HAZARD ANALYSIS CRITICAL CONTROL POINT (HACCP) IN A RED MEAT ABATTOIR

By

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Submitted in partial fulfilment of the requirements for the degree

M.Sc. Food Science

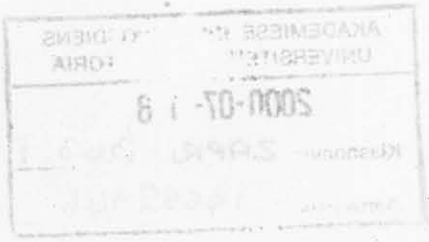
In the Faculty of Natural, Agricultural and Information Sciences

University of Pretoria

Pretoria

South Africa

October 1999



I declare that this thesis hereby submitted for the M.Sc. Food Science degree at the University of Pretoria had not been previously submitted by me for a degree at any other university.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to the following people and organisations for their contributions to the successful completion of this study:

Riette de Kock, Department of Food Science, for her able guidance in planning and executing this study and her valuable contributions and suggestions during the preparation of the thesis.

Director of Centre for Scientific and Industrial Research for the funding and the use of the Microbiology laboratory facilities.

Tracy Parsons and staff of microbiology Department Centre for Scientific and Industrial Research, for their suggestions in this study.

Dr. Gerhard Neetling, Director of the Red Meat Abattoir Association for his positive motivation and assistance with the training of the staff at the abattoir and the staff of the RMAA.

The Manager and staff of the Renbro abattoir for their co-operation during the study duration.

My uncle and auntie, Dr. J.A. Ogude and Professor N.A. Ogude without whose support and encouragement I would not have done the course.

My parents for their love, interest and encouragement, Omondi and Didi for their love.

My husband Fred and toddler daughter Dani for their support, encouragement and understanding.

Finally to the Lord almighty who made it all possible.

ABSTRACT

HAZARD ANALYSIS CRITICAL CONTROL POINT (HACCP) IN A RED MEAT ABATTOIR

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A hazard analysis critical control point (HACCP) programme was carried out in a class C abattoir in the Hammanskraal region of Pretoria, South Africa to determine whether the implementation of HACCP would minimise pathogens in meat at the abattoir level. The need to find methods to minimise these pathogens is due to the fact that the incidences of food poisoning and food-borne diseases are not declining. In addition, most animals are symptom-less carriers of these pathogens implying that their presence cannot be detected by the classical meat inspection procedures and assumed healthy animals can be a potential health hazard. This work is therefore important as it justifies the potential for use of the HACCP system in small abattoirs bearing in mind that various researchers have found pathogens within the meat chain of South Africa.

The experimental design involved a hygiene evaluation of the plant, a hazard analysis and microbiological analyses. The hygiene evaluation of the plant was carried out to determine whether the abattoir has basic good manufacturing practices in place, a foundation for a HACCP system. Thereafter, by following the processing line step-by-step, a hazard analysis was done to fully comprehend the impact of the slaughtering and hygiene procedures on the microbial loads of the carcasses. A critical control point work sheet was drawn up for the skinning, evisceration and chilling steps.

The main objective of the study was to determine the effect of HACCP on the microbiological status of carcasses. A non-destructive microbiological analysis was carried out on a total of 100 carcasses with 50 forming the baseline data and the other 50 forming the HACCP data. Analyses were done for aerobic plate counts, *Staphylococcus aureus,* total coliforms, *Escherichia coli, Clostridium perfringens,* and *Salmonella.*

The statistical evaluations of the data showed that all variables were significantly reduced ($p < 0.01$) after HACCP implementation except for the aerobic plate count data and *Escherichia coli* at splitting of carcass. However, after 24 hours chilling all the pathogens were significantly reduced ($p < 0.05$). A consistent positive hygiene trend was achieved for all the variables tested. Minimal detections as low as 40% of carcasses at splitting and 2% after 24 hours chilling for *Escherichia coli* and 14% at splitting and 0% after 24 hours chilling for *Clostridium perfringens,* were also recorded after HACCP implementation. *Salmonella* was isolated from only 2% of the carcasses during the baseline data collection at the splitting step. After HACCP implementation all the samples were negative at both the control points.

The main limitation of the study was that most of the personnel within the meat industry were illiterate and therefore training was difficult and took a longer time. The high employee turn -over also calls for constant training and can lead to hygiene fluctuations within the line. The workers remuneration also depended on the number of carcasses they processed hence chain speeds tended to be fairly high and these could compromise the hygiene of the product. Generally, there is also still a lack of minimum microbial standards for meat processing operations using the HACCP system.

The aim of the study, which was focused on reducing pathogen levels in a carcass, was attained. The study can therefore form a basis for implementation of HACCP in small red meat abattoirs in South Africa. However, it is recommended that a further similar study should be done for multiple abattoirs concurrently over a longer duration to get a more comprehensive picture of the South African abattoir industry viz. HACCP.

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CHAPTER 1

INTRODUCTION

Consumer protection has become the central issue of public interest world-wide due to incidences of food poisoning and food-borne diseases which have shown no marked tendency to decline in recent decades (Harrigan, 1998; Jouve, 1998; Steinmceblj, 1995). Unfortunately, meat and meat products which form a major part of the current diet have been associated prominently with these food illnesses (Bryan, 1980). Research work has therefore been focused on the meat distribution chain to try and identify points at which problems might be arising and can be prevented (Ayres, 1955; Kapsrowiak & Hechelmann, 1992; National Advisory Committee on microbiological Criteria for Foods (NACMCF), 1993; Nortjé, 1987).

From these works, it has been noted that factors that impact on the microbiological safety of raw beef, range from the farm to the consumer. These factors can be divided into four segments namely: live animal practices, slaughter and processing operations (abattoir), distribution and retailing and consumer food handling practices (NACMCF, 1993). The abattoir is the main contributor as it is the point at which the initial translocation of microorganisms from the environment to the product occurs. This implies that it is also the initial point at which food-borne pathogens would be introduced into the carcasses (Gregory, 1996; Hechelmann, 1995b; Kapsrowiak & Hechelmann, 1992; NACMCF, 1993). This has led to interest in the bacteriological (especially food-borne disease causing organisms) status of beef carcasses, and hygiene in the abattoirs (Harrigan, 1998; Roberts, Hudson, Whelehan, Simonsen, Olgaard, Labots, Snijders, Van Hoof, Debevere, Dempster, Devereux, Leistner, Gehra, Gledel & Fournaud, 1984; Wood, Holder & Main, 1998).

The abattoir's vulnerability to potential food-borne pathogens comes from the fact that unlike most of the other food processing industries it has no microbial destruction step and therefore any hygiene deficiencies can lead to considerable contamination of carcasses. This potentially high level of accumulative contamination on the raw material cannot be compensated for even by the most rigorous hygiene measures during later processing stages hence, compromising safety of the products (Buchanan & Whiting, 1998;

Untermann, Stephan, Dura, Hofer & Heimann, 1997). To incorporate safety in the production of meat products, the Hazard Analysis Critical Control Point system (HACCP) seems an excellent means. HACCP represents a proactive approach to averting health hazards as it allows for a more comprehensive approach to dealing with all significant hazards of either biological, chemical or physical origin (Bernard, 1998; Jouve, 1998; Peters, 1998; Sperber, 1991; Stier & Blumenthal, 1995; Synder, 1992). South Africa is in the process of legislating the mandatory use of HACCP in the food industry (Ms Verna Carolissen, Assistant Director Department of Health, South Africa, 1999 - Personal communication).

1.1 STATEMENT OF THE PROBLEM

In South Africa over the past and at present "hygienic slaughter" has been and is still being implemented through visual inspection and supervision of skilled operatives by suitably qualified personnel such as meat inspectors and veterinarians. The role of the meat inspectors includes ensuring slaughter of wholesome animals, maintenance of plant sanitation and prevention of carcass contamination (Dr. Gerhard Neetling, Director of Red Meat Abattoir Association, South Africa, 1999 - Personal communication; Nortjé, 1987; Riemann, 1973; Vorster, 1994). This method of meat inspection has had a very important function of reducing food-borne diseases. However, the disease pattern in livestock has changed and some of the most important food-borne diseases eludes the efforts of the meat inspector (Wood *et al.,* 1998). The main problem with these food poisoning organisms is that in most cases animals are symptomless carriers. Hence, assumed "healthy" animals can easily infect other animals (Wood *et al.,* 1998).

Carcass contamination can be via personnel, faecal material, contents of the animal's alimentary tract or by contact with the hide whose cleanliness is impossible to assure (Bell, 1997; Roberts *et al.,* 1984). Contamination of the raw product represents a potential risk due to the expansion of the meat distribution chain with resultant hygiene strain at each level and changes in eating habits, like less severe internal end point cooking temperatures (Kasprowiak & Hechelmann, 1992; Grijspaardt-Vink, 1996).

There is a need therefore to determine the major points in abattoirs at which control can be exerted, to minimise pathogens. The choice of a small meat abattoir is because high

volume beef slaughter establishments tend to control microbiological contamination more effectively than small volume establishments (Hogue, Dreesen, Green, Ragland, James, Bergeron, Cook, Pratt, & Martin, 1993; Jericho, Bradley, Gannon & Kozub, 1993). This project will therefore be a measure of the usefulness of a HACCP system in a small (class C) abattoir and therefore help to determine if there is any potential for use of HACCP in small abattoirs.

CHAPTER 2

LITERATURE REVIEW

2.1.1 EPIDEMIOLOGY OF FOODBORNE ILLNESSES ASSOCIATED WITH RAW BEEF

Food-borne diseases are increasingly being recognized as a major cause of morbidity in both industrialized and developing countries, and also of mortality in the latter. However, the full extent of the social and economic impacts is hard to measure due to underreporting of cases (Sofos, Belk & Smith, 1999b; Todd, 1989). Analogously, South Africa is also caught in the same web but the underreporting in this case can be attributed to the current health regulation. The regulation states that notification is required only when five or more cases are reported by one physician or at one medical institution (Department of National Health and Population Development, South Africa, 1989).

Studies done by various authors indicate that the foods of animal origin are the main vehicles of pathogens in human foods. These animal products mainly meat, fish, poultry, have been implicated in food-borne diseases as the live animals are exposed to a variety of potential sources of microorganisms at the various rearing points (Bean & Griffin, 1990; Sofos *et al.,* 1999b). The microorganism sources are diverse and include soil, water, feed, air and other animals (Ayres, 1955). In healthy animals the microorganisms are confined primarily to the gastrointestinal tract and exterior surfaces such as hide, hooves and hair. But during slaughtering and dressing the meat usually becomes contaminated with these microorganisms and the extent of contamination depends to a large degree on the basic good manufacturing practices of the abattoir (Ayres, 1955; Mackay & Derrick, 1979). However, there has been an increased safety concern with meat and meat products as there seem to be an increased occurrence of food-borne diseases with the consumption of these products (Bean & Griffin, 1990; Cross, 1996; Dean, 1991; Roberts *et al.*, 1984; Tompkin, 1990; Wood *et al.*, 1998).

In South Africa despite lack of pathological reports, work done by various researchers has shown the possibility of contamination of meat products by various pathogens. For instance, Vorster (1994) reported that South African meat and meat products are contaminated with bacterial pathogens though not at high levels, but could easily pose a health risk to immuno-compromised individuals. Prior to the study carried out by Vorster (1994), Nortjé (1987) in his study on the meat chain had made various recommendations, which could assist in lowering microbial loads in the meat products. These included a strict adherence to the maintenance of the cold chain throughout the meat production chain and an effective management of sanitation programmes from the abattoir to the markets, failure of which there would still be high levels of contamination on the South African meat products. Unfortunately this recommendations seems not to have been implemented as Vorster, Greebe & Nortjé (1994) showed the presence of pathogens such as *Escherichia coli* and *Staphylococcus aureus* in the retail meats of South Africa. A recent study done by Nortjé, Vorster, Greebe & Steyn (1999), have also shown the occurrence of *Bacillus cereus* and *Yersinia enterocolitica* in retail meats and further reiterated the need to implement methods to prevent the entry or proliferation of these pathogens in meat products.

The urgency to effect control of these microorganisms is to avoid outbreaks of food-borne disease associated with similar organisms, which have occurred in other countries. Reported food-borne outbreaks from other countries notably the United States have shown that if human illnesses are ranked by organ system gastrointestinal infections rank second in incidence. For instance in 1990 an average of 35,000 to 40,000 deaths were attributed to gastrointestinal infections with food being the vehicle in one-third of the total number of incidences (Jackson, 1990). A survey done by Todd (1992) in Canada indicated that microorganisms particularly *Salmonella, Staphylococcus aureus, Clostridium perfringens,* and *Bacillus cereus* were the main etiologic agents and the foods involved were mainly meats and poultry has which has a higher number (No) of cases (Table 2.1.).

Table 2.1: Foods associated with food-borne incidents in Canada 1975-1984 (Todd, 1992).

Food type	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	Mean	
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	%
Meat	276	219	192	185	191	165	128	209	195	228	199	23
Seafood	50	54	52	65	50	54	45	78	70	68	59	7
Poultry	74	74	63	79	81	96	82	94	100	146	89	10
Eggs	1	-	3	6	4	6	4	1	3	4	3	<1
Dairy foods	21	32	42	33	43	29	33	71	86	104	49	6
Bakery foods	67	74	67	65	69	53	47	81	70	81	67	8
Infant foods	5	11	11	12	15	12	10	22	13	16	13	2
Confectionery	12	14	12	15	11	9	10	12	8	22	13	1
Vegetable	34	43	29	53	35	31	42	32	31	48	38	4
Fruit	15	21	29	42	34	34	42	37	38	35	33	4
Chinese foods	39	52	58	49	54	61	37	50	62	67	53	6
Salads	22	24	28	32	24	17	19	29	21	42	26	3
Sandwiches	33	23	26	28	26	20	17	42	35	41	29	3
Beverages	28	16	24	28	19	32	35	53	37	55	33	4
Others or unknown foods	160	203	141	144	169	140	96	177	194	223	165	19
TOTAL	837	860	777	836	825	759	647	988	963	1180	868	100

Between 1968 and 1977, prior to the study carried out by Todd (1992), 58% of all reported outbreaks of food-borne illnesses were also associated with meat and poultry. This figure subsequently, dropped to 33% in 1977 to 1984 (Bryan, 1980).

