CHAPTER ONE:
INTRODUCTION AND PROBLEM STATEMENT

1.1 INTRODUCTION

Meat has been part of human diets for most of mankind's existence. Beef, is a high source of protein, which contains all the essential amino acids and iron. The iron found in meat is in the form of heme iron, which is better absorbed than the non-heme iron found in plants (Schnepf, 2007). Through the ages, a need existed for controlling the activities of producers, processors and manufacturers of food intended for human consumption. This need led to the development of food laws in South Africa (Department of Health, 2006). Furthermore, public awareness of meat associated health hazards, such as heart attacks (Erlinger & Appel, 2003) and fatal *E. coli* bacterial infections (Ethelberg, Smith, Torpdahl, Lisby, Boel, Jensen, Nielsen & Mølbalk , 2009) led to the development of stringent legislative requirements to ensure the supply of safe food products.

In the 1960s, there was a concern about poor hygiene at South African abattoirs. This inadequacy was addressed by creating the Abattoir Commission under the Abattoir Industry Act 54, 1976. This commission then developed hygiene norms and standards for abattoirs and became the only body with the authority to approve applications for building new abattoirs in South Africa (Department of Agriculture, 2007). As a result, the red meat industry became extensively regulated by the Abattoir Commission. The meat industry was characterized by the following: restrictions on the movement of meat between different areas, compulsory auctioning of carcasses according to grade and mass in controlled areas and supply control via permits and quotas (Jooste & van Schalkwyk, 1996). The South African meat industry has gone through many regulatory changes since 1994 (Dittmer, 1998; Red Meat Abattoir Association, 2006).

In South Africa, abattoirs are graded A, B, C, D or E according to throughput (number of animals slaughtered per day). For example, Grade A abattoirs have a throughput > 100 slaughter units, while grade E abattoirs have a throughput of one to eight slaughter units per day (DoA, 2007). To ensure standardized hygiene practices at all abattoirs, the DoA introduced the Hygiene Assessment System (HAS) in 1999. HAS is used to quantify the hygiene standards at abattoirs, against national standards.

The Gauteng Provincial DoA, Directorate: Veterinary Public Health (GPDoA: VPH) introduced a rating scheme, Abattoir Hygiene Rating Scheme (AHRS) in 2003 (Figure 1.1). Although participation in AHRS is voluntary, abattoirs that achieve high ratings receive special certificates and are made known to the public. AHRS ratings range from five stars (90 and above), four stars (75 to 89) and three stars (60 to 74). Abattoirs that score a rating below 60 do not receive any awards (DoA, 2003). The significance of a higher rating is the assurance that the products derived from such an abattoir are of high quality, with a longer shelf life from a hygiene point of view (RMAA, 2004). A rating awarded to an abattoir is valid for twelve months, after which the rating is reassessed.

South African abattoirs that export meat and meat products to the European Union are, in addition to HAS, are required to implement the HACCP system, based on European Union requirements (Van de Venter, 2000). The DoA allocates resident official state veterinarians to these abattoirs and the importing countries periodically carry out physical audits of the HACCP plans of these abattoirs.

1.2   PROBLEM STATEMENT

In 1992, the South African meat industry was deregulated leading to the Abattoir Industry Act 54, 1976 being repealed and the Abattoir Commission being disbanded. The removal of these restrictions brought about a major restructuring of the meat industry. For example, there was a rapid increase in the number of smaller abattoirs in the rural areas mostly on-farm facilities and transportation of livestock to abattoirs became less regulated and therefore easier, responding to the demand (Dittmer,

1998).The deregulation of the industry allowed wholesalers to source live slaughter animals directly from farmers or feedlots, assuming ownership of the animals before the animals are slaughtered. As a result, abattoir owners do not have control over the quality of animals presented for slaughter (RMAA, 2002). Most importantly, meat inspection became a private activity, allowing abattoir owners to employ their own meat inspection personnel (RMAA, 2002). This situation led to a decline in the management of hygiene practices, as adherence to stringent meat inspection procedures was compromised at abattoirs (RMAA, 2002).

To circumvent the declining control of hygiene practices at abattoirs during slaughtering, the RMAA offers training on good hygienic practices, good manufacturing practices and food safety systems at all registered abattoirs in South Africa. The European Commission recognizes the South African Bureau of Standards (SABS), as the competent certifying body for ISO/SANS including SANS 10330 which is a food safety standard. The HACCP certified abattoirs supply meat and meat products to both the export and the domestic markets. The use of different hygiene management systems at abattoirs may create a perception that fresh beef from abattoirs that use HAS + HACCP may be of superior microbiological quality compared to beef from abattoirs that use the HAS. Standardization of practices at abattoirs is further compounded by the lack of a single food safety system in the South African meat industry, as a result of the absence of a centralized food control government agency. Consequently, the regulatory approach is fragmented, located within a number of government departments and within the three tiers of government, namely, national, provincial and local (Jackson, 2006; Van de Venter, 2000). DoA, Directorate: Veterinary Services, is the competent authority for setting norms and standards pertaining to veterinary hygiene i.e. the minimum standards required for an abattoir to be registered, while the provincial and local governments are competent authorities for the enforcement of all legislative requirements.

Contamination of meat carcasses has pointed to many sources including abattoir workers (Wagude, 1999). In instances where, abattoir workers' remuneration is linked to the number of head of cattle slaughtered a day, workers tend to increase the

slaughter line speed (Wagude, 1999). A line speed that is too high implies inadequate time for slaughter operatives to carry out their functions, which may lead to increased chances of spillage during evisceration and inadequate sterilization of slaughter equipment leading to increased risk of contamination of carcasses. The generation of abattoir specific microbiological data can be used for training purposes and illustrations so that abattoir workers understand the link between their practices and meat safety.

Foodborne diseases commonly occur without being reported and South Africa is no exception. The first case of *E. coli* O157:H7 infection was reported in 1990 in South Africa (Browning *et al.*, 1990) followed by sporadic cases of bloody diarrhoea (Effler, Isaäcson, Arntzen, Heenan, Canter, Barret, Lee, Mambo, Levine, Zaidi & Griffith 2001; Galane & Le Roux, 2001). Low levels of *E. coli* O157:H7 have been isolated from meat and meat products in South Africa (Abong'o & Momba, 2009). Food security is more critical than food safety among both rural and urban poor. The lack of vigorous surveillance of food pathogens in South African meat and meat products presents a challenge for food hygiene trainers, as it becomes difficult to demonstrate the magnitude of contamination with these pathogens. There is a need to generate more data from abattoirs, supermarkets, street vendors and butcheries to ascertain the prevalence of *E. coli* O157:H7 in the meat industry and such information must be made available to the public.

Although little is known about consumer perception and awareness of food safety in South Africa, generally freshness of meat is often cited as one of the most influential variables impacting on consumers' decisions to purchase fresh meat (Glitsch, 2000).

It is assumed that there would be more control at abattoirs that use the HAS + HACCP system thereby reducing opportunities for contamination of carcasses during the process of slaughtering, resulting in carcasses of superior microbiological quality compared to carcasses from abattoirs that use only HAS. The aim of this study was therefore, to assess the effectiveness of the combination of the HAS and HACCP system in preventing the microbiological contamination of beef carcasses during

slaughter, as opposed to using HAS alone. *E. coli* O157:H7 has been isolated from faeces of slaughter animals at abattoirs, as well as in processed, ready to eat meat products in South Africa. As a result, the ability of spoilage microorganisms to inhibit the survival of *E. coli* O157:H7 on fresh meat, aerobically and under vacuum storage was also investigated.
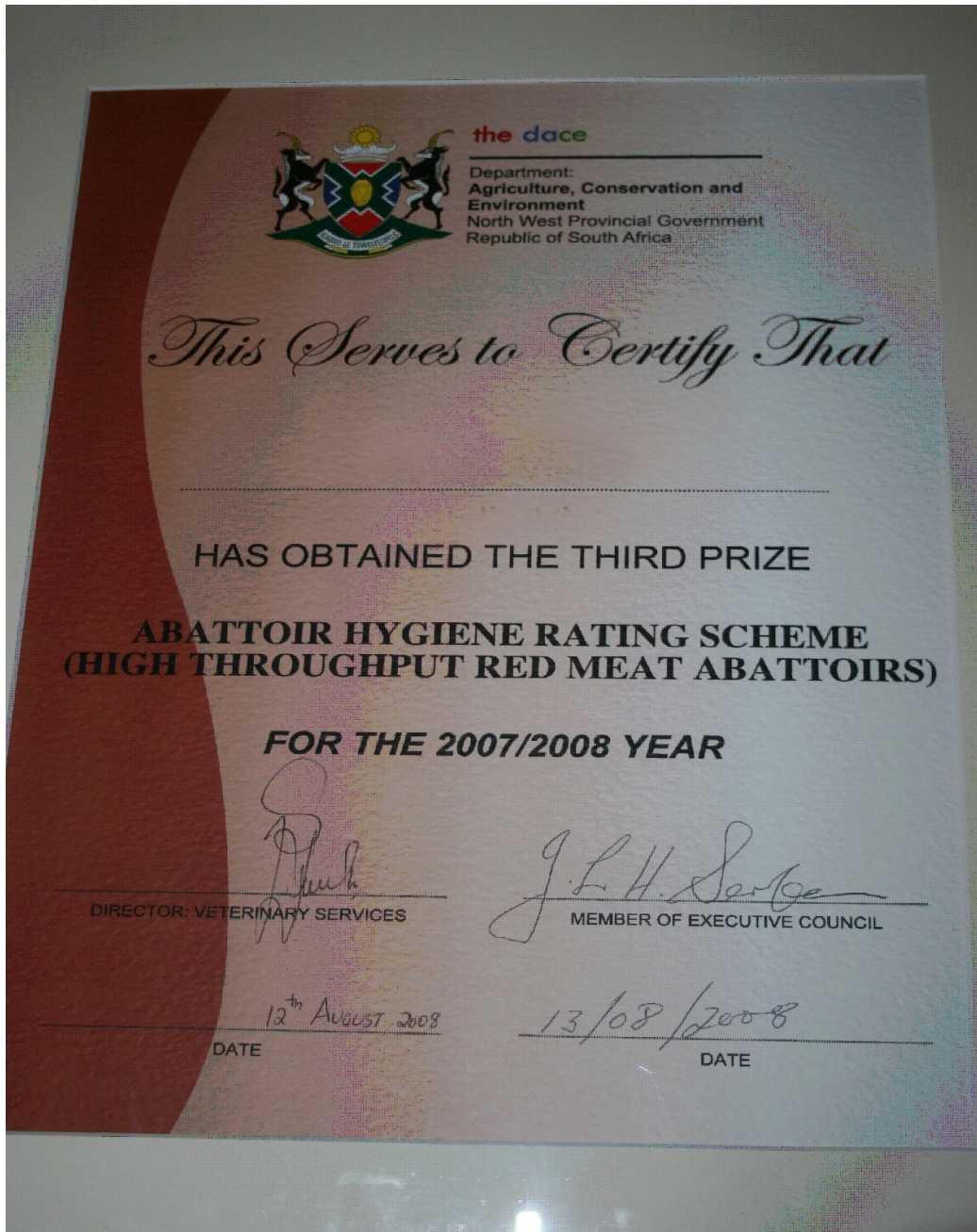
Figure 1.1:   Example of an abattoir hygiene rating scheme certificate

