CHAPTER FIVE:
GENERAL DISCUSSION

In South Africa, the red meat abattoir industry is divided into those abattoirs that are linked to the feedlot and the wholesale sectors (class A and B) and those which are owned by farmers (classes C, D and E). Classes A and B abattoirs comply with all statutory measures including continual training of slaughter operatives and proper temperature control, while it is questionable if this is the case with the majority of the latter abattoir classes. Insufficient training of slaughtermen can lead to contamination of carcasses during the slaughtering process with pathogenic bacteria, which may proliferate under poor temperature control conditions. Inadequate cooking of contaminated meat could potentially cause diseases when this meat is consumed.

5.1 REVIEW OF METHODOLOGY

5.1.1 Swabbing of carcasses

In this study, the swabbing method (Gill & Jones, 2000) was used to collect sponge swab samples from chilled beef carcasses. *E. coli* O157:H7 and *Salmonella* spp. were among microorganisms under investigation in this study. Food pathogens have a low prevalence and a heterogeneous distribution on beef carcasses (Bolton, 2003). It would have been possible to use the excision method (Gill & Jones, 2000) to collect samples for microbiological analysis. However, the swabbing method was preferred because it provided an opportunity to collect swabs from a larger surface area (whole forequarter) compared to the area that could have been covered by using the excision method. The excision method would only cover a smaller area, usually about 20 cm^2 per carcass. Contradictory observations have been made by previous researchers when comparing swabbing and excision methods. Martínez, Celda, Anastasio, García and López-Mendoza (2010) compared the recovered bacterial loads from fresh beef carcasses by the excision or swabbing method. Martínez *et al.* (2010) recorded higher APC (4.50 log_{10} cfu/cm^2) from excision samples compared to 3.53 log_{10} cfu/cm^2

recovered from swab samples. In addition, Martínez *et al.* (2010) observed that the bacterial count recovered from swab samples was influenced by the type of swab used. Based on their results, the use of cellulose swabs, as used in this study, could not have been a limitation factor on the results. Higher bacterial counts were recovered from swabbing with abrasive sponges compared to viscose ones (Martínez *et al.,* 2010). However, care should be taken that the pressure of sponging should be as if the sampler is removing dried blood from the carcasses, while not being so hard as to crumble or destroy the sponge (Anonymous, 2007). Trenton *et al.* (2010) observed that the fat cover on the sampled site may prevent adequate removal of bacteria by occluding the pores of the sponge, thereby reducing removal of bacteria.

5.2 ISOLATION OF ENTEROBACTERIACEAE FROM CHILLED BEEF CARCASSES

Enterobacteriaceae counts from abattoirs that use HAS and those that use HAS + HACCP were found to be similar. The presence of Enterobacteriaceae on carcasses suggests transfer of faecal material onto the sterile carcass during the slaughter process, which may suggest that the currently available dressing procedures at both HAS and HAS + HACCP abattoirs cannot be relied upon in preventing faecal contamination during slaughter. In this study, the Enterobacteriaceae was enumerated as a whole by using VRBG agar without taking a further step to identify the specific Enterobacteriaceae members prevalent on beef carcass at abattoirs using different HMSs. This additional information could have provided insights into the effects of HAS and HAS + HACCP on different types of Enterobacteriaceae on beef carcasses. Moreover, comparison could have been made between abattoirs using the same HMS. Stiles and Ng (1981) used biochemical testing to identify and characterise 2220 Enterobacteriaceae from meats and identified principal Enterobacteriaceae types to be differentiated in meat.

5.3 ISOLATION OF *E. COLI* O157:H7 FROM BEEF CARCASSES

Beef and beef containing products have been implicated in both VTEC (Sartz, De Jong, Hjertqvist, Plym-Forshell, Alsterlund, Löfdahl, Osterman, Ståhl, Hansson & Karpman, 2008) and non-O157 VTEC outbreaks (Ethelberg, Olsen, Scheutz, Jensen, Schiellerup, Engberg, Petersen, Olesen, Gerner-Smidt & Mølbalk, 2004). In this study, the target VTEC was only *E. coli* O157:H7, whereby a prevalence of *E. coli* O157:H7 of 20% was recorded from samples collected after evisceration, 20% after final wash and 0% after chilling from an abattoir with a combined HAS + HACCP system. The absence of *E. coli* O157:H7 after chilling could be due to the effects of chilling, a step in animal slaughter that is known to reduce the numbers of generic *E. coli* on carcasses. It is reasonable to assume that when present, *E. coli* O157:H7 is on the surface of carcasses, not in internal tissues of intact muscle that normally is protected from surface contamination during slaughter. A combination of rapid chilling of adequately spaced carcasses and surface dehydration during chilling should retard *E. coli* O157:H7 growth on carcass surfaces (Butler *et al.*, 2006).

