat products in abattoirs, subsequently causing food-related diseases (Sutton, 2004). Incidences of *Staphylococcus aureus* in bioaerosols of various abattoirs in this study were relatively low when compared to similar studies done. Shale *et al.* (2006) recorded counts ranging between 76 and 300 CFU.m$^{-3}$ in the deboning room of a Grade A (high throughput) red meat abattoir in South Africa. An investigation into the overall performance of the abattoirs revealed that the cattle abattoirs had the highest levels of APC and *S. aureus* counts, followed by pig and sheep abattoirs respectively. Similar findings were recorded by Sutton (2004) on the enumeration of total airborne bacteria and identification of *E. coli*, *Salmonella* spp., and *Staphylococcus* spp., in a beef and pork slaughter facility in the United States.

*Staphylococcus xylose* was only isolated in abattoirs A and B. This bacterium exists as a commensal on the skin of humans and animals and in the environment. *Staphylococcus capitis* is part of the normal flora of the skin of the human scalp, face, neck and ears (Shale *et al.,* 2006). This bacteria was isolated in abattoirs A, B, D and F. *Staphylococcus chromogenes,* which is associated with mastitis in dairy animals (Hogg & Lehane, 1999), was found in abattoirs C and D. *Staphylococcus cohnii* has commonly been known to live or occur on the skin of humans and chickens, and was isolated only from abattoir B along with *Staphylococcus lugdunensis*. The pathogenicity and virulence of *Staphylococcus lugdunensis* are similar to those of *Staphylococcus aureus*, however no data is available in terms of the potential of

*Staphylococcus lugdunensis* to cause foodborne diseases and little is known of its normal habitat (Quinn & Markey, 2004).

4.4.2.3 *Escherichia coli* and *Salmonella* species

*Escherichia coli* and *Salmonella* species were not isolated in any of the tested abattoirs. This is in contrast to the results of Sutton (2004), who reported mean counts ranging from 10-68 CFU.m$^{-3}$ and 0-77 CFU.m$^{-3}$ for *E. coli* and *Salmonella* spp. respectively in three pork abattoirs in the United States. However the recovery of these pathogens from aerial samples may also be influenced by the method and the medium used (Dobeic *et al.,* 2011). The reason for these results may be due to aerosolised bacteria being subjected to considerable stress leading to cell injury and/or death of bacteria (Heidelberg *et al.,* 1997).

4.4.2.4 Other bacterial species isolated from the environmental air

*Bacillus cereus* was isolated in abattoirs C and F. These bacteria can multiply and survive unfavourable conditions such as very low temperatures and pH as well as heat due to their ability to form spores (Quinn & Markey, 2004). This ensures their survival in the abattoir and poses a possible threat for foodborne diseases outbreak. *Bacillus cereus* has frequently been associated with foodborne disease outbreaks, with the earliest outbreak being recorded in 1906 (Sutton, 2004). *Bacillus cereus* causes two types of foodborne illnesses: the diarrhoeal type and the emetic type. The diarrhoeal type is caused by an enterotoxin produced by *Bacillus cereus* during its vegetative state in the small intestine (Quinn & Markey, 2004). The organism growing in food and

producing toxins causes this emetic type of foodborne illness. Due to its ubiquitous nature it seems impossible to obtain raw meat or raw products that are free from *B. cereus* (Nel, 2003).

*Bacillus subtilis, Microccocus luteus, Pseudomonas putida* and *Citrobacter braaki*, were isolated in abattoir B, while *Enterobacter sakazakii* and *Pantoea species* were isolated in abattoirs C and E. *Proteus mirabilis* was found only in abattoir A. These bacteria have a wide environmental distribution and are found in soil and on mammalian skin; they are frequently isolated from food products and the environment (Quinn & Markey, 2004). These bacteria could have entered the slaughtering facility on the hides of animals, in faeces or by the soil on animals. Their presence in the environment poses a risk of carcass contamination and can lead to meat spoilage and food-related diseases.

The study revealed that there are many sources of carcass contamination in the abattoir. The air could be an important source of carcass contamination; however further studies need to be done in order to verify air as a major or important source of contamination as the data generated in the study is not enough to establish the relationship.