CHAPTER TWO: LITERATURE REVIEW

2.1 *E. COLI* O157:H7, A FOOD PATHOGEN

2.1.1 Origin of *E. coli* O157:H7

Escherichia coli were first isolated by a German paediatrician, Theodore Esherich, in 1884 from faeces of human neonates (Khan & Steiner, 2002). The bacterium was then called *Bacterium coli commune.* Most *E. coli* strains are harmless commensals, however, some strains are pathogenic and cause diarrhoeal diseases. *E. coli* strains that cause diarrhoeal illness are categorized into specific groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes and distinct O:H serogroups. These categories include enteropathogenic *E. coli* strains (EPEC), enteroinvasive *E. coli* strains (EIEC), diffuse adhering *E. coli* strains (DAEC), enteroaggregative *E. coli* strains (EAEC) and enterohaemorrhagic *E. coli* strains (EHEC).

E. coli O157:H7 and many *E. coli* produce verotoxins, hence they have been named VT-producing *E. coli* (VTEC). The first confirmed isolation of *E. coli* O157:H7 in the United States of America (USA) was in 1975 from a Californian woman with bloody diarrhoea, while the first reported isolation of the pathogen from cattle was in Argentina in 1977, while the bacterium was first identified as a human pathogen in 1982 (Fernandez, 2008). The spread of *E. coli* O157:H7 in North America coincided with the importation of infected cattle from Argentina, where the rates of human infection were previously about three times higher than those found in North America (McMichael, 2001).

E. coli O157:H7 is usually a harmless bacterial strain present in the intestines of cattle. Probably, the potentially lethal variant of *E. coli* O157:H7 evolved in Argentinian cattle reservoir when the harmless variant of the bacterium acquired a gene from the deadly *Shigella* bacterium through a viral agent, resulting in the lethal

form (Heymann & Rodier, 1997). The resultant strain soon became prevalent in key cattle-growing regions throughout North America. However, for a new bacterial strain to become an infectious disease, it has to be able to survive the media through which it is transmitted to humans (Whittam, McGraw & Reid, 1998). In the Walkerton, Canada waterborne outbreak of 2000, which affected more than 2,300 residents and resulted in seven deaths (Hrudey & Hrudey, 2000), the pathogen had travelled from the intestines of cattle, through the surface water pathways, through the soils into ground water pathways and through the constructed drinking water system to be ultimately consumed by humans, where the pathogen survived the hosts' gastric acid defences (Whittam *et al.,* 1998). Contaminated food and water cause 700,000 deaths in Africa annually (Kertesz, 2009).

2.2 RECORDED *E. COLI* O157:H7 ASSOCIATED FOODBORNE OUTBREAKS

E. coli O157:H7 outbreaks have been reported most frequently in developed countries and in over 30 countries on six continents (Boyle, Swerdlom & Griffith, 1995; Chapman, 1994; Griffith & Tauxe, 1991; Mead & Griffith 1998; Nataro & Kaper, 1996; Water, Sharp & Dev, 1991). In Africa, *E. coli* O157:H7 associated outbreaks have been reported in South Africa (Browning *et al.,* 1990), Swaziland (Isaäcson *et al.,* 1993) and in the Central African Republic (Germanii, Soro, Vohito, Morel & Morvan, 1997), Kenya (Sang *et al.,* 1996), Gabon (Presterl, Zwick, Reichmann, Aichelburg, Winkler & Kremsner, 2003, Nigeria (Olorunshola, Smith & Coker, 2000) and the Ivory Coast (Dadie, Karou, Adom, Kette & Dosso, 2000). The outbreak that occurred in Swaziland in November 1992 was the largest recorded in Africa (Effler *et al.,* 2001). The infection rate was 42% of 778 screened residents (Raji, Minga & Machangu, 2006), many of whom had bloody diarrhoea and abdominal pains which distinguished these patients from those who had cholera. Symptomic cholera infection almost always manifests as profuse watery diarrhoea (Effler *et al.,* 2001).

The major problem with *E. coli* O157:H7 is that it is not detected by the usual methods used to isolate and identify traditional enteric bacterial pathogens.

Moreover, microbiology laboratories in many African countries do not routinely test *E. coli* O157:H7. Hence, many infections may go unrecognized (Raji *et al.,* 2006). Raji *et al.* (2006) suggested three public health measures (educating the public on dangers of eating undercooked meat, increasing awareness among clinicians of *E. coli* O157:H7 infection and mandating case reporting) to control infections associated with *E. coli* O157:H7 in Africa. The impact of gastroenteritis is dramatic for developing countries where an estimated 2.5–3.2 million children <3 years die each year (Bresee, Widdowson, Monroe & Glass*,* 2002).

2.3 CHARACTERISTICS OF *E. COLI* O157:H7

2.3.1 Acid tolerance

Ground beef has been implicated as the major primary vehicle of the transmission of *E. coli* O157:H7 (Griffith & Tauxe, 1991). *E. coli* O157:H7 has been implicated in outbreaks associated with high acid products such as apple cider (Besser, Lett, Weber, Doyle, Barrett, Wells & Griffith, 1993), mayonnaise (Weagant, Bryant & Bark, 1994) and yoghurt (Morgan *et al.,* 1993). The safety of high acid products and the acid properties of *E. coli* O157:H7 have been of concern. Acid tolerance is both growth phase dependent and inducible. Cells in the stationary phase of growth are more acid tolerant than cells in the exponential phase (Arnold & Kasper, 1995). Arnold and Kasper (1995) demonstrated that acid tolerance of *E. coli* O157:H7 was not dependent on prior exposure to low pH, as cells of a strain that had been grown overnight at pH 7.2 survived acid challenges. Furthermore, Arnold and Kasper (1995) observed enhanced acid tolerance in starved, late lag and stationary phase cells compared to mid lag phase cells.

2.3.2 Antibiotic resistance

Studies have revealed that *E. coli* O157:H7 are resistant to antibiotics (Galland, Hyatt, Crupper & Acheson, 2001; Wilkerson *et al.,* 2004; Zhao *et al.,* 2001). In a recent

study conducted in the North West Province, South Africa, Ateba, Mbewe and Bezuidenhout (2008) determined the antibiotic resistance of 76 *E. coli* O157:H7 strains from three types of species (pigs, cattle and humans) to nine different antibiotics. Their results demonstrated the prevalence of multiple antibiotic resistances (MAR) and resistance to three or more different classes of antibiotics in 93.4% of the *E. coli* O157:H7 isolated. Ateba *et al.* (2008) recorded that 83.8% to 100% of *E. coli* O157:H7 isolates from pig faeces were resistant to tetracycline, erythromycin and sulphamethoxazole. Resistance to these antibiotics was influenced by the location of the sample, i.e. all samples from two areas were resistant, yet none of the *E. coli* O157:H7 isolates from a third location were resistant to tetracycline. Moreover, none of the isolates from cattle faeces obtained from any of the farms were resistant to either neomycin or norfloxacin. In addition, all *E. coli* O157:H7 isolated from humans were resistant to erythromycin, tetracycline, ampicillin and sulphamethoxazole. These authors suggested that rainfall runoffs could be the mode of transmission of antibiotic resistant *E. coli* O157:H7 strains in the North West Province, particularly in communal set-ups where animals were seen drinking rainfall water and pigs sometimes feed on human excreta. Resistance of *E. coli* O157:H7 may have negative clinical implications by hampering clinical treatment of *E. coli* O157:H7 infections.

2.3.3   Carriage of a 60-MDa plasmid

E. coli O157:H7 isolates associated with human illness harbour a plasmid (pO157) of approximately 60 MDa that contains DNA sequences common to plasmids present in other serotypes of VTEC isolated from patients with haemorrhagic colitis. The plasmid is believed to play a role in the pathogenicity of disease (Fernandez, 2008).

2.4   TRANSMISSION OF *E. COLI* O157:H7

Cattle are the major reservoir of *E. coli* O157:H7 (Ateba *et al.,* 2008) and shed this organism more frequently when they are stressed (Armstrong, Hollingsworth & Morris, 1996). The 1992 Swaziland *E. coli* O157:H7 outbreak was preceded by

several years of drought, which led to a situation where cattle were grazing closer to the remaining sources of surface water and vegetation. With the onset of heavy rains (Mead & Griffith, 1998), water contaminated with cattle faeces (and presumably *E. coli* O157:H7) would have become readily available to both livestock and residents downstream (Effler *et al*., 2001). In addition, meat from dead animals was available for residents to consume. It was reported that much of the beef from dead animals would have been grilled leaving the possibility that portions remained undercooked. It is well documented that most *E. coli* O157:H7 outbreaks have been associated with the consumption of raw or undercooked foods of bovine origin (Fernandez, 2008; Raji *et al*., 2006).