The impact of the food-borne diseases on the meat industry worldwide has been multifold and is felt amongst all the stakeholders from the producer to the consumer (Food and Drug Administration (FDA), 1999). The South African meat industry being part of the global world market has not remained neutral. Drawbacks due to not only the incidences of food-borne diseases but other factors such as changes in eating habits have come into play affecting demand (Grijspaardt-Vink, 1996). For instance per capita meat production in South Africa dropped by about 25% between 1989 and 1997 (Food and Agricultural Organization (FAO), 1997).

In addition, to the microbial related food-borne diseases various other scandals have also rocked the meat market. These include the recent cases in Britain in which cows were found with bovine spongiform encephalopathy (mad cow disease) which is potentially lethal to man (Alisa, 1996; Bryan, Guzewich & Todd, 1997). More recently, dioxin contamination of animal feed in Belgium was reported (World Health Organization, 1999). Emergence of 'new' pathogens has also caused a threat to food safety (Cox, 1989; Farber, 1991; Palumbo, 1986). Hazard management is currently the best approach to the control of these emerging pathogens especially when they display similar ecological and physiological characteristics as the existing known pathogens (Harrigan, 1998; Tarrant, 1998). Hazard management more so is necessary for meat slaughterhouses as the operations lack a definitive step that can be relied upon to eliminate microbiological risks (Buchanan & Whiting, 1998).

2.2 MEAT BACTERIA OF HEALTH CONCERN

Meat is a nutrient rich substrate that can support growth of a wide range of microorganisms if not properly handled, processed and preserved. The microorganisms are introduced into the otherwise sterile interior of the meat surface by translocation of bacteria from the surface of the carcass. The ease of translocation depends on the degree of contamination and/or abusive conditions of handling and storage. These factors allow microbial proliferation, increase the potential for the presence of pathogenic bacteria and formation of toxins (Ayres, 1955; NACMCF, 1993; Nottingham, 1982; Sprenger, 1995; Sofos *et al.,* 1999a; Sofos *et al.,* 1999b).

In general, the presence of all these specific bacteria on the meat is dependent on the storage temperature, pH, moisture content, oxygen availability and the general handling of the carcass. It should be noted that as storage temperatures are lowered there is a significant decrease in the rate of microbial growth as well as a reduction in the diversity of the microbial flora. The moisture content of meat is also fairly high. With a water activity (a_w) of 0.99 this supports the growth of a

wide variety of bacteria. Growth of bacteria is also dependent on the pH of the substrate but growth is restricted at the lower end of the pH range. Therefore meat whose pH range is usually 5.3-6.5 would support the growth of a wide variety of bacteria. The pH of meat is influenced by various factors such as feeding and handling practices at the time of slaughter (NACMCF, 1993).

The nature and level of microbial contamination has important consequences in relation to public health, storage life and the type of spoilage likely to arise. Contamination with spoilage microorganisms may lead to product losses and therefore economic losses, whereas presence of pathogens or their toxins may be the cause of food-borne diseases (NACMCF, 1993; Nottingham, 1982; Sofos *et al.,* 1999a; Sofos *et al.,* 1999b). Raw meats are especially important sources of pathogens like *Salmonella* and *Clostridium perfringens,* that are often incriminated in outbreaks of food-borne diseases (refer section 2.2.1.2 and 2.2.2). Raw meat are also sources of *Staphylococcus aureus, Yersinia enterocolitica, Listeria monocytogenes, Campylobacter jejuni* and *Escherichia coli* (Bean, Griffin, Goulding & Ivey, 1990; Nottingham, 1982; Nortjé *et al.,* 1999; Vorster *et al.,* 1994).

The primary source of these carcass microbes starts from the animals' pre-slaughter environment as illustrated in Table 2.2.

Table 2.2: Sources of some bacteria of health concern in meat (Church & Wood, 1992).

Organism	Principle source
Staphylococcus aureus	Skin, mucous membranes of handlers
Clostridium perfringens,	Soil, intestinal tract
Listeria monocytogenes	soil, water, air or intestinal tract
Enteropathogenic *Escherichia coli*	Intestinal tract
Yersinia enterocolitica	Intestinal tract
Salmonella spp.	Intestinal tract

All these bacteria are of importance as they are usually associated with food poisoning of sorts (Figure 2.1). Food poisoning bacteria are ubiquitous and will be found in any food processing plant, which makes it imperative for all food processors to adopt a system of hygiene management to minimize or prevent the risk of illness from consuming contaminated food. The type of pathogens in a food is subject to the conditions to which the food is exposed to before and after processing. This can be emphasized by the study carried out by Nortjé, Nel, Jordaan, Naude, Holzapfel & Grimbeek (1989) on the retail markets of South Africa indicating that temperature abuse is usually prevalent in the supermarkets. Such abuse of storage conditions can allow growth of various pathogens such as *Clostridium perfringens.* The chance that the product might be inadequately cooked may lead to increased risks with consumption. The continuity of temperature maintenance of the cold chain is very important to reduce or stop the growth.

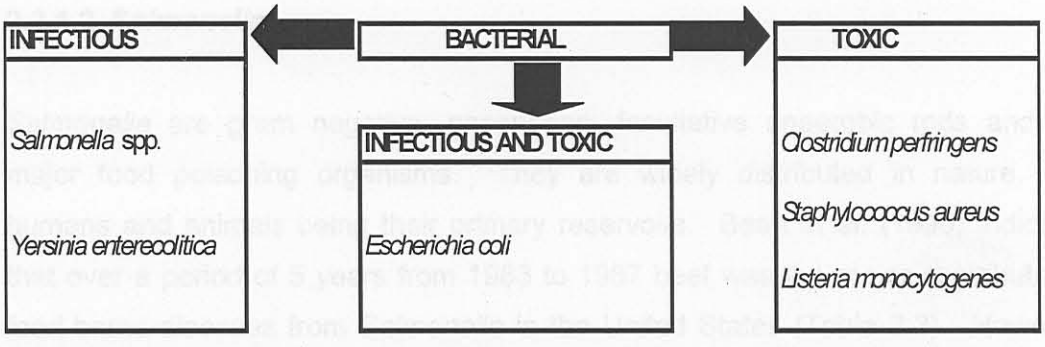


Figure 2.1: Types of food poisoning associated with meat pathogens (adapted from: Sprenger, 1995).

2.2.1 Enterobacteriaceae

The main Enterobacteriaceae species of interest in the meat industry are *Salmonella, Yersinia* and *Escherichia coli.* These are gram negative rods that cause food-borne gastroenteritis. They are mainly found in the animal intestine, soil and plants from where they can contaminate the food chain. They are regarded as indicators of faecal contamination when present in foods and are commonly isolated from hooves and hides of cattle (Stolle, 1981).

2.2.1.1 *Escherichia coli*

Escherichia coli is a gram negative, aerobic rod with certain strains that are pathogenic and produce an enterotoxin. Raw beef can be an important vehicle in the transmission of *E. coli* 0157:H7 (Doyle & Shoeni, 1987). The symptoms in man are usually a watery diarrhea and the disease is most commonly associated with travelers.

Escherichia coli generally, comprises a greater proportion of the total aerobic flora of the intestine than of the hide and its presence in meat is usually a result of faecal contamination or when the intestinal tract is punctured. Control of this pathogen is mainly assured by proper slaughtering techniques, hygiene during slaughtering and dressing together with prompt adequate cooling (Church & Wood, 1992; Nottingham, 1982).

2.2.1.2 *Salmonella*

Salmonella are gram negative, nonsporing, facultative anaerobic rods and are major food poisoning organisms. They are widely distributed in nature, with humans and animals being their primary reservoirs. Bean *et al.* (1990) indicated that over a period of 5 years from 1983 to 1987 beef was the major contributor to food-borne diseases from *Salmonella* in the United States (Table 2.3). However, NACMCF (1993) reported that currently the incidence rates of *Salmonella* on raw beef are generally, low (<5%). However, Hogue *et al.* (1993) and Stolle (1981) indicated that the levels are relative and can be fairly high depending on the health or handling of the animals during slaughter.

Salmonella species are maintained within the animal population by means of nonsymptomatic animal infections and in animal feeds. This leads to both sources serving to keep slaughter animals reinfected in a cyclical manner (Brown, 1982). The increased incidence of *Salmonella* in slaughter animals is also usually associated with transport of animals in overcrowded, dirty vehicles and poor hygiene in the abattoirs, resulting in spread of infection and an increase in contamination of carcasses by faecal material and intestinal matter. In slaughter

animals faecal matter is of great importance as it is usually the source of animal hide contamination. (Nottingham, 1982).

Table 2.3: Leading vehicle foods known for salmonellosis outbreaks in the United States, 1973-1987 (Bean & Griffin, 1990).

Rank	Vehicle foods	Outbreaks	Percentage
1	Beef	77	9.7
2	Turkey	36	4.5
3	Chicken	30	3.8
4	Ice cream	28	3.5
5	Pork	25	3.2
6	Dairy products	22	2.8
7	Eggs	16	2.0
8	Bakery products	12	1.5
9	Mexican foods	10	1.3
10	Fruits and vegetables	9	1.1

Salmonella species are of importance as they cause diarrhea and systemic infections, which can be fatal in particularly susceptible persons, such as the immunocompromised, the very young, and the elderly. Due to these serious side effects of *Salmonella* poisoning and potential fatalities (Table 2.4), its presence in food is usually expected to be negative. The *Salmonella* outbreaks, cases and deaths associated with foods in the United States over a period of 5 years are as shown in Table 2.4. Various factors, like increased mass food preparation, mass storing of food, international trade and decreased resistance to infection, have contributed to increased incidence of food-borne salmonellosis (FDA/ CFSAN, 1999; Nottingham, 1982).

The habit of eating raw or insufficiently heated foods, is the main cause of salmonellosis. This is because the food inspection methods in use in the meat

industry do not indicate *Salmonella* presence or absence as they are not visual (Brown, 1982). Therefore, control of this pathogen can only be assured by hygiene during slaughtering and dressing in addition, to prompt adequate cooling (Church & Wood, 1992; Nottingham, 1982).

Table 2.4: Salmonella outbreaks, cases and deaths traced to foods in the United States, 1983-1987 (Bean et al., 1990).

Years	Outbreaks/Cases/Deaths
1983	72/2427/7
1984	78/4479/3
1985	79/19660/20
1986	61/2833/7
1987	52/1846/2

2.2.1.3 *Yersinia*

Yersinia is a psychrotrophic, gram negative, facultative anaerobic rod. Interest in this specific pathogen has increased over the years due to the fact that unlike most pathogens, it is capable of growth at 5°C competitively with normal microflora. It can grow readily in beef held at 1-7 °C (Palumbo, 1986).

2.2.2 *Clostridium perfringens*

Clostridium perfringens is a gram-positive, spore forming anaerobe. The food poisoning strains of interest is mainly type A. It is widely distributed in nature and is found in soils water, foods, dust and in the intestinal contents of virtually every animal including man (Church & Wood, 1992; Nottingham, 1982; Sprenger, 1995). Meat and poultry animals are the most common vehicles of transmission (Smart, Robert, Stringer & Shah, 1979). This is because *Clostridium perfringens,* requires many amino acids or simple peptides and several vitamins which are provided easily by red meat and poultry (Craven, 1980). However, the foods mainly

involved in *Clostridium perfringens,* outbreaks are often meat dishes (Smart *et al.,* 1979). *Clostridium perfringens* gets into meats directly from slaughter animals or by subsequent contamination of slaughtered meat from containers, handlers or dust. Since it is a spore former it can withstand the adverse environmental conditions of drying, heating and certain toxic compounds (Brown, 1982).

Outbreaks have been fairly low which has been attributed to underreporting. The confirmed cases of outbreaks in the United States over a period of 5 years are as shown in Table 2.5. The typical symptoms of *Clostridium perfringens,* poisoning are usually abdominal pain, diarrhea, nausea and fever.

Table 2.5: Outbreaks, cases and deaths from *Clostridium perfringens,* food-borne gastroenteritis in the United States, 1983-1987 (Bean *et al.,* 1990).

Years	Outbreaks/Cases/Deaths
1983	5/353/0
1984	8/882/2
1985	6/1016/0
1986	3/202/0
1987	2/290/0

The microorganism is sensitive to cold temperatures (below 4°C) and food safety is therefore based largely on proper attention to time/temperature conditions of cooling and improvement of slaughter hygiene. It should be noted that dried spores can survive longer in frozen conditions (Brown, 1982; International Commission for Microbiological Safety of Foods (ICMSF), 1986; Smart *et al.,* 1979).

2.2.3 *Staphylococcus aureus*

This organism is a gram positive, facultative anaerobe. Its presence in food is usually associated with food handlers or mastitis in cows (NACMCF, 1993).