## 4.6 Conclusion

In conclusion, the results of this research reveal that there are high levels of *E. coli, Salmonella* spp. and *S. aureus* contamination on carcasses in various single species high throughput red meat abattoirs in the Free State. These bacterial contamination

levels were above the national safety limits as stipulated by VPN-15 on the carcasses. In addition, some of the APCs from the air samples exceeded the limit recommended by the APHA. Similar findings were also found in the air samples for *S. aureus*. These results suggest that there may be a lack of control strategies or failure in the HMS of tested abattoirs. Such failure may include improper slaughtering techniques, poor personal hygiene, sanitation and structural requirement. The HMS also lacks the inclusion of environmental temperature monitoring and ventilation evaluation, which could have contributed to the presence of foodborne pathogens in bioaerosols. The implications of this high count of indicator bacteria could result in possible foodborne disease outbreaks, deterioration of meat quality or shelf-life, with immense economic cost to the abattoir industry.

In order to decrease these high incidences of indicator bacteria, it is recommended that special attention be given to slaughter hygiene and sanitation, as well as structural design. Proper slaughtering techniques as prescribed by Standard Operating Procedures (SOPs), if applied correctly, appear to be advantageous in regulating the increase of undesirable bacterial contamination (Nel, 2003). In addition, it is also recommended that safety assurance and quality systems such as ISO 9001:2000, ISO 22000:2005 and the Global Standards for Food Safety be implemented in the abattoirs, over and above the current HMS. In South Africa there is no set standard for the level of bacteria in the air and their presence in the current study suggests the need for the inclusion of such guidelines in the existing regulations.

Bacterial counts were found to be higher after the final wash than before the final wash on several carcasses. This suggests that water could serve as a vehicle to spread the

contamination to other parts of the carcass. The study has also demonstrated the importance of chilling in microbial contamination control. This was confirmed by the change in bacterial levels at different processing stations; however this control is only a temporary measure and cannot be substituted for hygienic practices. Furthermore, the study found that the specific bacterial counts were different for the different animal species. *Salmonella* species was only found to be high in pig and sheep abattoirs. *Escherichia coli* were substantially higher in pig abattoirs, whilst *S. aureus* was isolated frequently in sheep and cattle abattoirs. This implies that the tested bacteria were not species specific.

There are no legal or legislated limits, guidelines or standards for indicator bacteria on carcasses in local abattoirs in South Africa. This has resulted in the use of many different microbiological standards by the abattoir industry and the interpretation of those standards is entirely the responsibility of the abattoir. It can therefore be concluded that there is an urgent need to develop microbiological standards for local abattoirs in the country. With reference to Chapter 3, the only legislative document for control and/or assessment of hygiene status in South African abattoirs is the HMS and HAS audits and it has been shown in the study that these control measures have many shortcomings when it comes to guaranteeing meat safety.

The data obtained during this study show firstly that indicator bacteria continue to serve important functions in abattoir industry testing programmes. Their role should be written into regulations guiding the production and provision of meat, since bacterial testing is currently not covered by the Meat Safety Act, Act 40 of 2000. Secondly, there is a need to introduce additional food safety systems over and above the current

compliance audits (HAS audits) that are done by VPHs and as required by the Meat Safety Act. Therefore the controlling authorities in South Africa need to develop and implement proper guidelines and limits in terms of bacterial levels on carcasses and in the air of food processing plants, and to re-evaluate the current Hygiene Management System as it fails to produce meat that complies with set guidelines.

## 4.8 References

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# CHAPTER FIVE

## Summative remarks

## 5.1 General discussion

In recent years, there have been heightened concerns about meat safety, not only from consumers but also scientists and economists that focus on the wider socio-economic issues associated with the safety of a country's food supply (Scheutz *et al.,* 2012). These concerns have arisen as a result of reports on emerging and re-emerging foodborne pathogens related to meat which have been associated with food safety scares, related illnesses and deaths (Govender *et al.,* 2013). In addition, the current food safety, quality system and meat inspection procedures are no longer effective in producing bacteriologically safe meat as accepted by various controlling bodies (Pinillos & Jukes, 2007). Therefore, most governments around the world are mandating the implementation of bacterial testing for meat production and processing, and validating the efficiency of the hygiene and safety systems. This situation is similar in South Africa, although control measures are not yet legislated and currently only HAS audits, which do not include bacterial counts on carcasses and in the production areas, are mandated by law.