2.5   CLASSIFICATION OF FAMILY *PSEUDOMONADACEAE*.

The *Pseudomonadaceae* family covers one of the most diverse and ecologically significant groups of bacteria that are Gram-negative, rod-shaped and contain polar flagella. Members of the genus are found in large numbers in a wide range of environmental niches, such as terrestrial and marine environments, as well as in association with plants and products of animal origin i.e. milk and meat that may become contaminated with pseudomonads during collection, storage and handling (Gennari & Dragotto, 1992). This almost universal distribution suggests a remarkable degree of genomic diversity and genetic adaptation (Kristoffer, Binnewies, Willenbrock, Hansen, Yang, Jelsback, Ussery & Friis, 2008). *P. fluorescens* encompasses a group of non-pathogenic saprophytes that colonize soil, water and plant surface environments and form biofilms on abiotic and biotic surfaces (Toutain, Caiazza & O'Toole, 2004). However, due to the ability of *P. fluorescens* to form biofilms on surfaces (Sillankorva, Neubauer & Azeredo, 2008; Talsma, 2007), this organism has been implicated in disease outbreaks derived from contaminated medical devices (Gershman, Kennedy, Noble-Wang, Kim, Gullion, Kacica, Jensen, Pascoe, Saiman, McHale, Wilkins, Schoonmaker-Bopp, Clayton, Arduino & Srinivasan, 2008; Pappas *et al.*, 2006). *P. fluorescens* is a group of obligate aerobes, except for some strains that can utilize nitrate (NO_3) as an electron acceptor in place of oxygen (Hedgecock & Costello, 1962; Rediers, Vanderleyden & De Mot, 2007).

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P. fluorescens also secrete pyoverdin, a fluorescent yellow-green siderophore under iron-limiting conditions (Meyer, Geoffroy & Baida, 2002).

2.6 CLASSIFICATION AND PHYSIOLOGY OF LACTIC ACID BACTERIA

LAB constitutes a group of gram-positive, acid-tolerant, generally non-sporulating, non-respiring rods (*Lactobacillus* and *Carnobacterium*) or cocci (all other genera) that are associated by their common morphological, metabolic and physiological characteristics. LAB produces lactic acid as the major end product during fermentation of carbohydrates (Axelsson, 2004). The genera that comprise LAB are *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus* and *Streptococcus*, which form the core group, while *Aerococcus*, *Carnobacterium, Enterococcus, Oenococcus, Sporolactobacillus, Tetragenococcus*, *Vagococcus* and *Weissella* are considered principal LAB (Stamer, 1976). *Weissella* is the only LAB genus that includes both rods and cocci (Collins *et al.*, 1993).

The classification of LAB into different genera is largely based on morphology, mode of glucose fermentation, growth at different temperatures, configuration of the lactic acid produced, ability to grow at high salt concentrations and acid or alkaline tolerance, of nonaerobic habit, but aerotolerant (Axelsson, 2004). The genus *Lactobacillus* is the largest of the genera included in LAB. Lactobacilli are widespread in nature and many species have found applications in the food industry. For example *Lactobacillus plantarum* is used in the brewery industry.

2.7   MEAT SPOILAGE

Meat is one of the most perishable foods and its composition is ideal for growth of a wide range of spoilage bacteria. The composition of the meat spoilage flora is greatly influenced by the storage conditions such as temperature and the type of packaging material (Nychas, Drosinos & Board, 1998; Tsigarida & Nychas, 2001). Spoilage of aerobically stored meat is dominated by *Pseudomonas* spp. (Mayr, Margesin, Klingbichel, Hartungen, Jenewein, Schinner & Märk, 2003). Mayr *et al.* (2003)

observed 83 to 100% *Pseudomonas* spp. on aerobically stored meat after 11 days of cold storage. In another study, Ercolini, Russo, Torrier, Masi and Villian (2006) studied microbial spoilage under three different conditions of modified atmosphere packaging, air, (ii) 60% oxygen and 40% carbon dioxed (MAP 1) and (iii) 20% oxygen and 40% carbon dioxed (MAP2) and low storage temperature (5 °C). Spoilage of meat, which varied according to the packaging conditions, occurred between 7 and 14 days of storage. *Pseudomonas* spp. were identified as acting during beef storage in air, while *Pseudomonas* spp and *L. sakei* were the main species found during storage using MAP3. When the numbers of pseudomonads reach around 100 million per gram, at the meat surface, they produce a putrid odour and slime forms (Food Science Australia, 2003). The main defects in meat are off-odours, and off-flavours, but discolouration and gas production also occur (Borch *et al.,* 1996).

The shelf life of meat is considerably increased by vacuum packaging. When oxygen impearmable packaging is used, the growth of gram–positive, mostly LAB is favoured because of increased carbon dioxed levels and lowered oxidative–reduction potential (Borch, Muermans & Blixt, 1996). LAB grow slowly at low temperature. LAB could grow to 10–100 million per gram after about 6 weeks of storage, but do not produce signs of spoilage. After 14–16 weeks, bitter or liver-like flavours develop (Food Science Australia, 2003).

2.8   SOURCES AND CONTROL OF CONTAMINATION OF MEAT AND MEAT PRODUCTS

Red meat animals can be infected or carry a wide range of microorganisms, which are potentially pathogenic for man. The most important of these are zoonotic bacteria, principally pathogenic serotypes of *E. coli*, such as O157:H7, *Salmonella* and *Camplylobacter* spp. (Humphrey & Jørgensen, 2006). However, the presence of *Salmonella* or *Campylobacter* on food does not necessarily mean that infection will result. Many *Salmonella* serotypes common in food animals have not been extensively implicated in human infection, probably because they are less virulent compared to serotypes like *S. enteritidis* and *S. typhimurium*, or maybe they do not

survive in the food chain (Humphrey & Jørgensen, 2006). Risks associated with the consumption of contaminated meat and meat products can be reduced by implementing systematic controls from farm-to-fork. At farm level, strategies including the application of probiotics in animals and diet management may reduce the shedding of *E. coli* O157:H7 by slaughter animals.

2.8.1 Animals presented for slaughter

Slaughter animals may arrive at an abattoir positive for different pathogens. Humane treatment of food animals impacts on meat safety and should receive increased attention worldwide (Gradin, 2006). Animal stressing may damage meat quality and lead to more contamination and cross contamination with pathogens due to resultant increased pathogen shedding. Excretion levels of pathogens such as *E. coli* O157:H7 can be higher after transportation, which is associated with stress in animal hosts, leading to the spread of faeces containing high levels of pathogenic organisms on the live animal hide, with subsequent contamination of carcasses during slaughter (Sofos, 2008). Furthermore, during hide stripping, some bacteria originating from the animal hide become suspended in the abattoir atmosphere. This contaminated air may come into contact with food products, i.e. carcasses, containers, equipment and other food contact surfaces during processing, where they may adhere strongly (Sutton, 2004). Nevertheless, the major source of carcass contamination is contact with the skin during hide removal or contamination by spillage of stomach contents during evisceration (Humphrey & Jørgensen, 2006). Meat safety can be assured through the development of an integrated control of pathogens, including pre-slaughter strategies, post slaughter or during processing in the plant, at retail and food services and at home (Sofos, 2008). Therefore, the target of control pre-slaughter should be to minimize sources, levels, access and transfer of contamination to animals and produce.

Challenges at pre-slaughter include the diagnosis of animals as carriers of pathogenic organisms. Diagnosing cattle on the farm or the feedlot as being "infected" by pathogenic bacteria is difficult because pathogenic bacteria often have little or no

effect on the health or production efficacy of animals. Therefore such animals are asymptomatic (Sofos, 2008). Zhao *et al.* (1998) observed that all experimental animals inoculated with *E. coli* O157:H7 in their study remained clinically healthy, with no evidence of diarrhoea. Cattle have been found to be insensitive to the deleterious effects of the toxins produced by *E. coli* O157:H7 and other EHEC (Pruimboom-Brees, Morgan, Ackermann, Nystrom, Samuel, Cornick & Moon, 2000). Detection of *E. coli* O157:H7 is also complicated by the fact that faecal shedding can be sporadic, with an animal testing positive for EHEC one day, but not again for several days or even weeks (Callaway *et al.*, 2004; Sofos, 2008).

2.8.2 Abattoir waste

The abattoir industry is one of the industries that contributes to the problem of possible foodborne disease and potential health hazards associated with food especially meat, by improper handling of condemned material (Adeyemi & Adeyemo, 2007). Abattoir waste is defined as waste or waste water from an abattoir which could consist of pollutants such as animal faeces, blood, fat, animal trimmings, condemned organs and carcasses, paunch content and urine (Department of Water Affairs (DWAF), 2001), together with inedible organs. Inedible offal includes skin, ears, gall bladder, foetuses, hair, hooves, snouts and horns (DoA, 2007).

Abattoir waste material carries a high level of microorganisms that may cause disease in humans and animals, such as *Salmonella* and *Escherichia coli* bacteria (Brown, 2006). According to South African legislation (DoA, 2007; DWAF, 2002), abattoir waste has to undergo pre-treatment before it can be disposed of. The Red Meat Regulations of 2004 (DoA, 2007) stipulate that any condemned material must be disposed of by means of a total incineration, denaturing and burial at secure sites. The burial sites have to be approved by the Provincial Executive Officer and the local government, wherein access should be controlled to avoid illegal removal of condemned material. Furthermore, in cases where condemned material is sterilised, the sterilization facility has to be registered. However, enforcement of these regulations is questionable because raw abattoir waste including animal carcasses has

been found on landfill sites in some provinces of South Africa (Makana Municipality, 2004). Roberts, de Jager and Blight (2009) demonstrated that the majority of the abattoirs in their study did not adhere to the legislation. For example, in cases where condemned material was disposed of by burying, it was evident that it had not been burnt before burying as a legislative requirement. Furthermore, incineration was not properly achieved because in most cases both abattoir managers and workers failed to adhere to the required temperature for proper incineration of abattoir waste. Moreover, in cases where municipal landfill site was used, it was found that access was not controlled.

South Africa is an upper-middle-income country (Federation of International Trade Associations (FITA), 2010). Despite its wealth, the experience of the majority of South African households is either one of outright poverty or of continued vulnerability to becoming poor (Agbola, 2003). This situation forces poor communities to seek any possible food source which includes landfill sites, dumping grounds and disposable sites used by abattoirs (Derbyshire, 2003). This situation creates an opportunity for human beings (scavengers) living off refuse sites to consume condemned abattoir products and carcasses, which could result in significant health problems leading to possible disease and death (Roberts & De Jager, 2004). Hepburn, McRae and Ogden (2002) demonstrated that *E. coli* survives in abattoir waste products stored at a low temperature and increases in numbers at abusive storage temperature. In addition, Avery *et al.* (2009) showed that heat treatment (60 and 50 °C for 10 min) of abattoir waste that has been artificially inoculated with *E. coli* O157:H7 did not achieve a complete kill of the pathogen. Therefore, it is imperative for the South African meat industry to develop strategies and mechanisms that may ensure proper handling of abattoir waste. In addition, governmental policing strategies need to be improved.