The occurrence of non-O157:H7 should have been investigated, which could have given a better picture of the prevalence of VTEC on beef. Arthur *et al.* (2002) isolated 361 non-O157 STEC isolates from beef carcasses where 49% of the O serogroups had previously been associated with diseases. It is well documented that many methods used in *E. coli* O15:H7 investigation, as was the case in this study, include the use of selective agents such as novobiocin or acriflavin to inhibit the growth of gram-negative organisms and a combination of vancomycin, cefsulodin and cefixime to suppress the growth of *Aeromonas* spp. and *Proteus* spp. However, Stephens and Johnson (1998) illustrated the toxicity of these agents towards stressed *E. coli* O157:H7 cells due to the presence of bile salts and antibiotics in selective enrichment media (Stephens & Johnson, 1998). Therefore, chilling may not have killed the cells that were isolated after evisceration and washing, but they may have been unculturable.

5.4 ISOLATION OF *SALMONELLA* SPP. FROM CHILLED BEEF CARCASSES

Meat carcasses may be contaminated with *Salmonella* spp during slaughtering (Marlony *et al.*, 2008). In this study, the prevalence of *Salmonella* spp from beef carcasses was only investigated after chilling, whereby, *Salmonella* spp. were not isolated from any of the samples. The chilling process can injure bacterial cells as a result of low carcass surface a_w and low temperature (McEvoy, Sheridan & McDowell, 2010). Therefore, an investigation of the prevalence of this organism during the different steps of animal slaughter could probably have given a better indication of the efficacy of the HMS used in preventing contamination of carcasses with *Salmonella* spp. In this study, the method used was both labour and growth media intensive and relied on *Salmonella* specific biochemical tests, such as the production of hydrogen sulphide, non-fermentation of lactose and sucrose or xylose to distinguish *Salmonella* from other members of the Enterobacteriaceae. This method could have been modified by including a thin layer agar (TAL) of a non-selective medium such as tryptic soy agar (TSA), over layered on pre-poured and solidified selective medium plates of xylose lysine decarboxylase (XLD) medium. This method would have simultaneously facilitated the recovery of injured *Salmonella* cells, while also providing the selectivity of isolation of *Salmonella* spp. (Kang & Fung 2000). The advantage of the TAL method is that it allows injured *S. typhimurium* to be resuscitated and grow on the TSA layer, while selective agents from the XLD medium diffused to the top thin TSA layer. Once the selective agents diffused to the top agar layer, the resuscitated *Salmonella* spp. cells would start to produce a typical reaction (black colonies) and other microorganisms would be inhibited by selective agents diffused from XLD.

Alternatively, chromogenic media could have been used. Chromogenic media are microbiological growth media that contain organism specific enzyme substrates, linked to a chromogen. The target populations are characterized by enzyme systems that metabolize the substrate to release the chromogen, which then results in a colour change in the medium. Perez *et al.* (2003) established that chromogenic media

demonstrate higher specificity compared to conventional *Salmonella* spp. growth media. Similarly, Schönenbrücher, Mallinson and Bülte (2008) recovered 100% *Salmonella* from artificially inoculated raw meat when using chromogenic media (Miller-Mallinson, AES *Salmonella* Agar Plate Agar and Oxoid *Salmonella* Chromogen Media Agar) compared to traditional media. Moreover, Schönenbrücher *et al*. (2008) found that a combination of the enrichment in Selenite Cystine broth for 24 hours and chromogenic plating media significantly increased the recovery of *Salmonella*. The higher specificity of the chromogenic media also reduces the need for confirmatory tests, thereby cutting technical time and reagent requirements.

5.5 SAMPLE PREPARATION FOR *E. COLI* O157:H7 SURVIVAL STUDIES FROM ARTIFICIALLY CONTAMINATED BEEF LOINS

In this study, a constant inoculation level 10^2 cfu/ml of *E. coli* O157:H7 was used together with either 10^2 or 10^6 of *P. fluorescens* or 10^2 and 10^4 cfu/ml of *L. plantarum*, respectively, to contaminate sterile beef loins. Although the infectious dose of *E. coli* O157:H7 is unknown (Food and Drug Administration, 1998), Paton and Paton (1998a) estimated that the infectious dose of *E. coli* O157:H7 ranges from 1 to 100 cfu/cm^2. Since the level of *E. coli* O157:H7 was held constant at 10^2 cfu/cm^2, it would have been interesting to see how a lower inoculation level could have been affected by the lower levels of *P. fluorescens* and *L. plantarum*, considering that natural contamination of these bacteria is lower than the levels used in this study. The reason for using higher levels in this study was to ensure that the effects of *P. fluorescens* and *L. plantarum* were more apparent.