This dissertation showed the total HAS scores (Chapter 3) and their possible relationship with different indicator bacteria on carcasses and in production areas (Chapter 4) of six single species abattoirs in the Free State province. This study was conducted to answer the following questions: Does the HAS score reflect the hygiene status of an abattoir? Can the score be regarded as an indication of the safety of the meat derived from that abattoir? To what extent do HAS audit scores and bacteriological tests mirror each other?

In Chapter 3, HAS audits were conducted to assess the compliance level of six single species high throughput red meat abattoirs in the Free State province to the set hygiene standards as required by the Meat Safety Act, Act 40 of 2000. The efficiency of the HAS audits checklist in determining the hygiene status of an abattoir was also evaluated. In principle, individual abattoirs were in compliance with the set hygiene standards because the overall performance of each abattoir was above the HAS score of 60 which is the score separating a poor and good score. The total HAS scores of individual abattoirs ranged between 68 and 94, which are scores rated as “fair” and “excellent” respectively. This study demonstrates that abattoir ownership and target market could have a direct impact on the HAS scores. The abattoir that had the highest HAS score of 94 was managed by a company associated with meat exports and the supply of meat to reputable outlets within the country, and which also imposes upon the abattoir further hygiene and quality systems such as ISO 9001:2000, ISO 22000:2005 and Global Standard, over and above the existing HMS.

The specialisation of functions and adherence to regulations in large company-operated abattoirs (in this study there were two such companies) appears to be advantageous, since technical, financial and marketing specialist tasks are designated to specialised teams accountable to management. In a single-owner abattoir (of which there were four in this study), the owner is often the manager, marketer and technician. Therefore, the owner is required to divide his/her time to the detriment of technical aspects, while marketing and general management are prioritised. Although institutionalisation of HAS audits and quality assurance programmes in the abattoir HMS also seems to guarantee better hygiene condition, in small or single-owner

abattoirs there is always a challenge due to competency and number of staff members dedicated to certain functions.

In the same chapter (Chapter 3), it was also revealed that within the HAS audit checklist there are a number of problematic categories in which a similar trend was observed across all abattoirs. Personnel practices, General conditions, Structural requirements and Meat inspection were the HAS categories with poor scores in most of the abattoirs. This suggests that these categories can serve as possible sources of bacterial contamination of the product. As this pattern was observed in most abattoirs, much focus must be placed on training and re-training of personnel with regard to personal hygiene, the hygiene management system and meat inspection. This demonstrates the need to review training methods and curriculum on a regular basis.

Furthermore, it was also found that the weighted score of individual categories does not measure risks posed by that category to the actual meat safety. This was demonstrated by the fact that most abattoirs' total HAS scores were regarded as good, yet there were several categories with varying effects on meat safety that had poor scores. Given that the total HAS score is cumulative of different category scores, it is possible that a category of great importance to meat safety may have a low score, but the combination of the other categories may falsely improve the overall HAS score which may result in the oversight of given poor categories. This highlights the fact that the weight allocation score of some categories within the HAS audits checklist needs to be reviewed to match the risk of contamination posed to meat safety. Such categories are Personnel practices, Offal processing, Meat inspection, General conditions and Chilling and dispatch.

The total HAS score of the various abattoirs unfortunately does not demonstrate meat safety risks, since it does not measure the impact of non-compliance in relation to meat safety. For example, an abattoir can comply 80% to legislative requirements, but the impact of the 20% non-compliance is not measured, and this could be a possible source of risk to meat safety. It is therefore apparent that meat safety cannot be assured based on compliance, but should be assured based on the impact of non-compliance. These results suggest that the HAS audits were not sufficient in ensuring meat safety especially with regard to possible microbial contamination.