2.8.3 On-farm control strategies

On-farm pathogen reduction programmes contribute to the control of food safety problems by reducing both the probability of pathogen presence and environmental

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pollution. Such control measures directly minimize water and produce contamination and direct animal-human transmission of pathogens (Sofos, 2008; Zhao, Doyle, Harmon, Brown, Muller & Parks, 1998). Different synergetic strategies can be used to reduce the levels of pathogens in live animals including the use of probacterial and antipathogen strategies and dietary and management strategies.

2.8.3.1 *Use of probiotics in animals*

Probiotics are defined as commensal (harmless or beneficial) bacteria used to reduce pathogenic bacteria in the gut (Fuller, 1989). Probiotics beneficially affect the host by improving its microbial balance. Such commensal organisms in the gut can be competitive or antagonistic to foodborne pathogenic bacteria. Probacterial strategies can be categorized into two groups: 1) the introduction of a "normal" (non-pathogen containing) intestinal microbial population (probiotics) or 2) providing a limiting substrate (prebiotic) that is not digestible by the host animal but which may allow an already existing microbial population to expand its niche in the gastrointestinal population. The concept of addition of an exogenous bacterial population to the intestinal tract is called Competitive Exclusion (CE). The CE culture may be composed of a single or multiple strains of a single bacterium, or even several different species of bacteria. The CE culture will limit the population of pathogenic bacteria by competing for limited nutrients, by binding sites along the gut epithelium or by producing toxic compounds (Crittenden, 1999; Nurmi, Nuotio & Schncitz, 1992; Steer *et al.,* 2000).

Zhao *et al.* (1998) carried out a study in which experimental animals were inoculated with probiotic bacteria and the control animals were not. These authors used *E. coli* strains, excluding *E. coli* O157:H7 for their probiotic population. Zhao *et al.* (1998) detected *E. coli* O157:H7 in rumen fluid of experimental animals for an average of 14 days post-inoculation with probiotic bacteria, whereas from the rumen fluid of control animals *E. coli* O157:H7 was detected for 26 days. Zhao *et al.* (1998) concluded that treatment of cattle with probiotic bacteria can reduce the level of carriage and faecal shedding of *E. coli* O157:H7 and may thereby reduce environmental contamination

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with *E. coli* O157:H7. In another study, Brashears *et al.* (2003) reported the effective use of *L. acidophilus* culture in the diet of finishing feedlot animals in decreasing *E. coli* O157:H7 shedding by more than 50%. Faecal shedding of *E. coli* O157:H7 is directly correlated with levels of carcass contamination (Elder *et al.,* 2000), emphasizing that the live animal is a critical link in the production chain.

2.8.3.2 Diet management effects on levels of E. coli O157:H7

Changes in feeding frequencies are associated with changes in the rumen microenvironment. Feeding cattle concentrated diets high in starch and soluble carbohydrates results in a more acidic rumen fluid, mainly because of increases in volatile fatty acids (VFA) (acetate, butyrate, and propionate) and lactate production by ruminal bacteria. In contrast, feeding cattle roughage diets high in cellulose, hemicellulose and pectin, results in only slightly acidic rumen fluid as a result of lower concentrations of VFAs (Church, 1979). Increased shedding of pathogens by animals has been linked to raising the fibre content of ruminant diets, fasting and other forms of stress (Nicholson *et al.,* 2000).

Cattle in the USA are often fed high-grain diets to maximize growth efficiency (Huntington, 1997). In South Africa, the feedlot industry produces approximately 75% of all beef (South African Feedlot Association, 2010) (Figure 2.1). Typically, grain finishing diets are high in energy and low in fibre. The abrupt switch of diets from high-grain diets to 100% hay-based diets resulted in a reduction of faecal populations (Diez-Gonzalez, Callaway, Kizoulis & Russell, 1998). Similarly, Keen, Uhlich and Elder (1999) compared a group of naturally infected cattle using high grain and hay as variables in their study. They recorded that of the grain-fed cattle 52% remained *E. coli* O157:H7 positive compared with 18% of the cattle abruptly switched to hay. Based on such comparative studies, it has been stated that "the most effective way of manipulating gastrointestinal counts of *E. coli* was to feed hay" (Gregory *et al.,* 2000). In contrast, Hovde, Austin, Cloud, Williams & Hunt (1999) observed longer *E. coli* shedding by hay-fed animals compared to grain-fed ones. In their study Hovde *et al.* (1999) exposed eight 1-to-2-year-old Holstein to different

diets: a typical finishing diet (82 to 90% grain); a medium-quality diet (100% alfalfa hay); and low-quality diet (100% timothy grass hay). After three weeks of adaptation to a particular diet, the animals were inoculated with *E. coli* O157:H7. Regardless of the diet, animals shed similar titers of faecal *E. coli* O157:H7. The average duration that grain-fed cattle were culture positive was 4 days, whereas cattle fed alfalfa or grass hay diets shed faecal *E. coli* for longer times, averaging 39 and 42 days, respectively. In addition, *E. coli* O157:H7 populations collected from the faeces of cattle in both dietary groups were equally acid tolerant. Animal rumen is a highly complex and competitive environment that allows the survival and proliferation of a specific anaerobic population, thereby creating a difficult environment for transient organisms to survive (Hungate, 1966). Thran, Hussein, Redelman and Fernandez (2003) reported that rumen pH as a function of fermentation of dietary components was less effective in decreasing numbers of *E. coli* O157:H7 entering cattle rumen. Thran *et al.* (2003) concluded that the reduction in *E. coli* O157:H7 was rather due to competitive exclusion by the rumen microorganisms.

Figure 2.1:   Feedlot animals during feeding and relaxation periods

2.8.4 Control measures at abattoir level

2.8.4.1 Animal coats in carcass contamination

Animal coats are a significant source of microbial contamination of the carcass (Hudson, Mead & Hinton, 1998). Many countries apply a subjective ordinal visual rating system to food animals presented for slaughter, categorizing animals by degree of coat cleanliness (Small, Wells-Burr & Buncic, 2005). Animals assessed as clean are normally processed for human consumption. However, research on cleanliness of the animal coat has demonstrated that visually clean cattle often carry pathogens, for example, *E. coli* O157:H7 and *Salmonella* spp (Avery *et al.* 2002; Small *et al.,* 2002) confirming that hides should always be considered as posing a serious risk to meat safety.

All operations during slaughter and dressing of food animals that involve penetration of the skin such as penetrative stunning of animals, also in South Africa, carry a risk of introducing pathogenic bacteria from the skin onto edible parts of the animal as penetrative stunning pistols are not sterilized between animals (Buncic, McKinstry, Reid & Anil, 2002). Buncic *et al*. (2002) demonstrated in their study investigating the role of the penetrative pistol in spreading microbial contamination to edible parts and the abattoir environment that microbial contamination association with penetrative stunning can spread from the brain to the edible parts of the same animal, including muscles via the blood circulation. In addition, Buncic *et al*. (2002) observed that positive detection of the marker organisms in their study varied between individual animals, between types of tissues/organs and between types of the marker organism. The authors then speculated that the variation could have been due to factors including: (a) differences in stunning-associated damage of the brain blood vessels between individual animals, which could cause different counts of the bacteria to enter the blood circulation, (b) differences in post-stunning rate and/or duration of the heart activity between individual animals, which could cause different transfer of bacteria through the animal bodies, and (c) difference in volume/mass of blood, tissue

and organs between individual animals, which could result in different "dilution" factors for the counts of the marker organisms. Blood and liver were most commonly contaminated (in 90% of animals), followed by the lungs and spleen (in 80% of animals), deep muscle (in 20% animals) and on the carcass surface (in 50% of animals). In this study penetrative stunning was also positively linked to the spread of contamination to the environment. The marker organisms were present in protective clothing samples collected from the slaughtermen conducting the stunning of animals and samples collected from the pelt (leg, breast and shoulder) of the stunned animals. Buncic *et al*. (2002) further demonstrated that penetrative stunning could spread contamination to subsequently stunned animals if equipment was not cleaned and sterilized between animals.

Small *et al*. (2005) evaluated the effects of pre-skinning hide decontamination on carcass contamination. They recorded a positive correlation between the microbial loads on skinned carcasses with those on the hide of the same animal. They concluded that pre-skin hide decontamination would reduce overall microbial loads introduced into the slaughter line environment and onto the dressed carcasses, and hence, improve meat quality and safety.

2.8.4.2   Effect of line speed on carcass hygiene

According to Roberts (1980), line speed may have serious implications in relation to carcass contamination. The faster the line operates, the more opportunities there are for mistakes to be made and hence for more contamination to occur. The relationship between line speed and carcass contamination is influenced by a large number of factors including operator fatigue, knife skills, length of working day, levels of boredom and the presence or absence of proper management structures such as HACCP. The most important aspect is whether or not the operatives have sufficient time to carry out their jobs. In some countries, the speed line is regulated by the number of carcasses that an inspector can examine in an hour (Roberts, 1980).

2.8.4.3   Abattoir workers

The hygienic status of dressed carcasses is largely dependent upon the general slaughterhouse hygiene and the skills of the workers (Mothershaw, Consolacion, Kadim & Ahmed, 2006; Rahkio & Korkeala, 1996. The interviews conducted by Mothershaw *et al.* (2006) revealed that 89% of workers at the abattoir where they conducted their study had no training in safe food handling, and as a result, personal hygiene standards were also found to be low. Furthermore, Desmarchelier, Higgs, Mills, Sullivan and Vanderlinde (1999) showed that the incidence of coagulase-positive staphylococcus (CPS) at one of the abattoirs in their study increased by 33.5% after evisceration compared to counts enumerated before evisceration. This increase corresponded to the heavy contamination of the hands of workers performing the evisceration task. Desmarchelier *et al.* (1999) found that the hands of 75% of workers at trimming of visible contamination step were contaminated with CPS. Therefore, Desmarchelier *et al.* (1999) concluded that workers' hands could have been a source of carcass contamination with CPS, hence a large increase in counts after chilling for 72 hours. The significance of workers' contribution to carcass contamination was also illustrated by Wagude (1999) who observed a great improvement in the microbiological quality of beef after training workers on sanitation, personal hygiene and hand washing techniques.