Approximately 40% of adults carry *Staphylococcus aureus* in the nose and throat and 15% on the skin, especially around the hands. Therefore coughs and sneezes may carry droplet infection which can easily spread not only to the environment but also to the food being handled. However, the two most important sources of contamination to foods are nasal carriers and individuals whose arms and hands are inflicted with boils and carbuncles and are permitted to handle foods (Sprenger, 1995; Troller, 1976).

The symptoms of staphylococcus food poisoning usually develop within 4 hours of ingestion of contaminated food and the symptoms include nausea, vomiting, abdominal cramps, diarrhea, sweating, headache, prostration and sometimes a fall in body temperature (Sprenger, 1995). Although the disease caused by this organism is characterized by low mortality and relatively short duration, the frequency of poisoning and the severity of the symptoms mark *Staphylococcal aureus* food poisoning as an important food-borne disease. The six leading foods, which have been associated with the incidence of Staphylococcal outbreaks, are indicated in Table 2.6.

Table 2.6: Leading food sources for staphylococcal gastroenteritis outbreaks in the United States, 1973-1987 (Bean & Griffin, 1990).

Food source	Number of Outbreaks
Pork	96
Bakery products	26
Beef	22
Turkey	20
Chicken	14
Eggs	9

However, with regard to general food vehicles, outbreaks have been reported in the United States from 1973 to 1987 (Table 2.7). With notable improvement in the good manufacturing practices in the United States the disease outbreak dropped from 16% in 1983 to 1% in 1987 of all the reported cases of outbreaks of food-

borne diseases. Unfortunately in South Africa *Staphylococcus aureus* is still a major problem to contend with in the meat industry (Voster *et al.*, 1994).

Table 2.7: Staphylococcal food-borne gastroenteritis outbreaks and cases in the United States, 1973-1987 (Bean & Grifffin, 1990; Bean *et al.*, 1990).

Years	Outbreaks	Cases	Percentage of all cases
1973-1987	367	17248	14.0
1983	14	1257	15.9
1984	11	1153	14.1
1985	14	421	1.8
1986	7	250	4.3
1987	1	100	1.0

Studies done by Bean & Griffin (1990), indicated that the main factors that usually lead to the outbreak of staphylococcal food-borne gastroenteritis are usually due to temperature abuse of the products, poor hygiene amongst personnel and within the establishments and consumer associated problems as indicated in Table 2.8.

Table 2.8: Leading factors that led to the outbreaks of staphylococcal food-borne gastroenteritis in the United States, 1973-1987 (Bean & Griffin, 1990).

Causes	Number of Outbreaks
Improper holding temperature	98
Poor personal hygiene	71
Contaminated equipment	43
Inadequate cooking	22
Food from unsafe source	12
Others	24

Control of this pathogen can be achieved by (Sprenger, 1995; Troller, 1976):

- Removal of the microorganism physically for example by trimming of carcasses,
- aesepsis which is the provision of the first line of defence by focusing on the human element who harbour and transfer this organism directly or indirectly to foods through careless acts and allowing conditions to exist in which the pathogen can proliferate,
- Temperature-time control which invariably prevents or delays growth and toxin production and finally,
- Killing of the microorganism using bactericides.

2.2.4 *Listeria monocytogenes*

Listeria monocytogenes, is a gram positive, psychrotrophic bacillus. It is found mainly in soil, water, air or the intestinal tract. The incidence of *Listeria monocytogenes* is approximately 30-50% (or greater) in raw meat although levels are usually < 100 cfu/g. Interest in *Listeria* has increased because like *Yersinia* it is also capable of growth at refrigeration storage temperatures. The duration of storage may also give it extra time to grow to potentially dangerous levels. It has also been reported to be more pathogenic when grown at low temperatures as it produces a toxin (listeriolysin O). Control can be effected by implementation of a HACCP system (Farber, 1991; Sprenger, 1995).

2.3 MICROBIAL CONTAMINATION OF BEEF CARCASSES

The microbial profile of beef carcasses is dictated by events that occur during the conversion of the whole animal into carcass halves for further processing (Siragusa, Cutter, Dorsa & Koohmaraie, 1995). The incidence and levels of both pathogenic and spoilage microorganisms on the carcass can either be from intrinsic or extrinsic contamination. However, whether or not the load of intrinsic microorganisms associated with the living animal contribute a significant share of the contamination

occurring in and on the carcass depends not only on the methods of handling but also on the defense mechanisms of the animal (Ayres, 1955; Nottingham, 1982). Generally, intrinsic contamination is limited as bactericidal activity continues at least for 1hour after death (Gill & Penney, 1979). This indicates that the main source of carcass contamination is usually from extrinsic contamination.

Sources of extrinsic contamination are:

- The slaughter stock
- Abattoir environment

2.3.1 Slaughter stock

The main source of microbial carcass contamination is usually from the slaughter stock. This is due to microorganisms present in the intestines or on the hide of the live animal. The live animals are often highly contaminated or asymptomatic carriers of pathogenic bacteria (Cray, Cassey, Boswath & Rasmussen, 1998; Hancock, Rice, Thomas, Dargatz & Besser, 1997; Lettelier, Messier & Quessy, 1999). Therefore any compromises made on the welfare and hygiene during pre-slaughter handling subsequently, affects the carcass hygiene (Ayres, 1955; Gregory, 1996; Sofos *et al.,* 1999a; Sofos *et al.,* 1999b).

The degree of carcass contamination depends a lot on the environmental conditions to which the live animal has been exposed to. These environmental conditions include climate, geographic location, husbandry conditions, method of transportation, holding conditions and animal feed (Cray *et al.,* 1998). For instance Smulders (1995) elaborated on the effects of husbandry indicating that intensive agriculture involves the rearing of young animals having immature gut flora and therefore lacking resistance to colonisation by pathogens. Other workers have also indicated the vulnerability of the younger animals. Sofos *et al.* (1999b) showed that they were much more prone to *Salmonella* contamination compared to the older bulls. Church & Wood (1992) also indicated that in cases where the animal was housed, the main microorganisms were those of intestinal origin, whereas animals raised on pastures tended to carry more bacteria of soil origin. However, irrespective of where the animal is raised it is important to prevent excess mud and dung accumulation on the animal. For

example, Church & Wood (1992) showed that in housed animals as little as 3g of soil was required to contaminate a whole side of beef carcass with 10^5 microorganisms per 6.45 cm^2. A study done by Bell (1997) indicated that soiled hides coming into contact with the carcasses would typically give rise to the aerobic plate counts and *Escherichia coli* counts of approximately log 5.13 and log 3.90 respectively. A recent study by Sofos *et al.* (1999a) also determined that the larger the amounts of mud on the hide of steers and heifers the higher the incidence of *Salmonella*.

The most stressful environmental condition occurs during transportation and during the holding period in the lairage at the abattoir. These areas have been identified as potential critical control points in the slaughter lines (Kapsrowiak & Hechelmann, 1992). During transportation dispatched animals should not have full paunches, otherwise it becomes very difficult to avoid the spread of faecal contamination. It has been noted that any undue stress caused to the animal during transport or at the lairage can lead to increased spread of *Salmonella* from infected animals to uninfected animals (Church & Wood, 1992; Cray *et al.,* 1998; Herriot, Hancock, Ebel, Carpenter, Rice & Besser, 1998). The amount of time the animal spends at the lairage and the sanitary conditions of the lairages are also important as lack of proper care at the lairage can lead to heavy soiling of the animals' hides. This can provide a major source of bacterial contamination during slaughtering operations as was indicated by Sofos *et al.* (1999a). Smulders (1995) consequently showed that the farm environment and transportation permits a recycling of excreted pathogens as a result of which livestock are trapped in a vicious cycle of infection, excretion and re-infection due to insufficient preventive measures.

2.3.2 Abattoir environment

The abattoir environment as defined by the Public Health Regulations of South Africa is composed of the plant and equipment, personnel, cleaning and sanitation and slaughtering and processing of animals (Meat and meat products hygiene act. 1967-act no. 87 of 1967). For any processing to be carried out the abattoir must comply with the stated regulation. Figure 2.2 gives an overview of a typical

slaughterhouse. This is essential as the interacting environmental factors and the processing methods employed play a large role in fixing the distribution or adding to the microbes brought in by the animal and also determine the hygienic state of the slaughterhouse. In the slaughterhouse the condition of the carcasses immediately after slaughter is usually a good index of hygiene (Church & Wood, 1992).

2.3.2.1 *Plant and equipment*

Within the abattoir environment, attention must be paid to the functional design, layout and management of the slaughterhouse. The layout of the carcass transport lines for the slaughter, skinning, evisceration and chilling steps will influence the contamination of carcasses. The product flow is especially important in an abattoir, because subsequent steps usually carry less pathogens. Within the product flow the skin, offals and personnel carry a large number of pathogens which can easily be distributed and redistributed (ICMSF, 1988; South African Bureau of Standards (SABS) 049,1989).

Segregation of clean from dirty operations is also vital, as it will reduce cross contamination of carcasses by splashing. The physical separation of these areas also restricts access of transport to and from the areas and hence reduces any chances of cross contamination by personnel. Any equipment to be shared by the sections must also be sterilized to ensure that it is not a source of contamination. Equipment for butchering meat is difficult to operate in a consistently hygienic manner because of continual recontamination by incoming raw materials, the build up of meat residues often containing growing number of bacteria and the risk of cross-contamination between more or less contaminated meats. Good design of equipment and satisfactory finishing of contact surfaces is essential, as it will permit easy and effective cleaning. The design must avoid dead spaces where products can accumulate and not be removed by routine cleaning (Brown & Baird Parker, 1982). It should also ensure that the equipment sterilisers are available and maintained at a temperature of 82.2 ^{0}C.

In addition, to the functional design and plant equipment other support structures, which form the foundation of the plant installation, like proximity to potential sources of contamination; sufficiency and quality of water supply; wastewater removal;

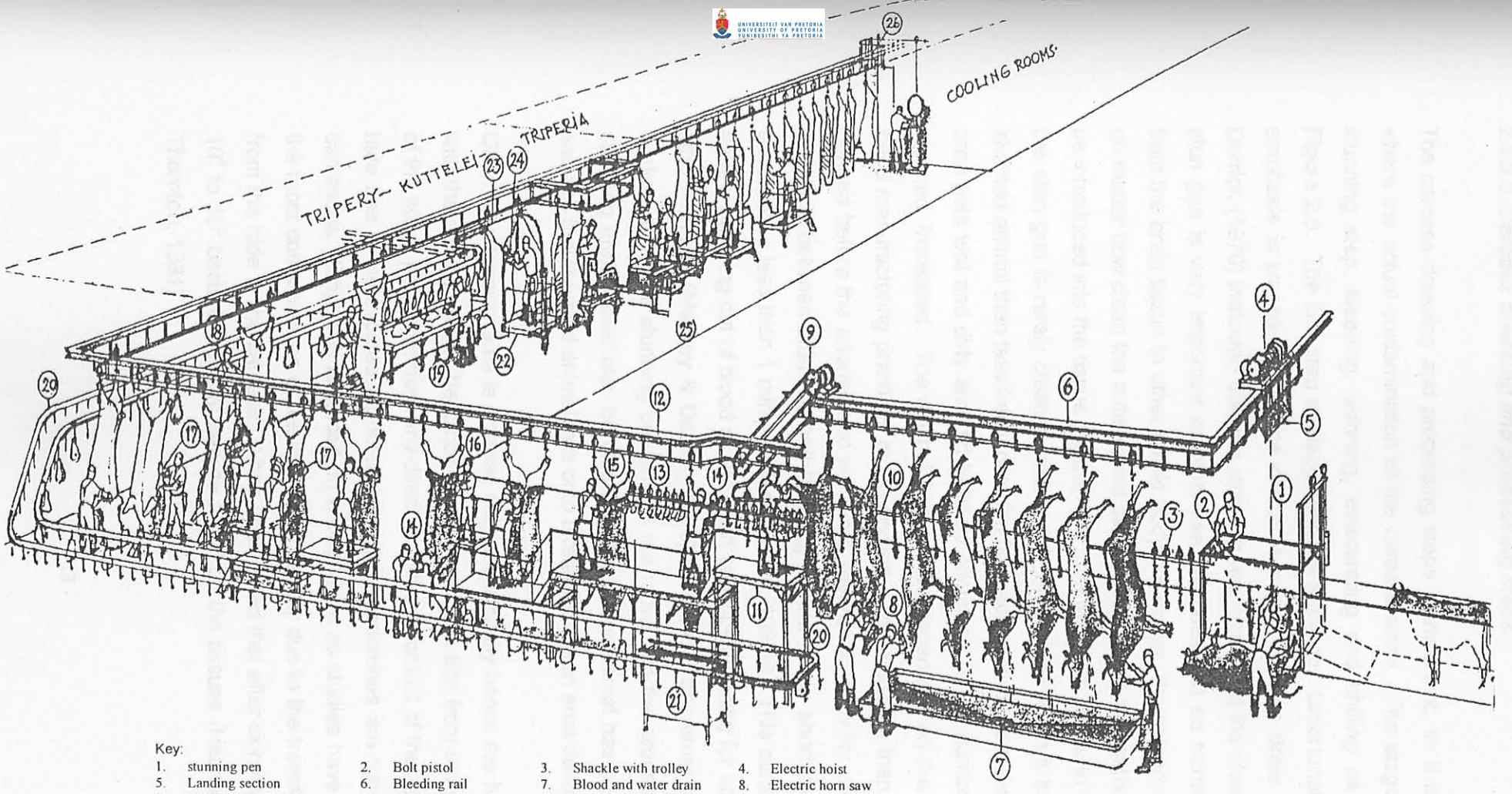
adequacy of power supply and availability of transportation must be considered (ICMSF, 1988; SABS 049,1989). This is important as micro-flora of processing plants can gain entry from the air, water, pests, people, dirt and equipment in addition, to those that gain entry through the raw material. The design of the plant can effectively restrict entry of pests, such as flies, rodents, birds, cats and dogs. They mainly contaminate meat with microorganisms by transferring microorganisms from one source to the next or from their droppings. Other factors such as equipping plant entries and exits with suitable barriers; fitting windows with appropriate screens to keep out insects, fitting air intakes with screens and filters for dust and appropriate environmental hygiene measures must also run hand in hand.