Chapter 4 on the other hand assessed microbial levels on carcass surfaces at randomly selected carcasses in cattle, sheep and pig abattoirs. Farm animals are major habitats of pathogenic bacteria, which are normally spread to meat during slaughter and processing. This study proved that carcasses carried zoonotic bacteria, and the species *E. coli*, *Salmonella* spp. and *S. aureus* were detected on different sites on the carcass surfaces. Ingestion of meat contaminated with these zoonotic bacteria has been associated with nausea, vomiting, cramps and diarrhoea in humans. Current results show the significance of bacteriological tests in meat safety assessment.

Traditionally, when one is faced with problems of microbial contamination, knowledge of sources of contamination, spreading and control of microbiological growth is crucial. This helps in the implementation of relevant hygiene measures to contain possible microbiological contamination, in order to maintain meat safety and subsequently reduce foodborne disease. Hence, it is important that microbiological testing be legislated as part of meat safety assessment in South Africa. From observations made in Chapter 4, possible sources of contamination were incorrect techniques for the

removal of hides and offal, personnel hygiene and structural designs. These factors or sources are covered and audited under the problematic categories (Personnel practices, General conditions, Structural requirements and Meat inspection) mentioned in Chapter 3, which justifies the fact that those categories served as sources of carcass contamination. Therefore, training and adherence to standard operating procedures appears critical in reducing the possibility of contamination. Another possible means of spreading of microbes within the abattoir could be associated with the cleaning of the animals being slaughtered. The more contaminated the animal presented for slaughter, the higher the possibility of bacterial load on the carcass. This highlights a need to develop and implement a policy or to find a way of handling dirty animals in the abattoir: a way needs to be found to clean off visual dirt present on the animal.

The bacterial count was found to be higher after the final wash than before the final wash on several carcasses. This suggests that water used under pressure can serve as a vector to spread the contamination to other parts of the carcass and/or the water used was not of acceptable quality (contaminated with bacteria). This requires further investigation into the quality of water used in abattoirs, an aspect which was not the focal point of the current study. The primary object of carcass washing is to remove bloodstains and improve appearance after chilling. Therefore, washing is no substitute for good hygiene practice during slaughter and dressing because water is likely to spread bacteria rather than reduce them. The study has also demonstrated the importance of chilling towards microbial contamination control, as samples collected after chilling had the lowest bacterial load. Thus the Chilling and dispatch category has a higher weight contributing to the total HAS score. Derbyshire (2011) indicates that

chilling inhibits the proliferation of the bacteria; however, it must not be used to as measure to compensate for bad hygiene practices during slaughter as temperature changes could allow the survival of bacteria. The role of air speed/flow rate of a wet carcass post washing on the slaughter line is also important to consider as it may have a great impact on bacterial contamination.

The most alarming issue uncovered in Chapter 4 was that the average counts of *E. coli*, *S. aureus* and *Salmonella spp.* levels found on the carcass surfaces exceeded the maximum limit stipulated in VPN-15. In addition, four virotypes (EHEC, EIEC, ETEC and EPEC) of *E. coli* and two serovars of *Salmonella* (*S. typhimurium* and *S. enteritidis*) associated with human diseases were identified. The bacterial load on the carcass surfaces and pathogenicity of isolated strains from the carcasses, suggests an inevitable possibility of the potential risk of food poisoning and transmission of zoonotic diseases to consumers. To make matters more challenging, South Africa does not have legislated limits for bacterial counts on the carcass surface for local abattoirs. Therefore, each abattoir uses its own standard that is not controlled and the interpretation of the standards is left to the abattoir owners, which could result in incorrect interpretation and manipulation of the results. Urgent attention from various role players in the red meat industry is needed to develop microbiological standards for carcass surfaces in the country. The government, however, should lead this initiative.

In addition to direct contact, which is regarded as the main source of carcass contamination, bacteria can also be transmitted through the air. Potential meat contaminants from air were also investigated in Chapter 4. This study has shown that

there was a presence of bioaerosols in the sampled abattoirs. An average APC ranging from 0 to 85.2 CFU.m$^{-3}$ was obtained for all the abattoirs, which was below the recommended limits by APHA. The reason for this is the utilisation of good air flow systems (ventilation) present in most abattoirs sampled. Of the three main bacterial species in the study, only *S. aureus* was found in the air. The average count ranged from 0.2 to 33.4 CFU.m$^{-3}$. The structural separation between clean and unclean areas, structural renovation that took place during processing, confined abattoir spaces, high levels of temperature and bacterial load on live animals presented for slaughtering may be possible sources of observed bioaerosol counts. Poor structural aspects as observed in Chapter 3 might have influenced the dispersal of bioaerosols. This suggests that air can serve as a possible source of carcass contamination. It was also revealed that there might be a relationship between direct carcass contamination and bioaerosols. However, further studies need to be done in order to verify this as the information generated in the study is not enough to establish the relationship.