2.9   HYGIENE MANAGEMENT SYSTEMS AT ABATTOIRS

Cattle slaughter operations, such as bleeding, dressing and evisceration expose sterile muscle to microbiological contamination that is present on the skin, in the digestive tract and in the environment (Gill & Jones, 1999; Sofos *et al.,* 1999). With a view to reducing the risks associated with the presence of food pathogens on carcasses, the need to achieve standardized control systems, and the desire to access international markets, the South African meat industry approached the government for assistance. The DoA co-ordinated a task team representing South African Meat Industries Company (SAMIC), RMAA and GPDoA: VPH. This task team developed the

Hygiene Assessment System (HAS) based on the United Kingdom's evaluation/audit system. HAS became a mandatory requirement for all registered abattoirs in 2000 upon its inclusion in the Meat Safety Act 40 of 2000 (RMAA, 2008).

2.9.1 Hygiene Assessment System

In the South African context, HAS is a tool, that is used in conjunction with the hygiene management system (HMS) to improve hygiene standards at abattoirs. The assessments are carried out using a HAS form (Figure 2.2) by hygiene managers (HM) at abattoirs and verified by provincial veterinary public health (VPH) inspectors. Has is a visual inspection of monitoring the 10 criteria on the HAS form to assess compliance to Meat Safety Act 40, 2000 (DoA, 2009). In South Africa, all animals presented for slaughter at any registered abattoir are examined before slaughter, anter mortem inspection. Antermortem is the first line of defence in protecting the public from potentially harmful meat products by accepting and allowing for slaughter only those animals that are healthy and capable of being converted into wholesome products for consumers (Du Preez, 2009). HAS monitors hygiene and animal welfare status of all registered abattoirs in South Africa to maintain uniform standards of hygiene performance (DoA, 2009). Abattoir operatives at dehiding and evisceration are subjected to training offered by RMAA to ensure that bacterial populations from the hide and intestinal contents are prevented from being transferred onto animal carcasses. Some South African abattoirs that export meat and meat products to the European Union member states, in addition to the use of HAS, have also implemented the Hazard Analysis Critical Control Point (HACCP) system.

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ABATTOIR:_____ ABATTOIR NO: _____ GRADE :_____DATE: _____

DAILY THROUGHPUT: C _____P _____S _____ Other_____

INSPECTION COMPONENT

RANK	NAME	DESIGNATED		EMPLOYER
		YES	NO	
VETERINARIAN:				
MEAT INSPECTORS:				
MEAT EXAMINERS:				
MEAT CLASSIFIER				

OWNER / MANAGER

NAME	CAPACITY	ADDRESS / PHONE / FAX

HYGIENE MANAGER

NAME	CAPACITY	ADDRESS / PHONE / FAX

HAS - SCORE SHEET

CATEGORY	CATEGORY SCORE	WEIGHT	WEIGHTED SCORE	VERIFICATION BY PROVINCIAL INSPECTOR
A. ANTE MORTEM		.10		
B. SLAUGHTERING AND DRESSING		.20		
C. MEAT INSPECTION / MARKING		.15		Date:……………………………………
D. CHILLING / DISPATCH		.20		
E. OFFAL PROCESSING		.05		Signature:………………………………
F. SANITATION / PEST CONTROL		.10		
G. PERSONNEL		.10		Comments:…………………………………………
H. GENERAL CONDITIONS		.05		……………………………………………………
I. RECORDS		.05		……………………………………………………
FINAL SCORE				……………………………………………………

		EXCELLENT
100 95 90		
85 80 75		GOOD
70 65 60		FAIR
55 50 45		POOR
40 35 30		BAD
25 20 15 10 5		CRITICAL

A 10	B 20	C 15	D 20	E 5	F 10	G 10	H 5	I 5	FINAL SCORE

Figure 2.2: A HAS form used at South African abattoirs (Du Preez, 2009)

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2.9.2 Hazard Analysis Critical Control Point

HACCP was jointly developed in the USA by the Pillsbury Corporation and the United States Army Laboratories as a system that would provide a degree of certainty that food was free from pathogens and toxins (Crossland, 1997). HACCP identifies the potential hazards (physical, chemical or microbiological) in the process and then designs the process and control systems to minimize the risks. The implementation of an HACCP system is based on seven principles: conduct a hazard analysis; identify the Critical Control Points (CCP); establish the critical limits; establish monitoring systems; establish corrective action; establish documentation concerning all procedures and records appropriate to these principles and their application; and establish verification procedures (Codex Alimentarius Commission, 1997).

HACCP is a preventative control system wherein hazards are identified, critical control points (CCPs) are determined and the methods for control and compliance are clearly specified (Kinsella, Sheridan & Rowe, 2006). International Standards Organization (ISO) 22000 and most other HACCP guides, specify that there are other prerequisites necessary before HACCP plans should be developed, including appropriate sanitation and hygienic practices and assembly of a multidisciplinary HACCP team, identification of products , process flow diagram, and controls already practiced. The decision tree technique should be used to identify CCPs followed by the prescription of corrective measures that should be implemented to control biological hazards. Misidentification of CCPs in a HACCP plan may render the prescribed standard operating procedures ineffective, resulting in an HACCP system that may give variable and inadequate control over microbiological conditions of raw meat (Bryant, Brereton & Gill, 2003). Finally, the implementation of HACCP systems at abattoirs has to be preceded by the establishment of microbiological data specific to the abattoir for the objective assessment of risks. Wagude (1999) compared the levels of bacterial contamination before and after the implementation of HACCP at a South African abattoir. After the implementation of HACCP, the author reported a reduction in the incidence of *Staphylococcus aureus* and *Salmonella* spp. after the carcass splitting step and further reductions in *S. aureus* count after chilling.

However, total bacterial counts and *Escherichia coli* counts remained similar after the implementation of HACCP. The author attributed most contamination of carcasses to a lack of training of abattoir operatives. Wagude (1999) reported that the unhygienic practices were aggravated by the link between the number of animals processed and the renumeration of operatives that resulted in the line speed being high.

A foodborne outbreak in the USA in late 1992, which was linked to the consumption of undercooked beef patties, triggered the introduction of stringent regulatory requirements by the United States Department of Agriculture (Eustace & Vanderlinde, 1999). The Pathogen Reduction Final Rule, gazetted in 1996, required the implementation by all meat and poultry processing plants of sanitation standard operating procedures, the adaptation of HACCP programmes and sampling of carcasses for generic *E. coli* and *Salmonella* for HACCP verification as part of the Rule. Furthermore, performance standards for those organisms were established for most slaughter classes. Although the final rule does not mandate decontamination treatments (acid washes, chemical dehairing, steam vacuuming, trimming, hot water spray washes and steam pasteurization), a large number of USA meat packers use these decontamination interventions. According to Smulders and Greer (1998), one of the advantages of the application of organic acids is that residual antimicrobial activity is demonstrable over extended periods of storage.

Contrary to the USA, the European Union advocates strict control of processing hygiene, to ensure the safety of meat and meat products. The European Union authorities view the inclusion of decontamination intervention as a way of masking evidence of inadequate hygienic processes (Eustace & Vanderlinde, 1999). As a result, exporting countries to the European Union, including South Africa could not use chemical decontamination interventions during animal slaughter, dressing and processing of primal cuts. However, according to the European Directive EC/471/2001, all red meat slaughterhouses within the European Community are obliged to operate according to HACCP principles (European Union, 2001). As a result of food crises and international trade requirements, the international community has had to review its food safety laws, with the sole intention of providing the highest

level of protection of human health (Hugas & Tsigarida, 2008). Similarly, at European level, Regulation No. 853/2004, which was brought into force in 2006, permits the use of substances other than potable or clean water to remove microbial surface contamination from foods of animal origin. However, the European Commission authorises the use of such substances only after the European Food Safety Authority (EFSA) has provided chemical and microbiological risk assessments.

2.10 COMMON CONTROL MEASURES USED AT HAS ALONE AND HAS AND HACCP ABATTOIRS IN SOUTH AFRICA

The South African meat industry employs trimming of visible contamination with sterile knives and chilling to control microbial growth on carcasses (RMAA, 2002). As an additional measure, one abattoir also uses spot steam vacuuming to remove localized faecal and ingesta contamination on carcasses.

2.10.1 Hot water sanitation of slaughter equipment

One common practice at most meat facilities, including those in South Africa, is to sanitize meat-cutting equipment (knives, neck splitters, bung tiers and saws) by dipping it into containers of hot water (82 °C) adjacent to processing lines to reduce the carcass-to-carcass spread of pathogenic and spoilage bacteria. Gill and McGinnis (2004) demonstrated the potential of tools used for carcass dressing to contaminate carcasses during slaughter and dressing. However, the presence of organic materials on slaughtering equipment reduces the antimicrobial activity of hot water. Hot water tends to coagulate protein, which allows organic material to adhere to equipment surfaces and leads to a greater difficulty in removing meat residues. Taormina and Dorsa (2007) found that brief (1 s) dip treatments of slaughter equipment had limited efficacy, compared to longer immersion time (5 s).

2.10.2 Trimming of visible contamination

During animal slaughter, contaminated carcasses are transferred from a processing to a detaining rail where visible contamination is removed a procedure called trimming. Trimming is an on-line process used to remove excess fat, small faecal spots and smears from beef (Sheridan, 2007). Trimming, which removes enteric pathogens associated with the contaminating matter (Bacon *et al.* 2000), is followed by visual inspection to ensure that contamination has been adequately removed after which the trimmed carcasses are returned to the processing line. Since, in South Africa, decontamination treatments such as acid washes are not used visible contamination is removed by trimming, followed by cold-water washing. Subsequently, carcasses are sent to the coolers. Gill and Landers (2004) documented the effectiveness of the trimming of visibly contaminated carcasses on the reduction of both total bacterial counts and of *E. coli* counts on beef carcasses.