2.3.2.2 *Personnel*

Health and hygiene of personnel is one of the factors, which determine the type, and quantity of pathogenic microorganisms transmitted to man from food. The Health of the worker is important as man harbours various pathogens and transmission can occur under various conditions; including during the incubation period prior to clinical manifestation of a disease. The incubation stage is of concern due to lack of discernible illness yet the individual is shedding pathogens (Sprenger, 1995). It has been shown that during acute illness a person suffering from acute salmonellosis may shed as many as 10^9cells/g of feaces. Purulent skin infections are often laden with *Staphylococcus aureus,* which are readily transferred to foods when infected persons handle foods. Hence persons ill with diarrhea, vomiting, colds or infected skin lesions should not handle foods. In addition, to the inherent microbes on the personnel, it has also been demonstrated that the hands of workers in the meat industry have more coliform bacteria and *Salmonella* than hands of workers in other food sectors. This is obviously due to extensive contact with products of animal origin (De Wit & Kampelmacher, 1982). Personnel must therefore wash their hands properly after any break, periodically while at the processing line, on change of job functions and after handling contaminated materials or objects.

The hygienic habits of the workers plays a significant role in the control of contamination. The habits are encompassed under basic good manufacturing

practices and range from basic clothing to hand washing and other processing steps and these factors all impact on each other. For instance, the clothing of workers must be clean, neat and without adornments. Uniforms have been noted to be an excellent means of controlling neatness and have a powerful psychological impact on workers attitudes towards good sanitation. In addition, strategic placement of facilities to ensure that they cannot be ignored is important. Such facilities include hand washing facilities and processing equipment, which ultimately play a role in maintenance of product flow (Nortjé & Van Holy, 1985). Studies carried out by the World Health Organization/Food and Agricultural Organization (WHO/FAO) (1983) concluded that routine health examinations is not useful as a control measure but education of food handlers, strict supervision and control of food hygiene offered a more effective alternative strategy. Thus, it is very important that the slaughter process follows proper sanitary guidelines at all times with emphasis on personnel health, general appearance; personal conduct, training and housekeeping practices (Bell, 1997; Samarco, Ripabelli, Ruberto, Iannitto & Grasso, 1997; Sierra, Gonzalez-Fandos, Garcia-Lopez, Fernandez & Prieto, 1995; Sprenger, 1995; Wood *et al.,* 1998).



Key:

1. stunning pen	2. Bolt pistol	3. Shackle with trolley	4. Electric hoist
5. Landing section	6. Bleeding rail	7. Blood and water drain	8. Electric horn saw
9. Declined conveyor	10. Shackle return rail	11. Transfering platform	12. Dressing rail
13. Trolley hooks	14. Electric feet saw	15. Aitch bone cutter	16. Tail puller
17. Pneumatic dehidingknife	18. Brisket saw	19. Viscera inspection table	20. Head conveyor
21. Head flushing cabinet	22. Elevating platform	23. Pneumatic spreader	24. Electric splitting saw
25. High pressure washer	26. Overhead track scale		

Figure 2.2: Layout of a cattle slaughter house (Atlas Danmark)

2.3.2.3 *Carcass dressing and processing steps*

The carcass dressing and processing steps is important, as it is at this level where the actual contamination of the carcass starts. The stages include the stunning step, bleeding, skinning, eviscerating and chilling as illustrated in Figure 2.3. The first step of slaughtering is stunning. Unfortunately, very little emphasis is placed on hygiene of the stun gun. Studies done by Mackay & Derrick (1979) indicated that the stunning technique and the cleanliness of the stun gun is very important as otherwise microbes can be transferred directly from the brain tissue to other edible body organs viz. the spleen. Implying that no matter how clean the subsequent slaughtering steps are, pathogens can still be introduced into the tissue organs right from the initial stage. In most abattoirs the stun gun is rarely cleaned and is only maintained when it backfires. The stunned animal then falls freely onto the floor adjacent to the stunning bay. The area gets wet and dirty and dirt build up continues as the numbers of animals stunned, increased. The dirt build up is also dependent on the adherence to good manufacturing practices in the section. The animal is then shackled and hanged before the arteries and veins are severed to allow for proper bleeding. The time between stunning and bleeding should be as short as possible and should take less than 1 minute (Hechelmann, 1995b). This duration allows for natural pumping out of blood as the heart continues beating for about 3 minutes after stunning (Mackey & Derrick, 1979). To avoid any cross contamination, workers from the stunning bay should not move into the processing hall. The stunning area should also be cleaned after every animal has been bled. This ensures that the next animal falls onto a relatively clean area devoid of soil.

Often the stunning area is cleaned only occasionally hence the hide on contact with the ground usually becomes impregnated with filth from the walls and floor of the killing pen. Therefore any direct or indirect contact of the carcass with the hide has to be avoided as otherwise a lot of microbes are transferred to the carcasses. Emphasis is placed on a clean hide as studies have indicated that the most contamination of the carcass is usually due to the transfer of microbes from the hide to the carcass. It has been noted that after skinning, as many as 10^4 to 10^6 bacteria per cm^2 can be found on the tissues (Hechelmann, 1995b; Thornton, 1981)

The gastrointestinal and respiratory tracts of live animals have also been implicated in contamination of the carcasses (Ayres, 1955; Bell, 1997; Roberts *et al.*, 1984). Pathogenic bacteria, which can contaminate carcasses during slaughter and dressing, inhabit the gastrointestinal tract. The transfer of these pathogens to the carcasses mainly occurs when the intestinal tract is punctured accidentally, consequently spilling the gut contents onto the dressed meats. This emphasises the need for not only good hygienic practices but also good slaughtering techniques in an abattoir (Nortjé & Naude, 1981). Incidences of contamination associated with respiratory tracts have decreased due to the suspending of the stunned animal hence, the mucus is released outwards (Ayres, 1955; Church & Wood, 1992).

In the evisceration step, intact viscera present little hazard but leakage from the gastrointestinal tract could cause widespread contamination (Church & Wood, 1992). The pathogens introduced onto the carcass are mainly those associated with faecal matter (Bell, 1997). Contamination during the slaughter operations of carcass skinning and evisceration can also occur by the introduction of the pathogens onto the meat surfaces via personnel hands, saws, equipment and clothing (Dickson & Anderson, 1992; Selgas, Marin, Pin & Cassas, 1993; Sierra, *et al.*, 1995).

At the completion of dressing, the warm (30-40°C) and wet carcass surface is ideal for the proliferation of pathogens and spoilage microorganisms (Church & Wood, 1992). The carcass surface must therefore be cooled to 7°C or below to minimize microbial growth before it becomes a hazard. Various researchers (Cox, 1989; Farber, 1991; Palumbo, 1986) have also reviewed the possibility of growth of emerging pathogens at the chilling stage. Temperature control and monitoring in an abattoir is therefore very important, as it is the final stage at which control can be effected (Figure 2.3). However, cooling too rapidly must also be avoided as otherwise cold shortening (toughening) of the muscle can occur (Church & Wood, 1992; Kapsrowiak & Hechelmann, 1992).

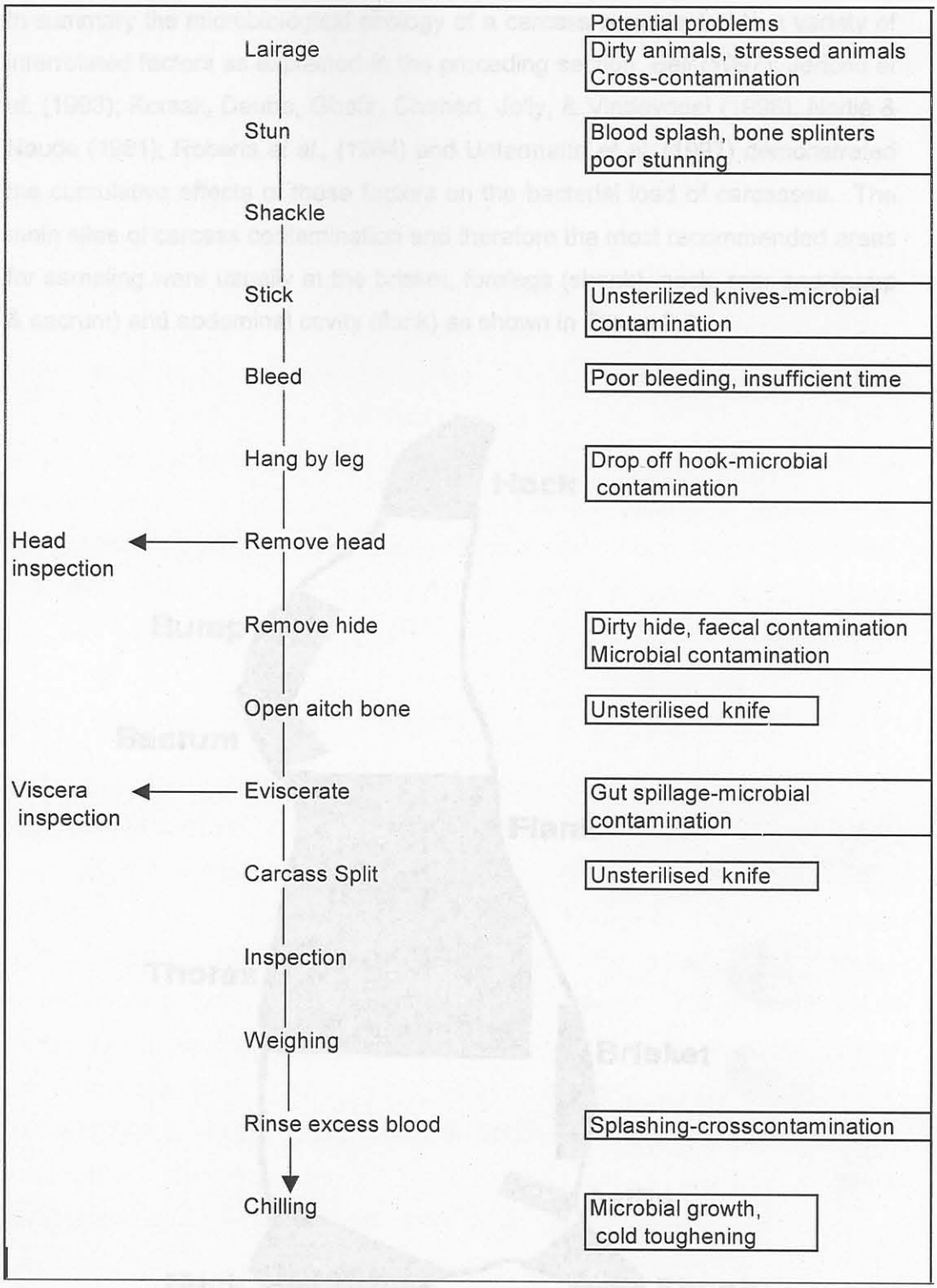


Figure 2.3: Typical cattle slaughter line and potential problems (adapted from: Church and Wood, 1992).

In summary the microbiological ecology of a carcass is subjected to a variety of interrelated factors as explained in the preceding section. Bell (1997); Jericho *et al.* (1993); Korsak, Daube, Ghafir, Chahed, Jolly, & Vindevogel (1998); Nortjé & Naude (1981); Roberts *et al.,* (1984) and Untermann *et al.* (1997) demonstrated the cumulative effects of these factors on the bacterial load of carcasses. The main sites of carcass contamination and therefore the most recommended areas for sampling were usually at the brisket, forelegs (shank), neck, rear end (rump & sacrum) and abdominal cavity (flank) as shown in Figure 2.4.

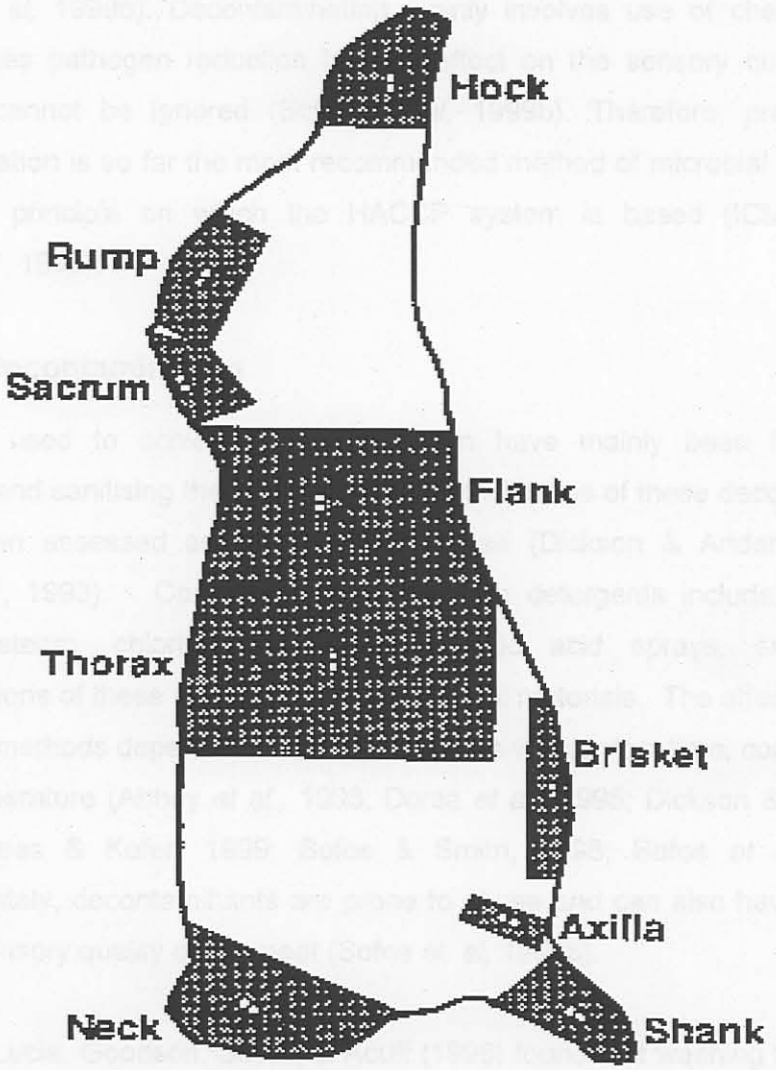


Figure 2.4: Lateral view of beef carcass depicting potential contamination points (adapted from: Jericho *et al.,* 1993).