The bacteriological safety of meat is determined by the number and type of bacteria isolated from the carcasses. Meat is considered bacteriologically unsafe when the presence of zoonotic bacteria is above maximum limits as stipulated in the local legislation and/or when pathogenic bacteria are isolated from the product (Yousuf *et al*., 2008).

Table 5.1 shows total HAS scores and total bacteriological test results of selected single species high throughput red meat abattoirs in Free State province. The results in the table show that total HAS scores obtained for respective abattoirs did not reflect the safety of the meat. There is no direct association between these two factors when

comparing abattoir A (94) and abattoir B (68), which shows that the highest HAS score had the lowest bacterial occurrence and vice versa. However, this trend does not occur when comparing abattoirs E and B, which have comparable bacterial occurrence even though their total HAS scores vary substantially. On the other hand abattoirs E and C have almost similar total HAS score but the bacterial incidences are different. Therefore abattoir E would be expected to have a lower bacterial presence.

In addition to the bacterial occurrence which did not show any pattern, the presence of pathogenic bacteria may further justify that total HAS score does not reflect meat safety. The current results show that a high total HAS score does not mean that there is guaranteed meat safety. Taking into account the bacteriological profile of abattoirs A and B, which are at opposite ends of the total HAS score range, observation shows that they both had *E. coli* virotype EHEC which is causes haemorrhagic colitis with bloody stools and thermbutic thrombocytopenic purpura syndrome causing brain damage and high mortality in humans (Quinn & Markey, 2004). Furthermore, when comparing abattoirs E, C, F and D to abattoir B which recorded the lowest HAS score, it was clear that those abattoirs had certain virotypes (EIEC, ETEC and EPEC) which were not found in abattoir B. Irrespective of HAS scores, pathogenic strains of *E. coli* were isolated in various abattoirs, which demonstrates that bacterial serotyping is critical in meat safety assessment because it will provide further clarity on how a patient can be treated and/or how to reduce contamination of a specific strain within processed meat.

Despite the fact that most abattoirs obtained a “good” HAS score, critical non-conformances were identified during the audit. Critical non-conformances are recorded

when the non-conformance has a direct influence on the safety of the product and therefore poses an imminent risk to public health (Derbyshire, 2011). This further proves that HAS audits cannot assure meat safety.

**Table 5. 1:** The total HAS scores and bacteriological test results of single species high throughput red meat abattoirs in the Free State province

| Abattoir | Total HAS score | Bacteriological test results | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *E. coli* | | | *Salmonella* species | | | *S. aureus* | |
| | | Present | *VPN limits | Serotype | Present | VPN limits | Serovars | Present | *EHO limits |
| A | 94 | 3 | 1 | EHEC | 0 | 0 | 0 | 8 | 6 |
| E | 80 | 14 | 1 | EHEC, ETEC | 5 | 5 | *S. heidelberg*<br>*S. typhimurium*<br>*S. enteritidis* | 13 | 13 |
| C | 79 | 5 | 1 | EHEC, ETEC | 0 | 0 | 0 | 10 | 4 |
| F | 74 | 13 | 7 | EHEC, EIEC, ETEC, EPEC | 4 | 4 | *S. heidelberg*<br>*S. typhimurium*<br>*S. enteritidis*<br>*S. muenchen* | 5 | 5 |
| D | 70 | 12 | 3 | EHEC, ETEC | 8 | 8 | *S. heidelberg*<br>*S. typhimurium*<br>*S. anatum*<br>*S. schwarzengrund* | 19 | 11 |
| B | 68 | 13 | 2 | EHEC | 0 | 0 | 0 | 19 | 18 |

*VPN limit – the number of carcasses that exceeded the VPN limit per bacterial species. *Environmental Health Officer

Limits are maximum acceptable levels of bacteria on the carcass.