2.10.3 Steam vacuuming

Steam vacuuming uses steam and/or hot water to loosen soil and kill bacteria. Then the application of a vacuum removes the wastewater and contaminants (Bolton, Doherty & Sheridan, 2001; Meat Industry Services, 2006). Steam vacuuming equipment is a hand-held device consisting of a vacuum wand with a hot spray nozzle delivering water at 88–94 °C to the carcass surface under pressure while simultaneously vacuuming the area (Dorsa, Cutter & Siragusa, 1996). Steam continually sanitises the device (Bolton *et al*., 2001). According to Kochevar, Sofos, Bolin, Reagan and Smith (1997), steam vacuuming reduced the mean aerobic plate counts (APC) and total coliform counts (TCC) by 0.72 and 0.26 $\log_{10}$ cfu/cm^2 respectively, from carcasses which did not have visible faecal contamination. A combination of knife trimming and steam vacuuming showed a higher reduction of the mean APC and TCC from visibly contaminated carcasses, by 1.38 $\log_{10}$ cfu/cm^2 and 1.59 $\log_{10}$ cfu/cm^2, respectively.

The effectiveness of steam vacuuming depends on the diligence of application of the operative and the operational status of the equipment. For example, Kochevar *et al.* (1997) observed increased reduction in APC after the operatives had been sufficiently trained in operating the steam-vacuuming device. Moreover, the curvature of some surfaces may make proper contact with the vacuum head difficult which can reduce the effectiveness of the treatment. Furthermore, at least 10 s is required for a pasteurising effect and operatives on-line may not have sufficient time for the job (Bolton *et al.*, 2001). Bolton *et al.* (2001) recommended critical limits for steam vacuuming systems (Table 2.1).

Table 2.1:   Critical limits for steam vacuuming systems (Bolton *et al.*, 2001)

Water temperature	$\geq$82 °C
Water pressure	3.4–10.3 Pa
Air vacuum	-0.093 Pa
Steam pressure	20.7–34.5 Pa
Area decontaminated	No more than 2.5 cm^2

2.10.4 Chilling

During animal slaughter, carcasses are pl aced in the chillers immediately after the final wash until the temperature of the deep round reaches 7 °C or lower to retard bacterial growth. Carcass chilling controls bacterial growth via extrinsic (temperature, relative humidity (RH), air speed and carcass spacing) and intrinsic factors water activity (a_w). Chilling is monitored by checking the deep round temperature of a number of randomly selected carcasses per rail in the chillers. There have been different reports on the effectiveness of chilling in controlling bacterial growth on beef carcasses. According to McEvoy, Doherty, Sheridan, Thomson-Carter, Garvey, McGuire, Blair and McDowell (2003), chilling is effective in controlling the growth of bacterial pathogens on beef carcass surfaces. In their study, three carcasses tested positive for *E. coli* O157:H7. After chilling, McEvoy *et al.* (2003) observed a change from positive for *E. coli* O157:H7 before chilling to negative after chilling on one beef carcass, whereas, with the other two carcasses,

there was an increase in the prevalence of *E. coli* O157:H7. These authors suggested three reasons for the negative result, namely, the irreversible attachment of bacteria to meat surfaces, the effect of chilling and probably the failure of the sampling method to recover the organism from the meat surface.

Chilling may stress bacterial cells because of the synergistic effect of low water activity and temperature. The low detection of *E. coli* O157:H7 from carcasses after chilling could be a subject of the analytical method used. For example, the presence of bile salts and antibiotics in the enrichment media (Hara-Kudo, Ikedo, Kodaka, Nakagawa, Goto, Masuda, Konuma, Kojima & Kumagai, 2000; Stephens & Johnson, 1998) may inhibit the growth of injured cells, resulting in the false presence of *E. coli* O157:H7. Nevertheless, McEvoy *et al*. (2003) observed an increase in *E. coli* O157:H7 prevalence on carcasses after chilling, which they conclude was a result of cross-contamination during chilling. In another study, Kinsella *et al.* (2006) did not find chilling effective in reducing the levels of artificially inoculated *Salmonella typhimurium* on beef after 24 h chilling. These authors recorded only 1 log or greater reduction in *S. typhimurium* count after 72 h. In general, the maximum chilling period of carcasses is 24 h to avoid weight loss (Kinsella *et al.,* 2006).

2.11 COMPETITION BETWEEN SPOILAGE AND FOOD PATHOGENS DURING BEEF STORAGE

2.11.1 Bacterial attachment to meat

Meat is a complex food ecosystem of which the chemical and physical properties can allow the colonization and development of a great number and variety of organisms (García-López, Prieto & Otero, 1998; Holzapfel, 1998). Bacterial attachment is probably the first step in the contamination of solid surfaces. Attachment is generally considered to be a two-stage process. The first stage is reversible attachment that occurs when the bacteria are trapped in a water film on the contact surface. The second stage is irreversible attachment that occurs as the bacteria form a more permanent physical attachment (Davies & Geesey, 1995). Benito *et al.* (1996)

demonstrated that attachment of LAB to beef muscle was influenced by both the immersion time and the cell concentration in the adhesion medium. Interestingly, with *E. coli* O157:H7, attachment to beef surfaces was rapid, occurring within the first few minutes of incubation with little increase in attachment occurring when exposure times were extended (Fratamico, Schultz, Benedict, Buchanan & Cooke, 1996). Bacterial surface structures that assist their adhesion to surfaces include outer membrane proteins, capsular polysaccharides, lipopolysaccharides, curli, non-hemagglutinating pili, fibrillae and flagella. However, Chen, Rossman and Pawar (2007) did not observe major differences in attachment by curliated and noncurliated cells of *E. coli* O157:H7 to beef.

2.11.2 Factors influencing growth of bacteria on meat

The shelf life of meat depends on the number and type of bacteria initially present and their further growth in the ecological conditions applied during storage, particularly the storage temperature, pH and gaseous atmosphere (Russo *et al.*, 2006). Meat stored aerobically at chill temperatures is dominated by *Pseudomonas* spp. (Dainty & Mackey, 1992; Gill & Newton, 1978), while the typical microbiota of vacuum-packed fresh meat products stored at chill temperatures consists of LAB and Enterobacteriaceae at levels of 10^8 and 10^6 $\log_{10}$ cfu/g, respectively (Sutherland, Patterson & Murray, 1975).

When different microbial species live in the same environment they certainly influence each other and in the case of meat, the interactions between microbial groups during storage play an important role in both the development and prevention of spoilage. This antagonistic activity is defined as antibiosis. Russo *et al.* (2006) demonstrated antagonistic activity of LAB against *Brochothrix thermosphacta* in vitro at 5 °C for 7 days. The authors concluded that the LAB antagonistic activity was not due to bacteriocins but rather to the effect of decreased pH and competition for substrate, as substrate competition and antagonism are important in the selection of microflora in any given ecological niche (Fredrickson & Stephanopoulos, 1981).

2.12 BACTERIAL INTERACTIONS ON MEAT

Competition between microflora is an effective way to preserve food and guard against pathogenic microbial growth (Sun & Ockerman, 2004). The inhibition of pathogens can be due to the production and excretion of substances that are inhibitory or lethal to other microbial cells, competition for attachment/adhesion sites, rendering the environment unfavourable/undesirable to other microorganisms, competition for oxygen and other nutrients or combinations of the above (Raghubeer, Campbell & Meyer, 1994). Jay (1996) suggested that low levels of background organisms do not have much influence on pathogens. Jay (1996) hypothesized that increasing levels of background flora may inhibit the survival of pathogenic microorganisms on meat due to microbial interactions among these microorganisms. Jay (1996) suggested protecting carcasses with appropriate mixtures of harmless bacteria, which may compete by occupying attachment sites, thereby reducing colonization by pathogens.

2.12.1 Interactions between *P. fluorescens* and *E. coli* O157:H7 when grown together on foods

Pseudomonas species is a spoilage causing bacterium present in a variety of food environments and foods (Robinson, Batt & Patel, 2000; Shaw & Latty, 1982). In the dairy industry, *P. fluorescens* is one of the most commonly isolated psychrotrophic bacteria that dominate the microflora of pasteurised milk at the time of storage (Ogier, Son, Gruss & Tailliez, 2002; Reddy, Bills, Lindsay, Libbey, Miller & Morgan, 1968). *E. coli* has been used as an indicator of pathogenic bacteria in beef products (Ray, Johnson & Field, 1984) and is also used as a faecal indicator because of its close association with intestinal waste materials (Beerens, 1998).

Pseudomonas spp. use several competitive mechanisms including bacteriocin production i.e. pyocin from *P. aeroginosa*, a phenazine antibiotic and non-nitrogen containing compounds and siderophore-mediated competition for iron (Henry, Lynch & Femor, 1991; Leong, 1986). They preceded other meat borne bacteria in the utilization of glucose, amino acids and lactic acid on fresh meat medium (Gill &

Newton, 1978). Sun and Ockerman (2004) used different levels (zero, 2 and 4 $\log_{10}$ cfu/ml) of *P. fluorescens* to investigate its antagonism and inhibition of *E. coli* on sterile ground beef. These authors used similar levels of *E. coli* and the inoculated samples were incubated at 4 and 25 °C for 14 days and 30 h, respectively. *P. fluorescens* showed an extended lag phase whereby growth was observed only at day 7 for 2 log treated samples and at day 10 for 4 log treated samples.

2.12.2 Interactions between LAB and *E. coli* O157:H7 when grown together on foods

LAB are known to have inhibitory effect against the growth of a wide range of foodborne pathogens (Lewus, Kaiser & Montville, 1991). Kalalou, Zerdani and Faid (2010) investigated the interaction between LAB and *E. coli* O157:H7 on camel meat. In their study these authors observed the growth of *E. coli* O157:H7 when inoculated alone and when inoculated with LAB on vacuum packaged camel meat stored at abusive temperature (10 °C) for 4 days. The LAB counts of inoculated samples dropped followed by the development of spontaneous lactic flora. Towards, the end of vacuum storage, *E. coli* counts were lower than those in the control (Lewus *et al.,* 2010).

E. coli O157:H7 has been associated with produce in 21% of the foodborne outbreaks that occurred between 1982 and 2002 (Amêzquita & Brashears, 2002). In the mid 1990s, fresh produce was recognized as a vector for foodborne illnesses caused by *E. coli* O157:H7 (Deliquis & Dinu, 2007). Gragg, Brook and Brashears (2010) compared the efficacy of LAB and water on the inhibition of *E. coli* O157:H7 on fresh spinash stored at 7 °C for 24 hours. Water alone reduced the level of *E. coli* O157:H7 by 0.88 $\log_{10}$ cfu/g, while the LAB cocktail showed a reduction of 1.03 $\log_{10}$ cfu/g. Gragg *et al*. (2010) concluded that LAB treatment was the most effective intervention in controlling *E. coli* O157:H7 in fresh produce.