2.4 MICROBIAL CONTROL

Elimination or reduction of microbial levels in meat has received considerable attention in recent years and the problem of reducing the number of bacteria from the carcasses has been approached in a variety of ways. The focus of microbial control by the various workers has been on either preventing contamination or decontaminating the carcasses (Abbey, Randall, Riemann, Kotrola, Wilson, Boyer & Brown, 1998; Dickson & Anderson, 1992; Dorsa, Cutter, Siragusa & Koohmarie, 1995; Pless & Kofer, 1999; Sofos & Smith, 1998; Sofos *et al,* 1999b). Decontamination mainly involves use of chemicals and emphasises pathogen reduction but the effect on the sensory quality of the product cannot be ignored (Sofos *et. al,* 1999b). Therefore, prevention of contamination is so far the most recommended method of microbial control and it is the principle on which the HACCP system is based (ICMSF, 1988; NACMCF, 1993).

2.4.1 Decontamination

Methods used to achieve decontamination have mainly been focused on washing and sanitising the carcasses. The efficiencies of these decontaminants have been assessed separately or combined (Dickson & Anderson, 1992; NACMCF, 1993). Commonly used sanitizing detergents include; hot water (82°C), steam, chlorine, short-chain organic acid sprays, and various combinations of these and approved bactericidal materials. The effectiveness of all these methods depend on a variety of factors viz. contact time, concentration, and temperature (Abbey *et al.,* 1998; Dorsa *et al.,* 1995; Dickson & Anderson, 1992; Pless & Kofer, 1999; Sofos & Smith, 1998; Sofos *et al,* 1999b). Unfortunately, decontaminants are prone to abuse and can also have an effect on the sensory quality of the meat (Sofos *et. al,* 1999b).

Castillo, Lucia, Goodson, Savell, & Acuff (1998) found that washing the carcass surface with hot water at 95°C brought about significant reductions on the microbial levels. They suggested that it could be used as a CCP in a HACCP system. However, this method has it flaws as shown by earlier studies carried out on hot water washing by Dorsa *et al.* (1995). They indicated that despite the positive achievements of the reduction in microbial levels, the hot water wash extended hydration of the carcass and therefore protected a limited number of

bacterial populations.

Various researchers have also reviewed the use of disinfectant solutions. Delazari, Iaria, Riemann, Cliver & Mori (1998) found that the efficacy of chemicals was dependent on the type of exposed tissue, lean or fat and the type of microorganism. Smulders and Greer (1998) further corroborated this by showing that some meat pathogens are sensitive to organic acids (e.g. *Yersinia enterocolitica*) while others are resistant to it (e.g. *E. coli*). The suggestion that the disinfectants are bacterium specific may also indicate that the choice of disinfectant used should ideally be broad spectrum and be able to act in any tissue type. It is also necessary to avoid reduced competition due to selective disinfection in the niche leading to dormant bacteria expressing themselves (Sprenger, 1995). Unfortunately, most of the broad-based disinfectants cannot be used on food products.

Dickson & Anderson (1992) reviewed works by various researchers on carcass decontamination by washing and sanitising systems. In all the systems production of pathogen free meat could not be guaranteed and the safety of the products still depended a great deal on good manufacturing practices. Smulders & Greer (1998) added that use of decontaminants could easily be abused and used as a means of concealing poor hygiene. But more important is the fact that these methods can only reduce the initial levels of bacteria and there is no control over future re-contamination of the product. This can even be in cases where such problems could otherwise have been prevented or avoided through proper design, processing techniques and good manufacturing practices (ICMCF, 1988; NACMCF, 1993; Pless & Kofer, 1999).

Van Netten, Valentijn, Mossel, & Huis In 'T Veld (1997) indicated that HACCP is a prerequisite for ultimately rendering meat carcass surfaces free of pathogens no matter what decontamination method was used. Therefore, various carcass intervention procedures such as washing, sanitizing agents and pasteurization can only be used as an adjunct to HACCP. These methods would therefore be used only to improve the bacterial condition of the carcass by intervention at specific points in the process (Dorsa *et al.,* 1995; Gorman, Morgan, Sofos & Smith, 1995; Gorman, Sofos, Morgan, Schmidt & Smith, 1995; Prasai, Acuff, Lucia, Hale, Savell, Morgan, 1991; Siragusa *et al.* 1995). Other schools of

thought also indicate that the decontamination of the carcass is not useful due to recontamination and growth during further processing (Smulders & Greer, 1998; Sofos & Smith, 1998). In addition, use of the decontaminants are also subject to several factors, including safety, product quality, efficacy, adaptability, need for decontamination and cost (Sofos & Smith, 1998).

2.4.2 Application of HACCP

The modern approach to abattoir contamination control relies on the HACCP system. The HACCP system provides the most effective means for minimizing microbial contamination on meat carcasses, thus reducing the risk of food-borne illness in humans (Bolton, Oser, Cocoma, Palumbo & Miller, 1999; Cross, 1996; Karr, Maretzki & Knabel, 1994; NACMCF, 1993; Yataro, Yutaka, Kimikazu & Tsutomu, 1999). Over the years researchers have noted that improvement of slaughtering techniques went a long way in controlling microbial levels better than use of decontaminants (Hechelmann, 1995a; Pless & Kofer, 1999; Sofos & Smith, 1998). This is especially important as the introduction of a HACCP programme adopts working patterns in which the processing techniques, design of the plant, flow of the product and personnel are all taken into consideration. With HACCP the main improvement of the microbial levels is achieved by identifying potential risk areas and the points are then monitored (ICMSF, 1988). In the case of abattoirs, monitoring mainly involves visual inspection to ensure that the slaughtering techniques are executed correctly and the product is well chilled. A study carried out by Bolton *et al.,* (1999) indicated that visual monitoring managed to reduce carcass contamination levels from approximately 8% to 1% over a period of 4 years with total aerobic counts falling by 99.8%.

2.4.2.1 *HACCP control measures*

HACCP control measures are any actions and activities that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level. The method of control is determined by risk analysis and depends to a great extent on the epidemiological data of the food-borne diseases of the specific food (refer 2.1). Before the control is effected the potential problematic areas have to be identified from the process flow chart. Figure 2.5 gives a general overview of the sites of contamination and the causes of contamination along the meat processing line from the live animal to the chilled carcass. It also shows

the points at which control can be effected within the beef slaughter and dressing process. This includes the carcass skinning, evisceration and chilling points (Kasprowiak & Hechelmann, 1992).

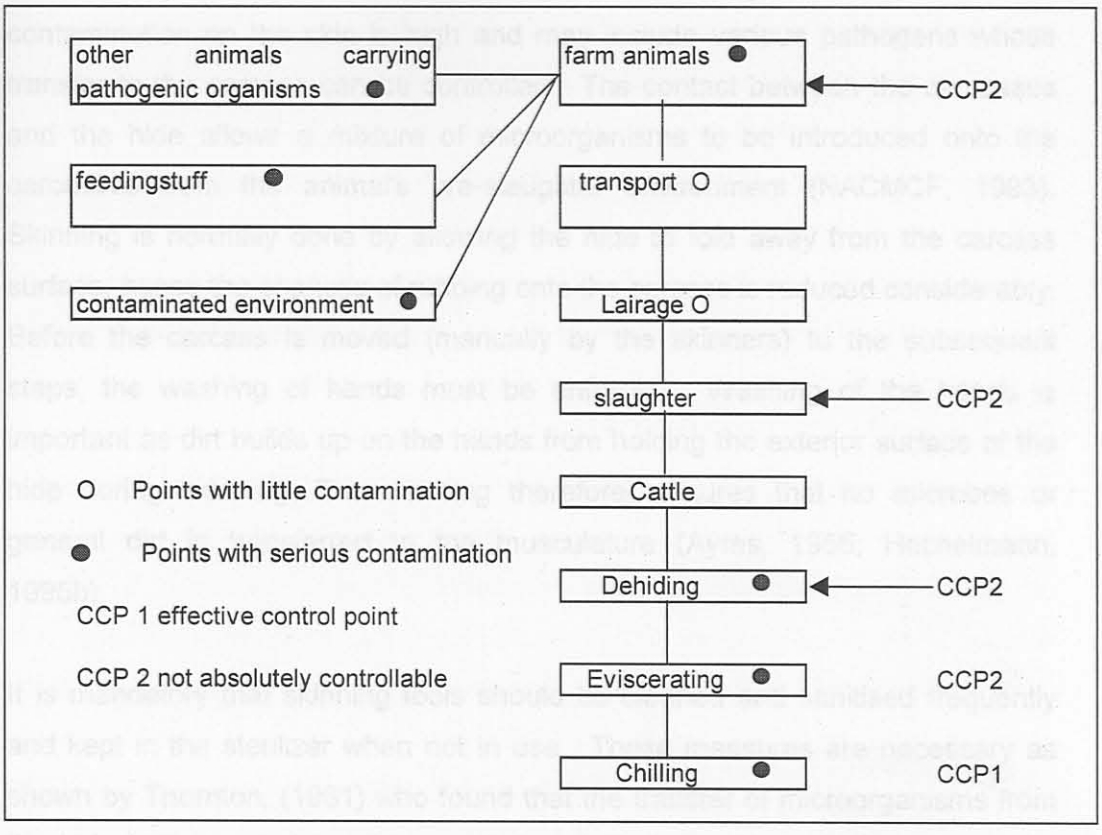


Figure 2.5: Causes of contamination and critical control points before and during the slaughter of cattle (adapted from: Kasprowiak & Hechelmann, 1992).

Control of microbiological hazards in an abattoir is usually carried out by:

- Temperature/time control of the chill room that can reduce levels of microbial growth;
- Strict adherence to the cleaning and sanitation programs, which can eliminate or reduce the levels of microbiological contamination;
- Personnel and hygienic practices, which can reduce the levels of microbiological contamination.

The processing should aim to ensure that each of the stages should be "cleaner" than the one before it and contamination from the previous process must

be avoided as much as possible (Bolton *et al.*, 1999).

2.4.2.1.1 Skinning

The skinning operation is considered as a critical control point because microbial contamination on the skin is high and may include various pathogens whose transfer to the carcass can be controlled. The contact between the carcasses and the hide allows a mixture of microorganisms to be introduced onto the carcasses from the animal's pre-slaughter environment (NACMCF, 1993). Skinning is normally done by allowing the hide to fold away from the carcass surface, hence the chances of rubbing onto the carcass is reduced considerably. Before the carcass is moved (manually by the skinners) to the subsequent steps, the washing of hands must be enforced. Washing of the hands is important as dirt builds up on the hands from holding the exterior surface of the hide during skinning. The washing therefore, ensures that no microbes or general dirt is transferred to the musculature (Ayres, 1955; Hechelmann, 1995b).

It is mandatory that skinning tools should be cleaned and sanitised frequently and kept in the sterilizer when not in use. These measures are necessary as shown by Thornton, (1981) who found that the transfer of microorganisms from the hide to the underlying tissues begins at the point of initial incision through the hide. The study indicated that this point tends to have the highest number of microorganisms with decreasing levels noted on the areas furthest removed from this region. Studies have shown that, either the blade, or contact of the carcass with loose skin, or personnel habits transfer these microbes (Ayres, 1955; Hechelmann, 1995b; Ridell & Korkella, 1993; Mackey & Derrick, 1979).

2.4.2.1.2 Evisceration

The process of evisceration is carried out as follows: the breastbone and the aitchbone are sawed through exposing thoracic, abdominal and pelvic cavities. The viscera is then loosened and removed in one continuous operation. Puncturing of the gastrointestinal tract by the knives leading to spilling of the gut contents on the abdominal cavity must be avoided. The evisceration step therefore also forms a very important control point in a HACCP program.

The carcass is then split into two halves, inspected and chilled (Hechelmann, 1995b).

2.4.2.1.3 Chilling

The chilling stage forms the final process at which control can be exercised in an abattoir. The step consists of lowering the carcass temperature below 10 °C and keeping it near 0 °C. Care must be taken to ensure that the chilled carcass temperature is at least below 10°C within a period of 24 hours. This method inhibits the growth of most pathogens causing food-borne diseases. For instance lowering the temperature to about 20 °C suppresses the growth rate of *Clostridium perfringens,* to levels were it is almost insignificant under practical conditions but total inhibition of growth is attained at 6.5 °C. In the case of *Salmonella* and *Staphylococcus aureus* growth is inhibited at 5.2 °C and 6.7 °C respectively. It should be noted that chilling does not kill microbes therefore the raw material must be of sound quality, cooled soon after inspection to prevent growth of mesophiles and ensure that there is minimal handling of the carcasses. Maintenance of the cold chain should continue up to the consumer level. Within the chill room the carcasses should be kept from touching each other to allow for proper airflow and avoid any chances of cross contamination (ICMSF, 1988; NACMCF, 1993; Rosset, 1982).