5.6 EFFECT OF HAS ALONE AND HAS COMBINED WITH HACCP ON THE MICROBIOLOGICAL QUALITY OF FRESH BEEF

HACCP systems identify all the steps throughout the chain where contamination deviations may occur, including measures taken on the farm, the quality of animal feed and handling of animals from farm to the abattoir. In the abattoir the HACCP system seeks to identify all steps in the slaughtering process where biological hazards

can either be minimized or eliminated during slaughter. These steps are referred to as critical control points (CCPs). The HACCP system, therefore, allows for quick corrective measures in those situations where deviations from the set controls in the CCPs are noted, thus assuring the safety and quality of the final product (Pinillos & Jukes, 2008). This study demonstrated that the microbiological quality of beef derived from HAS combined with HACCP abattoirs is not microbiologically superior compared to that of fresh beef derived from HAS abattoirs. APC counts from all abattoirs were similar. The highest Enterobacteriaceae count was recovered from one of the abattoirs that use the HAS + HACCP system. In addition, *E. coli* O157:H7 was isolated from that same abattoir. The positive isolation of *E. coli* O157:H7 from an abattoir that uses the HAS + HACCP system is in agreement with Josling (2004) who found that though risk assessment is a good preventive system, it leaves room for substantial uncertainty over the incidence of risk. Furthermore, Sperber (2005) attributed the occurrence of foodborne outbreaks in the USA, despite the widespread use of HACCP, to failures of cleaning and sanitation, or the lack of management awareness and commitment to provide the necessary training and resources to support HACCP plans.

5.7 SURVIVAL OF *E. COLI* O157:H7 CO-CULTURED WITH DIFFERENT LEVELS OF *PSEUDOMONAS FLUORESCENS* AND *LACTOBACILLUS PLANTARUM* ON FRESH BEEF

In this study, *E. coli* O157:H7 was challenged with different levels of *P. fluorescens* and *L. plantarum*, yet in reality *E. coli* O157:H7 may occur simultaneously with other VTEC on beef. The results of this study showed that 18 h cultures of *E. coli* O157:H7 and *P. fluorescens* required 2 days to adapt and start growing on meat stored aerobically at 4 °C. When bacteria are deposited onto meat surfaces, they undergo a period of adjustment in their new environment, where cell damage is repaired and bacteria adapt to utilize nutrients available before they can start to grow (Sentance & Husband, 1993). Temperature influences the lag phase and growth rate of *E. coli* including serotype O157:H7 (Duffy *et al.* 1999). There are instances where the cold chain may be broken, exposing raw meat to temperatures that promote the growth of

both pathogenic and spoilage bacteria. The challenge study with the same levels of *E. coli* O157:H7 and *P. fluorescens* at abusive temperatures (10 °C) would provide an indication of the length of the lag phase of this organism on beef loins. An antagonistic interaction between *E. coli* O157:H7 and *P. fluorescens* was observed, as evidenced by *E. coli* O157:H7 that stopped growing while *P. fluorescens* continued to grow to its maximal growth density at day 7. Nevertheless, the results of this study showed that *P. fluorescens* is unable to affect the growth of *E. coli* O157:H7 on aerobically stored beef, regardless of the levels in the inoculums. Therefore, improper handling of meat and meat products, as well as the lack of maintenance of the cold chain, which is more prevalent under non-commercial conditions in South Africa, may support the growth and spread of *E. coli* O157:H7. It would have been interesting to investigate the survival of *E. coli* O157 in the presence of a low level of a non-O157:H7 strain together with *P. fluorescens*.

Vacuum packaging is a widely used practice in meat marketing. Prime meat cuts are vacuum packaged for distribution and extension of storage life. However, in South Africa, vacuum packaging is used in only the commercial sector, yet the South African meat industry comprises both the commercial and the informal sector, with slaughter of own-use also being allowed. In this study, *L. plantarum,* under vacuum storage resulted in total kill of *E. coli* O157:H7 on beefsteaks. The onset of inhibition was influenced by the level of *L. plantarum* in the initial inoculums, with total kill being recorded earlier when the level of *L. plantarum* in the inoculums was 10^6 cfu/ml. The effect of spoilage bacteria on survival and growth of *E. coli* O157:H7 on meat depends on the type and levels of microorganisms present and storage conditions. *L. plantarum* regardless of the level completely eliminated *E. coli* O157:H7. It would have been interesting to see how an acid-adapted *E. coli* O157:H7 strain could have been affected by *L. plantarum*.

5.8 AREAS FOR FUTURE RESEARCH

Further research work needs to be conducted using molecular techniques to assess the effect of HAS alone and HAS + HACCP on the microbiological quality of beef.

Molecular techniques e.g. are rapid, specific and more sensitive compared to traditional methods, which are lengthy and media intensive. Rapid methodologies may allow for more visits to abattoirs under investigation.

The Enterobacteriaceae family includes indicator organisms for faecal contamination. A study using molecular techniques should be conducted to identify the specific organism within the Enterobacteriaceae family to elucidate the influence of HMS on the range of Enterobacteriaceae species occurring on beef carcasses.