2.13 INFLUENCE OF STORAGE CONDITIONS OF MEAT ON ITS MICROBIOTA

Meat is a good support of bacterial growth due to its composition: 75% water and many different metabolites such as aminoacids, peptides, nucleotides and sugars (Labadie, 1999). It is, however, a relatively poor source of sugar for bacteria, but an important source of proteins (Labadie, 1999). The types and proportion of bacteria growing on meat during storage result from the type of contamination introduced by the processing of meat and from the physico-chemical factors applied during storage. These factors (temperature, pH, nutrients, A_w and composition of the atmosphere) constitute hurdles that play a crucial role in the activity and growth of microorganisms. Following attachment, packaging influences the microflora of meat during storage (Chenoll, Macián, Elizaquível & Aznar, 2007).

2.13.1 Microbiota of aerobically chilled stored beef

In aerobically stored meats, milk, fish and vegetables, members of the *Pseudomonas* genus are the microorganisms that rapidly dominate the flora. Although the species that grow on different food products are often different they share important common features (Labadie, 1999). When bacteria coexist within a given ecological niche, substrate competition and antagonism are important in the selection of the resultant microflora (Gram, 1993). Gram (1993) demonstrated the antibacterial activities of 209 *Pseudomonas* strains isolated from spoiled and fresh fish in agar assays against organisms including food pathogens. The antimicrobial activity was more pronounced in strains that produced siderophores. However, the addition of iron to the substrate resulted in the elimination of antibacterial activity of two-thirds of those strains. Therefore, the inhibitory activity of those strains may have been siderophore-mediated competition for iron.

In another study Jurkevitch, Hadar and Chen (1992) suggested that the sequestering of iron by *Pseudomonas* spp. was an advantage to other bacteria that were able to utilize the siderophores.   For example, Gram (1993) observed dense growth of *L.*

monocytogenes and *S. aureus* around the wells containing *Pseudomonas* spp. Gram (1993) then suggested that antagonistic *Pseudomonas* spp. could have created a more advantageous nutritional composition, either through the supply of iron (Jurkevitch *et al.*, 1992) or by increasing the availability of low-molecular weight nutrients (Cousin & Martha, 1977). In addition, even among the *Pseudomonas* species competition exists. For example, *Pseudomonas fragi* does not synthesize pyoverdine (the green fluorescent pigment) but is able to synthesize its receptors on the outer membrane. This feature enables *P. fragi* to compete for *sidorephore* produced by the growth of other *Pseudomonas* species (Labadie, 1999).

2.13.1.1 *Effect of storage temperature on the competitive activity of P. fluorescens on beef*

Low storage temperature increases the lag phases of both spoilage and pathogenic bacteria on meat (Sun & Ockerman 2004). Sun and Ockerman (2004) investigated the influence of different levels of *P. fluorescens* on the growth of *E. coli* O157:H7 when co-cultured on sterile meat. These authors used different inoculation levels (zero, 2 and 4 cfu/ml) for both bacteria and incubated the inoculated samples under 4 and 25 °C for 14 days and 30 h, respectively. Under low temperature storage (4 °C), Sun and Ockerman (2004) observed an expected interaction between storage time and the *P. fluorescens* level in the inoculum. *P. fluorescens* count increased as storage period increased. The capacity of *P. fluorescens* to grow faster than their competitors at temperatures below 2 and 15 °C is due to their ability to utilize glucose, amino acids and lactic acid (Gill & Newton, 1978). However, Sun and Ockerman (2004) also observed a slow growth rate by *P. fluorescens* with significantly ($p < 0.05$) higher counts enumerated at day 7 for 2 log *P. fluorescens* group and at day 10 for log 4 *P. fluorescens* groups. The authors concluded that the extended lag phase was due to low incubation temperature. For the first 7 days of storage at 4 °C, *E. coli* counts remained the same for samples treated with both 2 and 4 $\log_{10}$ cfu/ml.

The *E. coli* O157:H7 growth curve showed a slight decline at day 7, followed by an increase in growth recorded at day 14. Low temperature could have been responsible

for the lack of growth shown by *E. coli* while decline in growth at day 7 could have been due to limited nutrient availability (Sun & Ockerman 2004). *P. fluorescens* have the ability to change their metabolism to utilize amino acids and lactic acid upon the depletion of glucose (Gill, 1996), therefore increasing availability of nutrients for *E. coli* to utilize. Moreover, oxygen could have been depleted due to high numbers of *P. fluorescens* recorded at day 14, creating a favourable environment for *E. coli*. Hence there was a recorded subsequent increase in *E. coli* counts at day 14.

At higher storage temperature (25 °C), *P. fluorescens* counts increased and reached 10^7 $\log_{10}$ cfu/cm^2 by 20 h, while there was a gradual increase in the growth of *E. coli* O157:H7. The level of *P. fluorescens* in the inocula enhanced the growth of *E. coli* on ground beef stored at 25 °C by way of degrading meat, therefore providing available nutrients for *E. coli* O157:H7 (Jurkevitch *et al*., 1992). Gram (1993) observed that the addition of iron did not eliminate the antibacterial activity of all siderophore producing *Pseudomonas* spp. Therefore, Gram (1993) concluded that the activity of such strains could be attributed to the production of other bacterial inhibitors such as antibiotics or bacteriocins.

2.13.2 Micro biota of vacuum packaged chilled stored beef

Meat and meat products are frequently commercialized by packaging under vacuum or in modified atmosphere to extend their shelf life. Hurdle technologies are frequently used to extend the shelf life of meat. For example, the combination of vacuum packaging and chilling produces an environment where only those organisms able to grow at low temperatures in the absence of oxygen can grow (Jones *et al.*, 2008). Storage under vacuum restricts the oxygen supply, depending upon the permeability of packaging film and thus has a selective effect on the microbial population (Labadie, 1999). As the composition of the gaseous phase changes during vacuum storage, the microbiota undergoes selection towards CO_2-tolerant growing species such as *Carnobacterium*, *Lactobacillus* and *Leuconostoc* species (Chenoll *et al*., 2007). Chenoll *et al.* (2007) did not detect LAB from samples analysed close to the date of packaging, but towards the end of storage LAB counts reached 10^8 $\log_{10}$

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cfu/g. Similarly, Jones (2004) had also observed an increase in LAB count at the end of vacuum-packaged storage (16 weeks) compared to the beginning of storage. Furthermore, Jones (2004) observed succession dynamics of LAB on chill-stored vacuum-packaged beef, which was correlated with the production of fermentation products. This author concluded that succession dynamics could be used in developing strategies to improve meat storage properties (Jones, 2004). LAB competes by producing metabolites that may inhibit the growth of their competitors (Ouwehand & Vesterlund, 2004).

2.13.2.1 Low molecular weight acids, lactic and acetic acids produced by LAB

LAB organisms have the ability to inhibit the growth of spoilage bacteria, and occasionally pathogens such as *S. aureus* and *L. monocytogenes* (Schillinger & Lücke, 1989; Stiles & Holzapfel, 1997), yet they also contribute to flavour development and the preservation of fermented sausages (Hammes, 1986; Liepe, 1983). In most cases, the formation of starter-derived inhibitors such as lactic and acetic acids from carbohydrates and the resulting decrease in pH are responsible for the antagonistic effect (Schillinger & Lücke, 1989) coupled with competition for nutrients (Ray & Daeschel, 1992). Fermentation reduces the amount of available carbohydrates and results in a range of small molecular mass organic molecules that exhibit antimicrobial activity, the most common being lactic, acetic and propionic acids (Blom & Mörvedt, 1991). Thus, the type and amount of fermentative products produced differ among LAB species. For example, in an anaerobic environment, homofementative LAB produces only lactic acid from glucose whereas heterofermentative species produce a variety of organic acids, ethanol and carbon dioxide (Stamer, 1976). LAB is used widely as starter cultures in the manufacture of dairy, meat and vegetable products.

When a mixture of acids is present in a food ecosystem, lactic acid contributes mainly to the reduction in pH, while acetic and propionic acids, which become dissociated, are the actual antimicrobial agents. Furthermore, in the presence of oxygen (O_2), LAB also generates hydrogen peroxide (H_2O_2), carbon dioxide and diacetyl. The

bactericidal effect of H_2O_2 is due to the scavenging of O_2 during some H_2O_2 producing reactions, which creates an anaerobic environment that is unfavourable for certain organisms (Egan, 1983; Ouwehand & Vesterlund, 2004).

2.13.2.2 Other low molecular weight antimicrobial substances produced by LAB

LAB produce a variety of antagonistic factors that include metabolic end products, antibiotic-like substances and antibacterial proteins called bacteriocins (Cleveland *et al.*, 2001; Klaenhammer, 1988) and protect themselves from desiccation, bacteriophages and protozoan attack by the production of exopolysaccharide substances (Axelsson, Chung, Dobrogosz & Lindgren, 1989). Bacteriocins are ribosomally synthesized, a heterogeneous group of biologically active proteins or protein complexes. They display bactericidal activity towards closely related species (Oliveira, Oliveira & Glória, 2008; Tagg, Dajan & Wanamaker, 1976). Bacteriocins produced by LAB differ in molecular weight, genetic origin and biochemical properties (Abee, Kröckel & Hill, 1995) and are sensitive to proteolytic enzymes such as pepsin and trypsin.

When proteolytic enzymes are added to bacteriocins, the latter lose their inhibitory effect (Chang, Lee & Chang, 2007; Lash, Mysliwiec & Gourama, 2005). The mode of action of bacteriocins may include the disruption of the membrane integrity of the target organism (Kramer *et al.*, 2004) or the disruption of enzyme functions (Kussendrager & Van Hooijdank, 2000). For example bacteriocins can inhibit the cell wall synthesis of the target cell (McAuliffe, Ross & Hill, 2001). Class IIa bacteriocins show strong antilisterial activity. They have a narrow inhibitory spectrum and thus do not kill starter cultures (O'Sullivan, Ross & Hill, 2002). Class III bacteriocins have only been isolated from members of the genus *Lactobacillus* (Klaenhammer, 1993).