*Salmonella* species were not recovered in abattoirs with the highest and lowest total HAS scores. Similar results were apparent when comparing abattoirs C and B. Furthermore, when comparing abattoirs D, E and F to abattoir B, it was noticeable that *Salmonella* spp. were present in abattoirs with high HAS scores, while absent in abattoir B which obtained the lowest HAS score. This further emphasises the fact that HAS score does not demonstrate meat safety. *Salmonella enteritidis* and *Salmonella typhimurium* are major causative agents of human salmonellosis in the world, with over 1.7 million cases occurring annually. This shows that meat obtained from abattoirs D, E and F might not be safe compared to meat obtained in abattoir B, considering the type of *Salmonella* serovar isolated.

Microbiological standards serve as a guideline to the meat industry on the bacterial load that is acceptable on the carcass. This is done to prevent and/or reduce the occurrence of foodborne diseases in the human population and to increase the shelf life and quality of the meat. South Africa does not have such standards for local abattoirs, therefore VPN-15, the standard set for export abattoirs, was used for the current study. Table 5.1 above further shows that, regardless of the total HAS scores, most carcasses in tested abattoirs exceed the maximum limits. No significant difference was found between the highest total HAS score abattoirs and the lowest score abattoirs in terms of number of carcasses that exceeded the limits with the exception of *S. aureus*.

The correlation between the aerobic plate count (APC) and other microbial types was also investigated in the current study. A strong relationship was found between *E. coli* and *S. aureus* and APC. This suggests that APC could be used to estimate the

amount of *E. coli* and *S. aureus* present in the meat. Therefore APC was used to establish if any association exists between HAS score and bacterial counts.

In addition to the above findings, an association between total HAS score and APC also shows that there is no relationship between these variables (Figures 5.1 and 5.2). This further demonstrates that HAS score cannot be used to reflect meat safety. For example, at the highest HAS score (94) the mean APC was higher than at the lowest HAS score (68). At the HAS scores of 70 and 78, the APC was the highest from the sample collected before the final wash. A similar pattern was apparent from the samples collected after the final wash. Furthermore at the lowest HAS score (68), the mean APC was higher than at highest HAS score (94) for the samples collected after chilling. Similar findings have been made by the Britain Food and Veterinary Office (2000), as cited by Pinillos and Jukes (2007). This implies that there is no association between HAS score and the APC from carcass samples, and air samples as such the two variables should both be included in the hygiene and safety evaluation system.