In South Africa, there is a need to conduct an investigation on the occurrence of VTEC on live animals both at feedlots and free range beef animals compared to the levels of VTEC on beef carcasses and retail samples. This must include both *E. coli* O157:H7 and non-O157 *E. coli* that have also been implicated in sporadic foodborne outbreaks. This investigation could be expanded to other animal species such as sheep, goats and pork.

L. plantarum inhibited the growth of *E. coli* O157:H7 on vacuum packaged beef loins. Further research needs to be conducted to determine the level of *L. plantarum* that inhibits *E. coli* O157:H7 without adversely affecting the sensory qualities of vacuum packaged beef.

CHAPTER SIX:
CONCLUSIONS AND RECOMMENDATIONS

The HAS system has been shown to be an effective tool for assessing hygienic practices at abattoirs, as statistically similar bacterial counts, i.e. to those at HAS + HACCP abattoirs, were recorded. However, to provide an increased assurance that pathogens are detected rapidly and corrective action taken, a HAS + HACCP system would be a preferred hygiene management system, which would also function as a base for a proper traceability system. In South Africa, presently, only a single abattoir is listed as an exporter of beef to the European Union. Strengthening of hygiene management systems could improve beef export opportunities.

The level of *P. fluorescens* and low storage temperature do not inhibit the survival of *E. coli* O157:H7 on beef. *E. coli* O157:H7 survives at levels that can cause foodborne illness regardless of the level of *P. fluorescens* present on meat. Slaughter animal farmers, therefore, need to strengthen preventive strategies to eliminate the entry of *E. coli* O157:H7 into the food chain.

Under vacuum packaging, the combination of the influence of the level of *L. plantarum* and low storage temperature inhibited the survival of *E. coli* O157:H7 on beef loins. The higher the cell suspension of *L. plantarum* is, the earlier the onset of the inhibition of *E. coli* O157:H7 survival on beef.

In South Africa, information on food poisoning outbreaks implicating *E. coli* O157:H7 is scanty. Yet there are reports (Ateba *et al*., 2008; GPDoA, 2004) illustrating that *E. coli* O157:H7 was isolated from patients as early as 1988 in Pretoria (Geyer, Crewe-Brown & Greeff, 1993). Moreover, a study carried out in 1993 at Dr George Mukhari Hospital (previously known as Ga-Rankuwa Hospital) in Pretoria revealed that the most prevalent organism in paediatric patients diagnosed with gastroenteritis was ETEC (Sooka, Du Plessis & Keddy, 2004). In addition, *E. coli* O157:H7 has been positively isolated from live animals (both commercial and

communal), their faeces and carcasses and even from ready-to-eat meat products (Ateba *et al*., 2008). The revelation that *E. coli* O157:H7 is prevalent in South Africa does not correspond with the lack of reported cases, a situation that could probably be attributed to inadequate surveillance for this organism in South Africa, where testing is still voluntary. Food regulatory authorities, such as the DoA in South Africa, need to enforce "zero tolerance" towards *E. coli* O157:H7 in raw meat to protect the entry of this pathogen into the domestic market and also to be on par with prospective international trading partners. Contamination of raw meat with *E. coli* O157:H7 is a vehicle of transmission into the food chain. Improved vigorous mandatory testing for VTEC from farm-abattoir to retail needs to be established so that areas that need more control can be identified, such as slaughtering *E. coli* O157:H7 shedding animals separately from non-shedding animals.

Abattoir owners need to invest in training of slaughter personnel to ensure that all workers including management take ownership of hygiene practices during animal slaughter and during further processing. Contamination of raw meat can occur as a result of inadequate cleaning of surfaces, equipment and hands of food handlers. Moreover, it has been reported that at some abattoirs, remuneration of slaughter operatives is illegally based on the number of cattle slaughtered per day. This practice encourages slaughter operatives to increase the line speed to achieve a desired number of cattle slaughtered but unfortunately compromises hygiene. A too high line speed may lead to spillages and cross contamination during evisceration.

One of the reliable means of ensuring safety of meat is thorough cooking. The South African meat industry needs to embark on awareness campaigns for consumers to ensure that raw meat is handled properly at all levels of the food chain. In cases of animals not slaughtered at registered abattoirs (ritual slaughter, slaughtering of animals for own consumption and traditional slaughtering for special occasions such as weddings) cattle are often transported in unsuitable vehicles, a condition that may result in stressed animals leading to increased shedding of pathogenic microorganisms. This situation may increase contamination of animal carcasses with pathogenic bacteria and cross contamination of ready-to-eat foodstuffs. In this regard,

awareness could be created if the relevant authorities worked closely with communities and encouraged the presence of environmental health practitioners to render meat inspection services at community level.