In conclusion proper control and monitoring of these critical control points are essential to avoid any transfer of microorganisms to the carcasses as illustrated in Table 2.9. In its entirety, improvement of slaughtering techniques can therefore be relied upon to achieve better microbial controls on the slaughter line. However, even with HACCP, zero tolerance in an abattoir is not practical and cannot be attained (NACMCF, 1993).

Table 2.9: Generic HACCP plan: Critical Control Points for Beef Slaughter (NACMCF, 1993).

Process/step	CCP	Critical limits	Monitoring procedure/frequency	Corrective action	Records	Verification
Skinning	CCP 1	≤20% of carcasses with dressing defects	Operator observes effectiveness of skinning process for each carcass. Visual analysis conducted under adequate lighting	Add operators Reduce chain speed Conduct carcass trimming	Random post - skinning carcass examination	Examination of random carcasses after skinning is complete using sampling plan sufficient to assure process control Survey of review records Initially conduct microbiological analysis for aerobic mesophiles and/or enterobactericiae to establish baseline data on expected bacterial numbers. Periodic follow up analysis to verify process control Review control charts to confirm that sampling frequency is sufficient to detect 20% defect criteria

Table 2.9: Generic HACCP plan: Critical Control Points for Beef Slaughter (NACMCF, 1993) continued.

Process/step	CCP	Critical limits	Monitoring procedure/frequency	Corrective action	Records	Verification
Evisceration	CCP	0% 0ccurence of the following defects for a single carcass: faecal material, ingesta, urine or abscesses		(1)Trained employee immediately trims defect area on carcass (2) Add operators (3) Reduce chain speed (4) sanitize soiled evisceration tools with 82.2 ° C water (5) sanitize soiled clothing with 48.9 ° C water or appropriate sanitizer	Random post-evisceration carcass examination	Supervisory review of records and operations Random examination of carcasses after evisceration using sampling plan sufficient to assure process control
Chill	CCP	Deep muscle (6in) temperature of 7 ° C within 36 h, reaching 10 ° C after the first 24h Carcass spaced a minimum of 1-2 in apart	Continual confirmation of environmental conditions (temperature) Monitor carcass spacing upon arrival to chill coolers Conduct random temperature monitoring of carcasses after appropriate chill time	Adjust carcass spacing Adjust chill cooler temperature Alert maintenance if cooler unit is not functioning properly Continue chilling carcasses until internal temperature reaches 10 ° C	Chill log	Supervisory review of records Review thermometer calibration Periodic monitoring of cooling rates of deep muscle tissue through the use of temperature recording devices

2.4.2.2 *Benefits of HACCP in the meat industry*

Benefits of HACCP in the meat industry are multifold and the benefits can be reaped across board. Primarily, it places the responsibility for ensuring food safety on the personnel. This is due to the well-organized and documented plan, which comes with a HACCP system. A HACCP system also consistently ensures safe food production. By assuring safety during processing with a HACCP system, the resources expended on the finished products can be devoted to process control and optimizing the process. In addition, to safe production of food, the effective monitoring of CCP's can lift the whole operation hence ensuring improved quality (Stier & Blumenthal, 1995). Perceived sense of enhanced safety and quality always follows from the management to the workers. This is normally a result of enhanced understanding of the operation or increased worker involvement, which are inherent features of a HACCP system. This leads to process optimization as employee morale is boosted, leading to greater efficiencies as the personnel feel they are part of the program (Stier & Blumenthal, 1995).

Secondly, in operations like abattoirs which are mainly supplier or producer oriented, a HACCP system makes them more desirable trading partners due to assured safe and quality products. Implementation of a HACCP in one system within a food chain also always sparks a chain reaction, as the specific entity in question, expects the same standards to be maintained by his suppliers of raw materials and distributors of the completed product. Therefore as one link in the food chain adopts a particular quality management system its influence on other units cannot be overlooked. For instance expectations of adoption of a similar approach by the farm production unit would increase to ensure a steady supply of better animals (Gardner, 1997; Stier & Blumenthal, 1995).

Other factors like reduced product liability cannot be overlooked. Scarlett (1991) summarized the HACCP approach to product liability as follows; "to a food technologist HACCP is a food safety tool which has well developed methods for preventing mistakes from happening. To a lawyer, however, HACCP is a damage control procedure whereby the potential defendants in a multimillion-dollar lawsuit can try to minimize the possibility that their food product will be responsible for dozens, or even hundreds, of sick, very angry and extremely

vengeful plaintiffs". Interviews conducted by Stier & Blumenthal (1995) amongst food processors indicated that HACCP could allow them to reduce insurance rates.

Finally, it has an added advantage as it permits more government oversight in food production. This places the producers in a position where they are part of a rule making process should regulations demand its implementation. For instance, a government investigator can easily determine and evaluate both current and past conditions that are critical to ensuring the safety of food. It may also assist food companies to compete effectively in the world market (Culler, 1997; Jouve, 1998).

2.5 SUMMARY

Previous and current data on epidemiology of food-borne illness associated with meat indicate that meatborne diseases are still part of the meat processing chain. This is inspite of the hygiene measures in place in most abattoirs and the various modern decontamination procedures currently available in the market. It is in light of this that this project was proposed.

The HACCP system gives a distinct possibility of reducing pathogens as previously confirmed in other food industries as it focuses on controlling entry of microorganisms into the product. The other inspection methods mainly focuses on removal of the microorganism, this is especially a difficult feat in the meat industry as a total microbial destruction step is not available.

2.6 OBJECTIVES

The main objective of the study was to determine the effect of HACCP implementation on the microbiological status of carcasses in a specific C-class abattoir.

The following specific objectives were formulated:

To determine the effect of HACCP implementation on aerobic plate counts, total coliforms counts, *Staphylococcus aureus* counts, *Escherichia coli* counts, *Clostridium perfringens,* counts and *Salmonella* presence or absence on bovine carcasses at critical control points on the slaughter line.

To determine the effect of chilling on aerobic plate counts, total coliforms counts, *Staphylococcus aureus* counts, *Escherichia coli* counts, *Clostridium perfringens,* counts and *Salmonella* presence or absence on bovine carcasses before and after HACCP implementation.

CHAPTER 3

MATERIALS AND METHODS

3.1 EXPERIMENTAL DESIGN

The study was carried out in a class C[1] abattoir which processes on average 30-40 heads of cattle per day in the Hammanskraal region of South Africa. The study was carried out during the winter season. The experimental design that was followed is given in Figure 3.1. This involved a hygiene evaluation of the plant, a hazard analysis and a microbiological analysis. The hygiene evaluation was done using standard hygiene evaluation sheets from the Red Meat Abattoir Association of South Africa (RMAA) in conjunction with FAO hygiene assessment forms of abattoirs adapted from Church & Wood (1992) as shown in Appendix 1. The hazard analysis was done by studying the step by step practices employed in the processing and assessing them in detail to identify sources and mechanisms of contamination so as to be able to determine critical control points. All this was done concurrently with collection of microbial baseline data. The information then formed the guidelines for the training programme for personnel on the processing line. Subsequently, microbial data (HACCP data) was collected and compared with baseline data to assess whether training had any significant effect on microbial loads.

3.1.1 Hygiene evaluation

Hygiene is basic to the safety and quality of food throughout the world and is the responsibility of every one at work and every individual has a role in keeping the plant in good sanitary condition (Sprenger, 1995). A system of controlled movement of staff, product equipment and materials should be developed and organized so that contamination is not carried from one area to the next (Church & Wood, 1992). The need for the hygiene evaluation is because basic hygiene or good manufacturing practices form the foundation for a HACCP system. This ensures that the system once fully

[1] Class C abattoirs can slaughter a maximum of 50 herd of cattle a day.

implemented is focused on the product and any potential food illness or food spoilage can be controlled as emphasized in the previous sections (Lee & Hathaway, 1998).

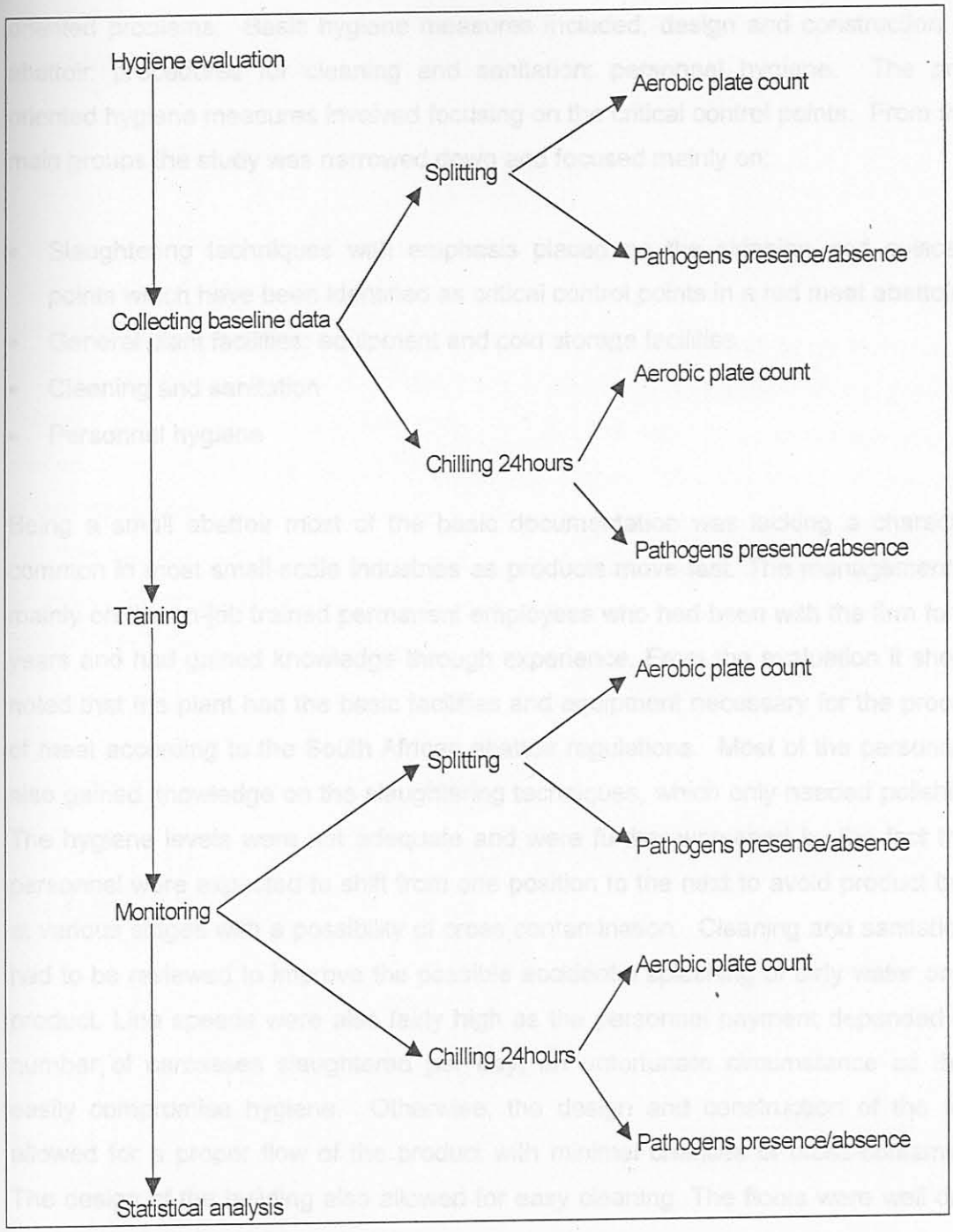


Figure 3.1: Experimental design for the study.

Note: Sampling was done after splitting and not at skinning and evisceration of the carcass to avoid reducing the chain speed of the carcasses.

Hygiene evaluation of the plant was carried out from the lairage to the dispatch over a period of 6 weeks. The evaluation was divided into basic hygiene problems and process oriented problems. Basic hygiene measures included; design and construction of the abattoir; procedures for cleaning and sanitation; personnel hygiene. The process oriented hygiene measures involved focusing on the critical control points. From the two main groups the study was narrowed down and focused mainly on:

- Slaughtering techniques with emphasis placed on the skinning and evisceration points which have been identified as critical control points in a red meat abattoir
- General plant facilities: equipment and cold storage facilities
- Cleaning and sanitation
- Personnel hygiene

Being a small abattoir most of the basic documentation was lacking a characteristic common in most small-scale industries as products move fast. The management relied mainly on the on-job trained permanent employees who had been with the firm for many years and had gained knowledge through experience. From the evaluation it should be noted that the plant had the basic facilities and equipment necessary for the processing of meat according to the South African abattoir regulations. Most of the personnel had also gained knowledge on the slaughtering techniques, which only needed polishing up. The hygiene levels were not adequate and were further worsened by the fact that the personnel were expected to shift from one position to the next to avoid product build up at various stages with a possibility of cross contamination. Cleaning and sanitation also had to be reviewed to improve the possible accidental splashing of dirty water on to the product. Line speeds were also fairly high as the personnel payment depended on the number of carcasses slaughtered per day, an unfortunate circumstance as this can easily compromise hygiene. Otherwise, the design and construction of the abattoir allowed for a proper flow of the product with minimal chances of cross-contamination. The design of the building also allowed for easy cleaning. The floors were well drained, washable and of suitable material.