2.13.3 Production of bacteriocins by *L. plantarum*

L. plantarum produces an antibacterial agent that inhibits the growth of some gram-negative bacteria including *E. coli* and gram-positives including *S. aureus* (Fricourt, Barefoot, Testin & Hayasaka, 1994; Lash *et al*., 2005). Lash *et al*. (2005) confirmed that the inhibitory substance produced by *L. plantarum* was a bacterion. These authors assessed the antimicrobial activity of that compound against a range of bacteria representing both gram-positive (*Staphylococcus aureus*, *Listeria innocua*, *Staphylococcus epidermidis*, *Micrococcus luteus)* and gram-negative bacteria (*E. coli, P. aeruginosa*, *Serratia marcescens*, *Shigella flexneri* and *S. typhimurium*) and also determined the effects of both changes in temperature and pH, its molecular weight and the effects of proteolytic enzymes on the inhibitory substance.

Lash *et al.* (2005) observed a greater inhibition of gram-negative, measured as more than 90% compared to the control, an unusual observation because inhibitory effects of gram-positive strains on gram-negative bacteria are less prevalent. In addition, the antimicrobial agent also inhibited Gram-positives although to a lesser extent at less than 90% compared to Gram-negatives. This compound exhibited a wide-range inhibitory effect on non-taxonomically related species including food-borne pathogens. *L. innocua*, used in the food industry as an indicator of potential *L. monocytogenes* contamination was also inhibited (Lash *et al*., 2005).

The characterization of this compound revealed that its inhibitory effect was heat labile, losing activity when the temperature was raised to 30 °C, and also lost at pH values below 4 and above 5, indicating a narrow pH range for inhibitory activity. Lash *et al*. (2005) established that the inhibitory compound had a large molecular weight, which they used to explain both the heat sensitivity and narrow pH activity. These authors suggested that due to the size of the compound, it could have been more rapidly degraded by subtle changes in temperature and pH.

2.13.4 Activity spectrum of bacteriocins

Major classes of bacteriocins produced by LAB include: lantibiotics, small heat stable peptides, large heat labile proteins and complex proteins whose activity requires the association of carbohydrate or lipid moieties (Klaenhammer, 1993). Bacteriocins of LAB are generally considered to be active only against gram-positive bacteria and not gram-negative ones and yeasts (Abee *et al*. 1995; Jack, Tagg & Ray, 1995; O'Sullivan *et al*., 2002). The inability of bacteriocins to act against gram-negative bacteria could be due to the fact that gram-negative bacteria possess an additional layer, the outer membrane (OM). This OM is composed of phospholipids, proteins and lipopolysaccharides (LPS) and functions as a permeability barrier that is able to exclude macromolecules such as bacteriocins or enzymes (Gong, Meng & Wang, 2010). The porins on the OM allow the free diffusion of molecules with a molecular mass below 600 Da. The smallest bacteriocins produced by LAB bacteria are approximately 3 kDa and are thus too large to reach their target, the cytoplasmic membrane (Klaenhammer, 1993; Stiles & Hasting, 1991). However, lactic acid is a disintegrating agent that causes LPS release, therefore permeabilizes the OM allowing bacteriocins and enzymes to effectively penetrate the Gram-negative bacterial OM (Alakomi *et al.*, 2000). These compounds kill the target cell by interference with energy transduction occurring at the cytoplasmic membrane and also inhibit biosynthesis processes of macromolecules such as DNA, RNA, proteins and polysaccharides (Sahl & Brandis, 1982). Héchard and Sahl (2002) suggested that some bacteriocins may interact with the cytoplasmic membrane leading to both pore formation or localised disruption of the membrane.

Several researchers have reported action of bacteriocins from LAB against Gram-negative bacteria and or yeasts, in combination with pH values below 5.5 (Gänzle, Weber & Hammes, 1999; Lash *et al*., 2005; Messi, Bondi, Sabia, Battini & Manicardi, 2001). In addition, Schillinger and Lücke (1989) demonstrated the inhibitory activity of the bacteriocins to lactic acid bacteria only, with the exception of *L. monocytogenes*. These authors attributed the apparent sensitivity of *L. monocytogenes* to bacteriocins as due to the fact that taxonomically, *Listeria* is closely

associated to the genus *Lactobacillus*. These authors reported that the activity of the antibacterial compound produced by *L. sake* was destroyed by protease treatment (trypsin and pepsin) but was resistant to heat. There was no reduction in antibacterial titer after heating for 20 minutes at 100 °C. These authors then concluded that *L. sake* antibacterial compound was a heat-stable peptide. Several LAB bacteriocins offer potential applications in food preservation and the use of bacteriocins can help to reduce the addition of chemical preservatives as well as the intensity of heat treatments, resulting in foods which are more naturally preserved and richer in organoleptic and nutritional properties (Gálvez, Abriouel, López & Ben, 2007).

2.13.5 Role of bacteriocin-sensitive LAB strains on the bacteriocin production of other LAB strains

LAB produces bacteriocins in an environmentally dependent manner (Anderssen, Diep, Nes, Eijsink & Nissen-Meyer, 1998; Barefoot *et al.*, 1994; Biswas *et al.*, 1991; Chang *et al.*, 2007; De Vuyst, Callewaert & Crabbé, 1996; Franz, Stiles & Belkum, 2000). Maldonado, Ruiz-Barba and Jiménez-Díaz (2003) demonstrated that the production of plantaricin NC8 by *L. plantarum* NC8 was induced in the presence of different gram-positive bacteria. In addition, in recent years, Chang *et al.* (2007) showed that kimchicin GJ7 (a bacteriocin produced by LAB isolated from a fermented Korean vegetable product) production was significantly enhanced by the presence of a sensitive *L. plantarum* strain. Temperature and pH also influence bacteriocin production (Oliveira *et al.,* 2008).

2.13.6 Effect of heat on bacteriocins

Oliveira *et al.* (2008) determined the influence of heat treatment and pH on the inhibitory effect of cell free supernatants (CFS) derived from selected LAB on *L. acidophilus*. *L. acidophilus* was found to be the most sensitive strain. Overall, the inhibitory compound present in the CFS were resistant to heat treatment at varying intensities. CFS retained 72% inhibitory activity after exposure to heat at 100 °C for 10 min. Moreover, CFS from some LAB isolates showed even increased heat

resistance by keeping 76 to 86% of original activity when heated at 100 °C for 30 min, while the inhibiting substance from different strains of *Pediococcus acidilactici* showed different resistance to heat treatment.

2.13.7 Effect of pH on bacteriocins

The CFS obtained from isolated LAB strains showed antimicrobial activity in a wide pH range (Table 2.2). Three LAB strains showed inhibitory activity up to pH 9. These results showed the possible application of LAB in food products with high pH like frankfurters. The combination of a wide pH range and high temperature tolerance of bacteriocins is a remarkable property to be used for biopreservation because many food procedures involve a heating step (Oliveira *et al.,* 2008).

Table 2.2: Influence of pH on the inhibitory activity of CFS of selected LAB isolated from vacuum-packaged beef (n=9) against *L. acidophilus,* using the agar well-diffusion test (Oliveira *et al.,* 2008).

pH	Diameter of inhibition zone (mm)/CFS from selected LAB isolates			
	Pediococcus acidilactici	*Lactobacillus delbrueckii* subsp. *bulgaricus*	*Lactobacillus casei*	*Pediococcus acidilactici*
4.0	10.1 ±0.7[a]	11.0±0.7[a]	11. ±30.6[a]	12.5±0.7[a]
5.0	9.8±1.2[ab]	10.0±1.2[b]	11. ±20.7[a]	12. ±01.5[a]
6.0	9.5±1.1[ab]	9.0±0.7[c]	11. ±01.0[a]	11.5±0.8[a]
7.0	9.2±0.9[ab]	-	9.9±1.0[b]	10.3±1.2[b]
8.0	8.9±0.7[b]	-	8.3±0.7[c]	8.6±0.4[c]
9.0	6.7±0.3[c]	-	6.6±0.2[a]	6.9±0.4[d]

- = no inhibition zone

Mean values (± standard deviation) with different letters in the same column are significantly different at p<0.05

2.14 HYPOTHESES

- Beef from high-throughput abattoirs where food safety systems are in place, will have reduced levels of pathogenic bacteria, compared to beef from similar abattoirs, but without such systems. During the implementation of a food safety system, microbial hazards are identified and reduced to an acceptable level by controlling critical control points, such as evisceration and chilling during the slaughtering process. Food safety systems minimize the incidence of pathogens on meat.

- Selected *E. coli* serotypes will be able to compete more effectively with spoilage bacteria such as *Pseudomonads* spp. and LAB on beef with low (10^2 $\log_{10}$ cfu/cm^2) spoilage bacteria contamination levels than on beef with high (10^6 $\log_{10}$ cfu/cm^2) spoilage contamination levels. High levels of spoilage microorganisms in a food matrix will out-compete pathogens. Pathogen growth rates and maximum densities are a function of the total microbial community composition and density in the food due to competition for nutrients, the production of inhibitory substance and overall density (Powell, Schlosser & Ebel, 2004).

2.15 OBJECTIVES

- To determine the incidence of total bacterial counts, *Pseudomonas* spp.*,* Enterobacteriaceae, LAB *E. coli* serotypes, *Salmonella,* and *S. aureus* on fresh beef after chilling, from high throughput abattoirs with food safety systems and beef from high throughput abattoirs without safety systems.

- To determine the relationship between different levels, high ($10^6\log_{10}$ cfu/cm^2) and low ($10^2\log_{10}$ cfu/cm^2) of spoilage bacteria, i.e. *Pseudomonas* spp. on the survival and growth of *E. coli* O157:H7 isolated from beef and inoculated at a constant level of $10^2\log_{10}$ cfu/cm^2 on beef stored aerobically for 7 days at 4 °C.

- To determine the relationship between different levels, high ($10^4 \log_{10}$ cfu/ cm^2) and low ($10^2 \log_{10}$ cfu/cm^2) of spoilage bacteria, i.e. lactic acid bacteria on the survival and growth of *E. coli* O157:H7 isolated from beef and inoculated at a constant level of 10^2 cfu/cm^2 on beef under vacuum storage for 2 months at 4 °C.