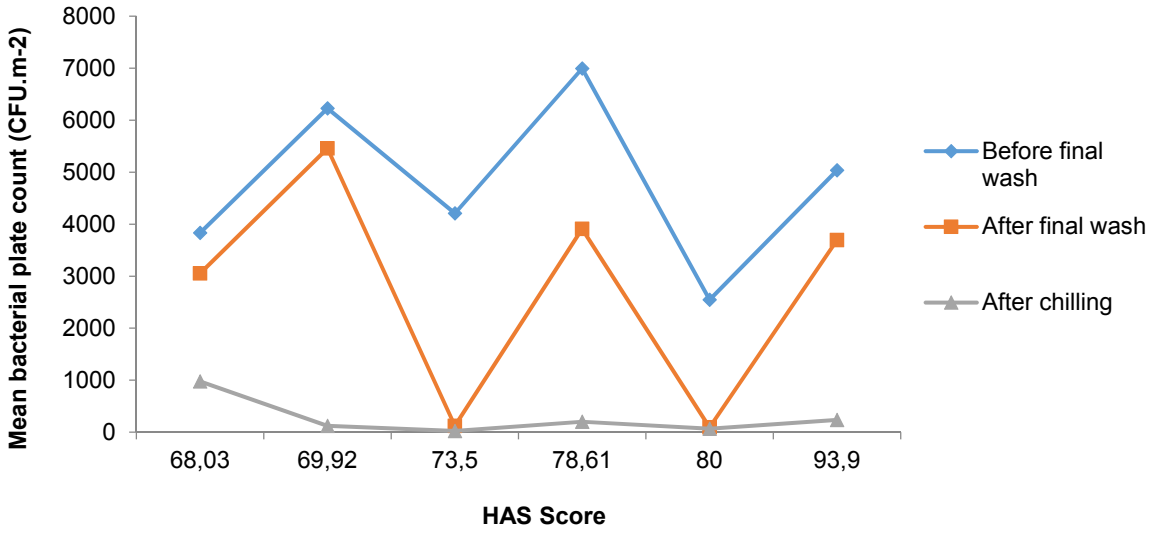


**Figure 5. 1:** Association between mean aerobic plate count and the final HAS score

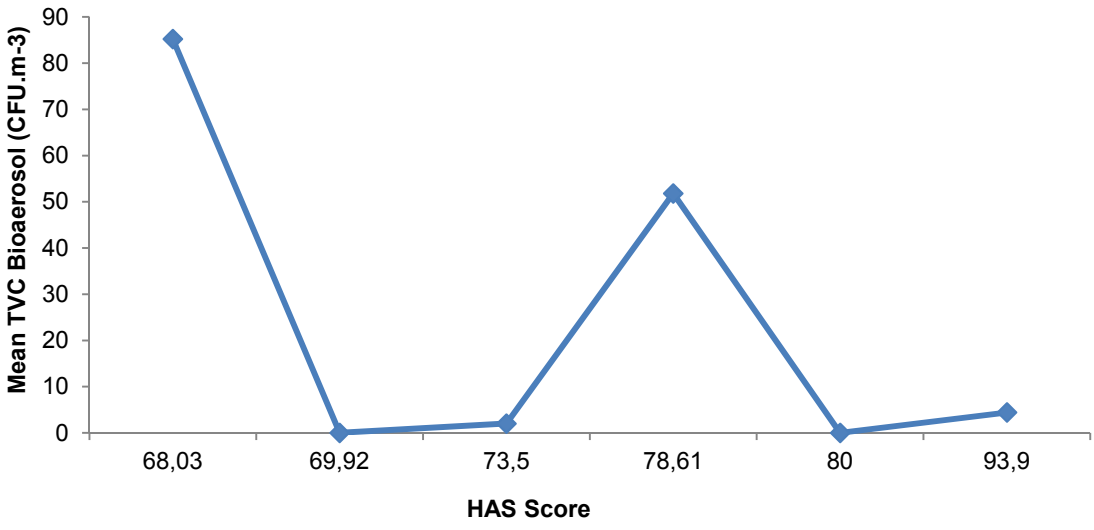


**Figure 5. 2**: **A**ssociation between mean total viable count, bioaerosols and final hygiene assessment system score

The reason for the variation in the HAS score and APC trends could be as a result of the impact of individual HAS categories. The lowest scored category (Structural requirements and Maintenance) in the abattoir with the highest final HAS score of 94 might have a direct effect on bacterial growth, and this could have resulted in the high APC. Abattoirs with high APC were found to have scored low in the following categories: Meat inspection, Personnel, General conditions, Structural requirements and hygiene management system. Given that the category scores are allocated based on their importance to and influence on meat safety, it should be noted that these categories account for a large proportion of the final HAS score. Hudson *et al.* (1996) found that a negative relationship existed between independent categories within the HAS and the APC. The present study did not investigate the impact of individual categories on the APC, but results show that this might have influenced the bacterial contamination.

The current study reveals that, irrespective of the total HAS score, pathogenic strains of bacteria were present on the carcasses at levels above recommended limits. It can therefore be concluded that the total HAS score does not reflect bacteriological meat safety and the score cannot be interpreted as a measure of meat safety; it can only be used to measure compliance to the set regulations as stipulated in the Meat Safety Act, Act 40 of 2000. The HAS audits are compliance audits and the score cannot mirror or be linked to bacteriological safety of the meat derived from a particular abattoir. It is important that bacteriological analysis of meat and air samples be included in the hygiene and food safety evaluation system, or other systems should be introduced or legislated. Therefore this study found that there is no relationship between the total

HAS score and bacteriological test results in single species high throughput red meat abattoirs in the Free State province.