While the evaluation was ongoing baseline data on the microbial levels of the plant was also being collected over a five weeks period. The baseline values were used to

develop preliminary HACCP plans for specific critical control points and to provide reference values to evaluate the impact of the HACCP program. Temperature profiles of the chilled carcasses were taken after 24 hrs using probe thermometers. The study was done according to National Advisory Committee on Microbiological Criteria for Food guidelines on HACCP for raw beef (see Table 2.9).

3.1.2 Hazard analysis

The hazard analysis consisted of inspection of the plant to identify sources and modes of contamination. Potential critical control points at which the hazards could be controlled were identified. To fully comprehend the impact of the slaughtering techniques and hygiene procedures on the microbial loads of the carcass the flow of the product was followed step-by-step as outlined in Figure 3.2. The skinning, evisceration and chilling stages are depicted Figure 3.3, 3.4 and 3.5 respectively. Single operations involved either individuals or a group of workers. The total number of people in physical contact with the carcass between slaughter and chilled storage were 12. These were divided as follows: two at stunning, four skinners, two at evisceration, one at splitting, one at inspection and two at chilling stage.

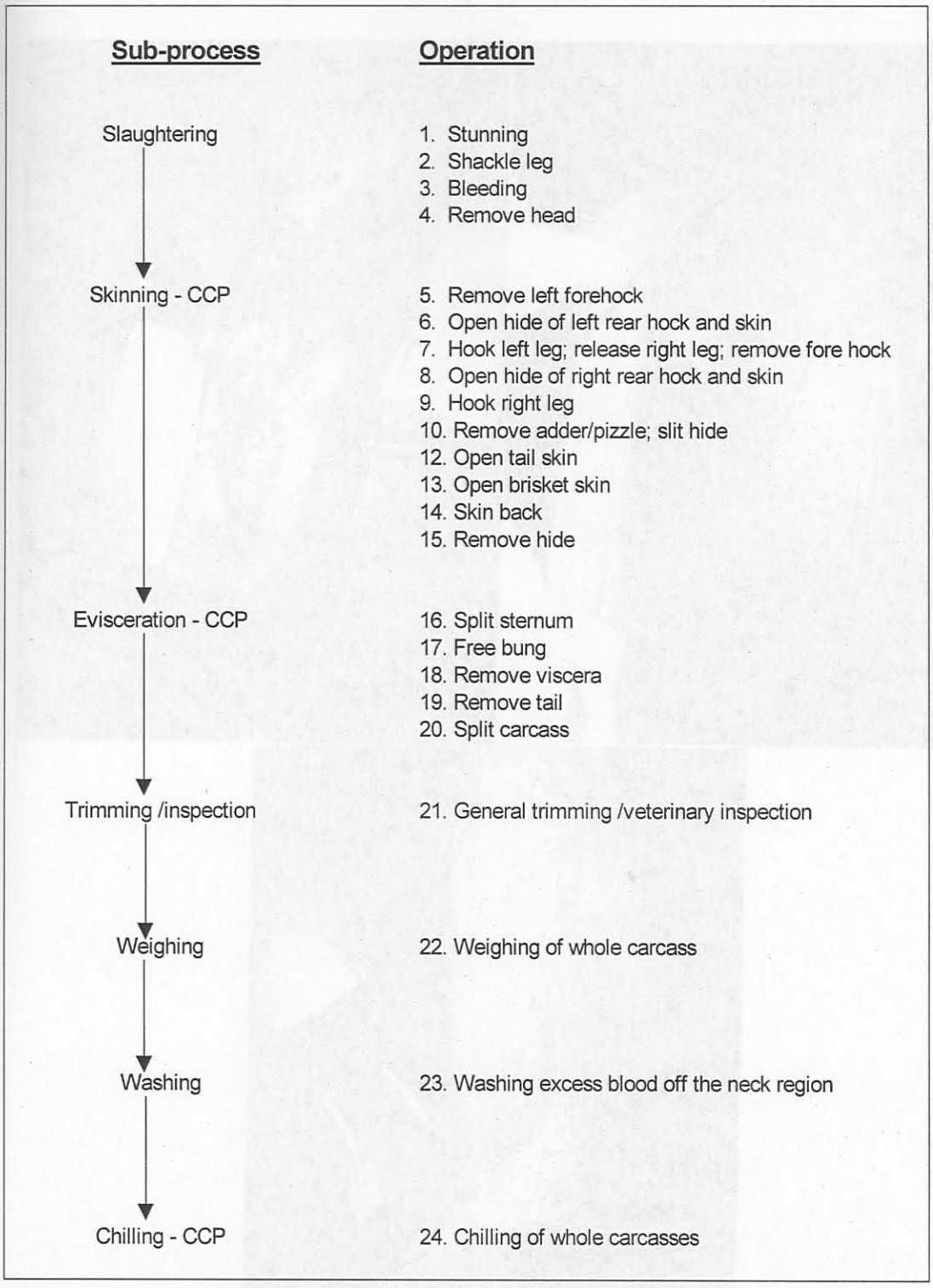


Figure 3.2: Flow chart and critical control points of beef processing steps for the abattoir under study.

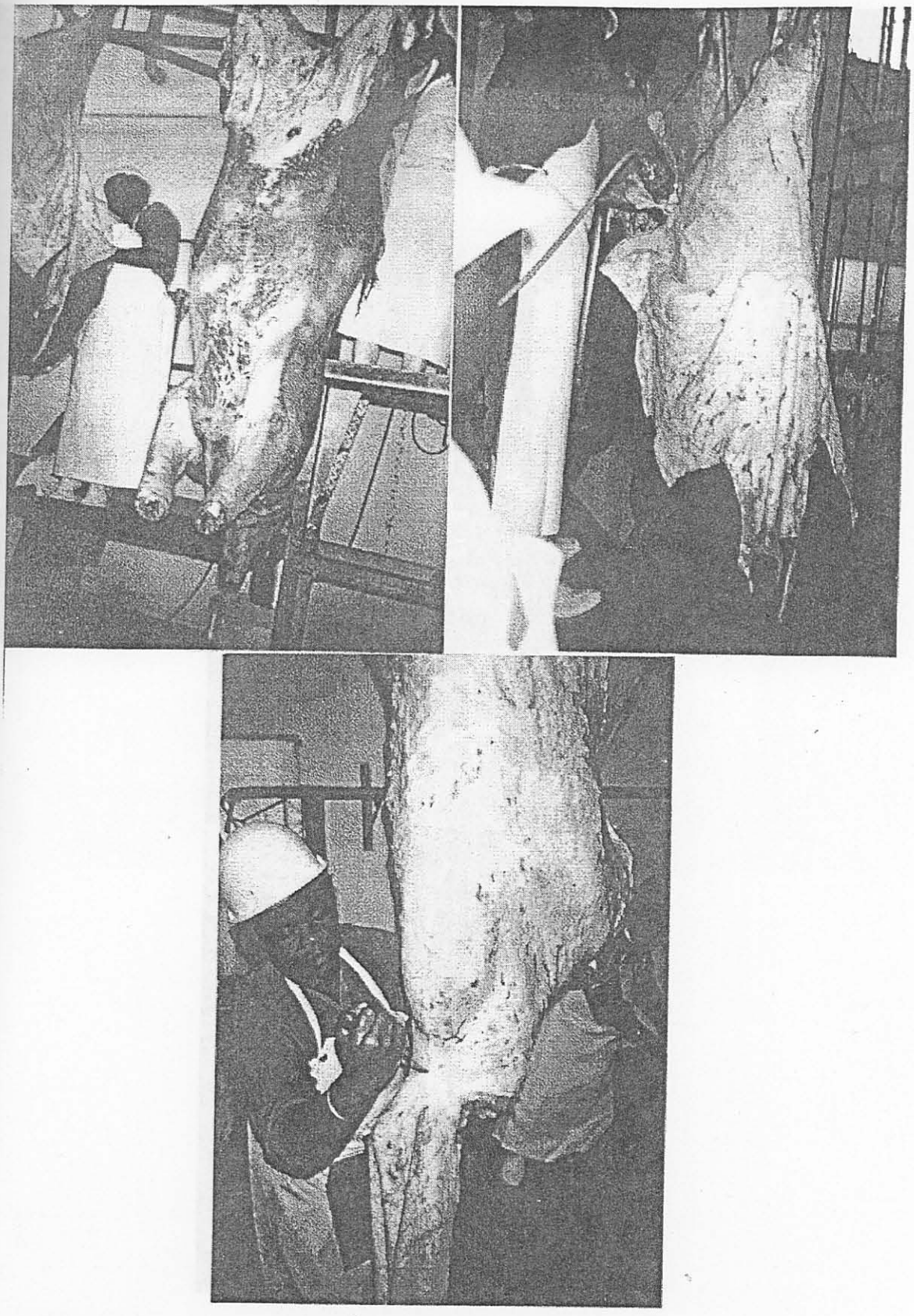


Figure 3.3: Pictorial presentation of the skinning steps.

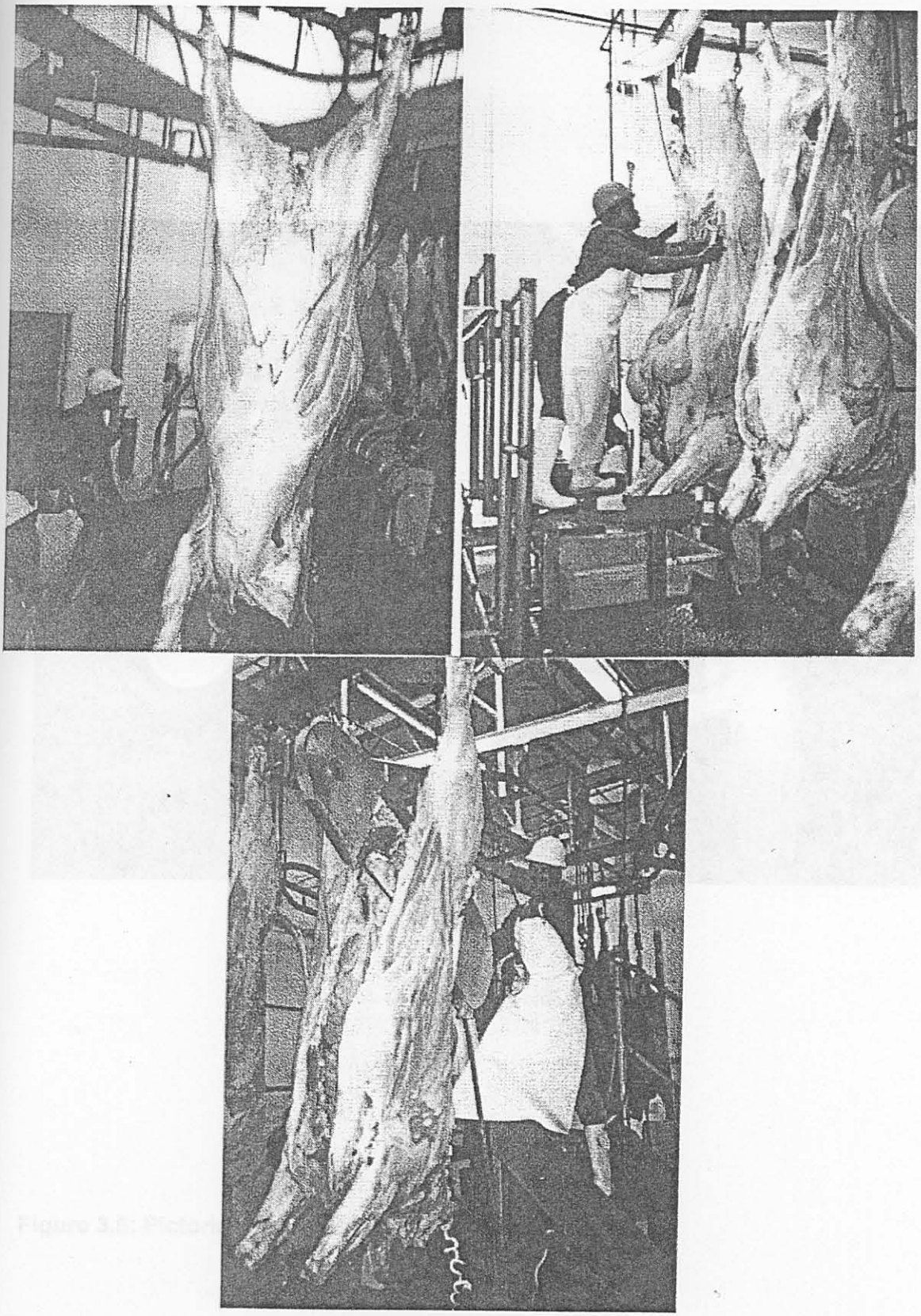


Figure 3.4: Pictorial presentation of the evisceration steps.