## 5.2 Recommendations

The following recommendations are made:

- A formula needs to be developed to reflect the actual impact of the respective categories on the final HAS audit scores on meat safety instead of the overall percentage that may mislead or not reflect the actual problems. This will also include the re-evaluation of the category weights.
- The institutionalisation of the combination of HAS audits and quality assurance programmes into the abattoir HMS to guarantee better hygiene conditions.
- The legal mandating of the inclusion of bacteriological analysis of meat and air samples into the hygiene and safety evaluation system at a prescribed frequency.
- The role of indicator organisms to be included into the regulation guiding the production and provision of meat safety, since they are currently not covered by the Meat Safety Act, Act 40 of 2000.
- The abattoir owner needs to invest in training of personnel to ensure that all workers including management take ownership of hygiene practices during animal slaughtering and further processing.
- Government should develop and facilitate the implementation of proper guidelines, standards and limits in term of bacterial levels in the carcass and air contaminants for the abattoir industry.

## 5.3 Research prospects

- Conducting similar studies in other abattoir species such as poultry and game.
- The finding in this study shows that HAS audits measure non-conformances and do not measure the impact of non-compliance. Therefore, further work needs to be done to assess the impact of non-compliance in relation to meat safety.
- Further studies need to be done on the occurrence of other airborne bacteria associated with single species abattoirs.
- Further research work need to be conducted to establish the relationship between independent HAS categories and bacteriological counts of the raw meat.
- Further studies need to be done using molecular techniques to determine the effect of HAS audits on the microbiological quality of raw meat as well as identifying sub-species for proper processes in case of possible food poisoning and/or outbreaks.
- The finding in this study show bacterial carcass contamination levels in the investigated abattoirs to be high. Therefore, further work need to be done to assess meat quality after it has left the abattoir to gain insight into the quality of meat that finally reaches the kitchen.

## 5.4 References

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# Annexure A

## Microbiological standards for export meat

| Category | Micro-organisms | Sampling Plan | | Limits | | Method |
|---|---|---|---|---|---|---|
| | | n(1) | c(2) | m(3) (log value) | M(4) (log value) | |
| **Carcasses and meat cuts of cattle, sheep, goats and horses** | Aerobic colony count | 35 | 7 | 3162 cfu.cm-$^2$ (3.5 log) | 100 000 cfu.cm.$^2$ (5.0 log) | ISO 4833 |
| | *E.coli* | 35 | 7 | 1 cfu.cm.$^2$ (0 log) | 10 cfu.cm.$^2$ (1 log) | |
| | *Salmonella* | 50 | 2 | Absent in area tested per carcass | Absent in area tested per carcass | EN/ISO 6579 |
| **Carcasses and meat cuts of wild cloven hoofed game and wild solipeds** | Aerobic colony count | 35 | 7 | 100 000 cfu.cm.$^2$ (5.0 log) | 550 000 cfu.cm.$^2$ 5.7 log) | ISO 4833 |
| | *E.coli* | 35 | 11 | 50 cfu.cm.$^2$ (1.7 log) | 500 cfu.cm.$^2$ (2.7 log) | |
| | *Salmonella* | 50 | 2 | Absent in area tested per carcass | Absent in area tested per carcass | EN/ISO 6579 |
| **Carcasses and meat cuts of ratites** | Aerobic colony count | 35 | 7 | 3162 cfu.cm.$^2$ (3.5 log) | 100 000 cfu.cm.$^2$ (5.0 log) | ISO 4833 |
| | *E.coli* | 35 | 7 | 1 cfu.cm.$^2$ (0 log) | 10 cfu.cm.$^2$ (1 log) | |
| | *Salmonella* | 50 | 2 | Absent in area tested per carcass | Absent in area tested per carcass | EN/ISO 6579 |

(1) '**n**' is the number of individual samples in a sampling plan (also called a sampling window)

(2) '**c**' is the number of marginal samples allowed in '**n**' samples

(3) '**m**' is a defined value separating a good result from a marginally acceptable result (values between **m** and **M** are considered to be marginally acceptable)

(4) '**M**' is the maximum value for a marginal result (values greater than **M** are unacceptable)

# Annexure B

**Figure 5.4:** Sample processing in the laboratory


**Figure 5.5:** Microscopic examination of bacteria on Gram stain