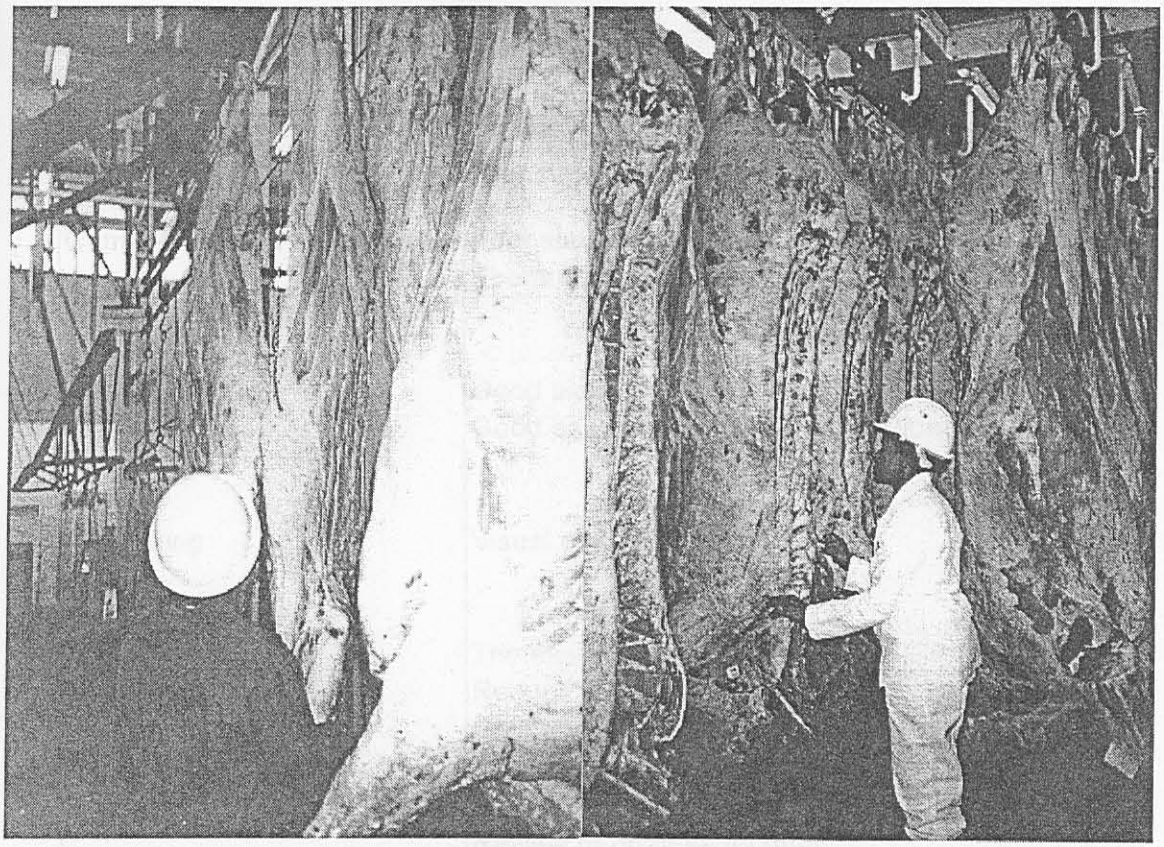


Figure 3.5: Pictorial presentation of the chilling step.

The critical control point work sheets for skinning, evisceration and chilling were prepared as indicated in Tables 3.1,3.2 and 3.3 respectively.

Table 3.1: Critical control point work sheet for the skinning step.

Process step	Skinning
Hazard	Contamination and growth of pathogens
Cause of hazard	Poor slaughtering techniques Poor sanitation of personnel and equipment
Control measure	Good slaughtering techniques Good sanitation of plant and equipment
Monitoring	Visual observation
Corrective action	Trimming Reduce chain speed GMP
verification	Microbiological analysis Review of process records
Records	Deviation/corrective action

Table 3.2: Critical control point worksheet for evisceration.

Process step	Evisceration
Hazard	Contamination and growth of pathogens
Cause of hazard	Poor slaughtering techniques-spilling of gut Poor sanitation of personnel and equipment
Control measure	Good slaughtering techniques Good sanitation of plant and equipment
Monitoring	Visual observation
Corrective action	Trimming Reduce chain speed GMP
Verification	Microbiological analysis Review of process control
Records	Deviation/corrective action

Table 3.3: Critical control point work sheet for the chilling step.

Process step	Chilling
Hazard	Growth of pathogens
Causes of hazards	Insufficient Time/Temperature maintenance
Control measures	Maintain temperature/time < 10oC within the first 24 hours
Monitoring	Periodic monitoring of carcasses and chill room temperature
Corrective action	Evaluate cause and prevent reoccurence Chill further
Verification	Periodic calibration of thermometer Review of temperature records Microbiological analysis
Records	Thermometer calibration log Deviation or corrective action

To be able to attain the targets for the CCPs, the RMAA trained the personnel on slaughtering techniques and the proper hygienic handling of the carcasses. The overall objective was to ensure that personnel were able to fully comprehend the "why" of hygiene to be able to fully respond to the "how" of hygiene and understand that hygiene and good housekeeping are a prerequisite for low microbial loads on the carcass.

3.2 MICROBIOLOGICAL ANALYSIS

Non destructive sampling (swabbing) was used as the study was carried out in a commercial abattoir where carcass excision is not practical as this affects the physical appearance of the carcass (Dorsa, Cutter & Siragusa, 1996). The nature of the swabbing method is such that the swab is rubbed on to the surface to collect the sample. The disadvantage with this method is that it does not always give precise results, as it cannot remove bacteria firmly attached to the surface (Benito, Pin, Marin, Garcia, Selgas & Casas, 1997). However, comparative studies done by Dorsa *et al.* (1996); Korsak *et al.* (1998) and Gill & Jones, (1998) on excision versus swab sampling showed that despite this disadvantage, swab sampling has more advantages than excision sampling. These includes not only maintaining the carcass surface intact but also saving on time and a considerably larger number of swabs can be examined with the same expenditure on cost.

Studies done by Untermann *et al.* (1997) also showed that there is a considerable variance of colony counts that occur within a carcass on the same day of slaughtering (section 3.3). This therefore makes this technique a practical method to examine a much more representative area of a single carcass than by excision of the carcass for hygiene monitoring of carcasses along the line. Untermann *et al.* (1997) further indicated that to get a useful trend on hygiene at least five to six swab samples from at least ten to fifteen carcasses would be necessary.

Jay (1992) indicated that for raw meat products potential safety and quality could be estimated with the use of indicator microorganisms including aerobic plate count, coliform count and *Escherichia coli* count. Coliform count and *Escherichia coli* count provide estimates of faecal contamination and poor sanitation during processing and has been recommended as appropriate for assessing the carcass dressing process for HACCP system purposes (Gill, Mcginnis, & Badoni, 1996).

3.2.1 Collection and treatment of samples

A total of 100 carcass sides were sampled from the plant throughout the study period. Of these, 50 carcasses formed the baseline data and the other 50 was sampled in the third phase of the study after training of the personnel. A total of 10 half carcasses were sampled per day (5 after splitting and 5 after chilling for 24 hours). The sampling was done on the carcasses within one hour of stunning after splitting and after 24 hours of chilling. It is important to sample the carcass soon after slaughtering, as bacteria tend to adhere to the carcass surface firmly with time. The ease and firmness with which the bacteria attach to the carcass play an important role in the sanitary quality of the meat. It should be noted that attachment is the first step in contamination and may lead to subsequent surface colonisation if conditions permit. The degree of attachment is important as it affects the swab sampling results (Benito *et al.,* 1997; Selgas *et al.*, 1993).

Samples were collected from four sites on the carcasses: the right or left brisket, right or left neck, the right or left fore leg and the right or left flank. These problem areas had the advantage of accessibility while the carcasses are on the line. The sampled points are illustrated in Figure 2.4 (Section 2.3.2.3). The choice of these sites as potential problem sites of carcasses during skinning and evisceration was based on observation during the first phase of the study (hygiene evaluation) in this particular abattoir. These sites compared well with studies by Bell (1997), Gill, Mcginnis & Bryant (1998); Gill *et al.* (1996); Korsak *et al.* (1998) and Untermann *et al.* (1997).

Sampling was done by swabbing a 10 cm^2 area of each of the four sites. A sterile gauze swab (10 x 10 cm^2 eight ply, curity gauze sponge: Johnson & Johnson, South Africa) which had been pre-moistened with 2% w/v peptone water was used (Dorsa *et al.*, 1996; Gill & Jones, 1998). The swab was rubbed horizontally and then vertically over the carcass surface (sterile gloves were worn over hands and changed between carcasses). A 100cm^2 sterile template was used to define the specific areas making a total of 400cm^2 per 1/2 carcass. The swab was then placed in a stomacher bag containing 10 ml of chilled 2% w/v peptone water. The samples were then placed in insulated containers and stored on ice while being transported

to the Council for Scientific and Industrial Research (CSIR) laboratories for microbial analysis within four hours.

3.2.2 Reagents and Materials

In the laboratory the swabs used for each carcass were pooled to form 40ml of the 2% w/v peptone water and then homogenised in a stomacher laboratory blender (Model 400 type BA 7021) for 2 minutes. A 1-ml portion of each homogenate was used to prepare 10-fold dilutions, to 10^3, in 2% w/v peptone water (Merck C134). The dilutions were spread on duplicate plates of various media except for *Salmonella* for which rapid method analysis was done for confirmation of either presence/absence. Conventional plate count analysis was carried out for the following bacteria: Total aerobic count, Total coliforms, *Escherichia coli, Clostridium perfringens,* and *Staphylococcus aureus.*

3.2.2.1 *Buffered peptone water*

Buffered peptone water (Merck C134) was used as a diluent.

The composition of the buffered peptone water used to achieve a 2% w/v of peptone water was as follows:

Peptone	10g
Sodium Chloride (NaCl)	5g
Disodium hydrogenorthophosphate dodecahydrate ($Na_2HPO_4.12H_2O$)	9g
Potassium dihydrogenorthophosphate (KH_2PO_4)	1.5g
Distilled water	1000ml

The buffer was prepared as follows:

The already premixed powder was dissolved in the distilled water by boiling. Then 10mls measured buffer quantities were prepared for swabbing as well as 9ml quantities for dilutions. The buffered peptone water was sterilised for 15 min at

121°C. The 10ml buffered peptone water was sterilised in Schott bottles whereas the 9ml buffered peptone water was sterilised in test tubes with aluminium caps. An Everlight vertical type autoclave TA-630 was used for the sterilisation of the buffer as well as the other media. The consecutive dilutions in the test tubes were made by diluting 1ml of each sample in 9ml sterile buffered peptone water.

Summaries of the dilutions are as follows:

2 swabs in 20 ml buffered peptone water	Homogenate
1ml in 9ml	(1/10 or 10^{-1})
1ml in 9ml	(1/100 or 10^{-2})
1ml in 9ml	(1/1000 or 10^{-3})

Every dilution was made after mixing of the sample in the test tube using a Vortex (Vortex mixer VM-300 from Gemmy Industrial Corporation).

3.2.2.2 *Aerobic plate count*

Nutrient agar (Merck C1) was used to determine the aerobic plate count of organisms in the samples. The medium was prepared as follows:

Nutrient agar (23g) was suspended in 1 litre of distilled water and brought to boil. This was done using a hot plate and stirred with a magnetic stirrer. The agar was then sterilised at 121°C for 15 min in sealed Schott bottles. The sterilised agar was left to cool to 50°C before plating.

Pour plates were made by mixing 1ml of 10^{-3} diluent sample with about 20ml of nutrient agar in sterile plastic petri dishes. These petri dishes were then incubated at 30°C for 48 hours before evaluation (Nortjé, Jordaan, Badenhorst, Goedhart, Holzapfel & Grimbeek (1990a). Incubation done at 30°C for 48 hours tended to yield higher counts of both the mesophiles and psychrotrophs (Nortjé, Nel, Jordaan, Naude, Goedhart, Holzapfel, & Grimbeeek, 1990b).

3.2.2.3 *Staphylococci species*

Baird Parker agar (Merck C41), a highly selective medium for the isolation and counting of coagulase-positive *staphylococci* was used.

The *Staphylococcus* species media was prepared according to the instructions by Merck. Baird-Parker agar base (63g) was suspended in 1 litre of distilled water and dissolved by boiling on a hot plate. Portions of 90 ml of the prepared agar were then dispensed into Schott bottles. The agar was sterilised at 121^{0}C for 15 minutes and cooled to about 50^{0}C.

At this stage the following were added:

i. 1ml of 1% sterile potassium tellurite (Bx15). This is a suspension of potassium-tellurite in distilled water and the ration is 0.5g: 49.5g respectively.

ii. 5ml 50% egg yolk emulsion (Bx14). This is a composition of 50ml-egg yolk added to 50ml sterile saline solution (0.85% NaCl in water)

The BX14 and BX15 were purchased already prepared.

The final medium contained Lithium Chloride and Tellurite, which inhibit the growth of accompanying microbial flora like coliforms, whereas pyrovate and glycine selectively stimulate the growth of *staphylococci*. *Staphylococci* show two characteristic features when grown on this opaque medium:

i. Characteristic opaque zones and clear rings outside the opaque zone are formed in the opaque medium as a result of the proteolysis (lecithin production) and lypolysis (lipase production) of the egg yolk in the medium, and

ii. Reduction of the tellurite to telluride by *Staphylococcus aureus* produces a black colouration of the colony.

Duplicate pour plates were made by surface plating the 0.2 ml of 10^{-2} diluent sample on the 20ml of the Baird-Parker agar in each sterile plastic petri dish. The plates were then incubated aerobically at 37^{0}C for 24hrs.

Staphylococcus aureus gave black, glossy, convex colonies (diameter 1-1.5 mm), with a narrow opaque margin surrounded by a clear zone, 2-5mm broad. Confirmation of the colonies was done using staphytect plus test DR 850 as follows:

1. The plates with the black glossy, convex colonies indicating presence of *Staphylococcus aureus* were tested.
2. A drop of the test latex was placed within one circle on the test card
3. A loop full of the colonies were removed and placed on the medium
4. The culture and the test latex was then mixed thoroughly and the test card gently rocked

Only agglutination, which was observed within a maximum of two minutes, was considered as positive presence of *Staphylococcus aureus.* Lack of agglutination indicates presence of other *Staphylococcus* species. Agglutination was an indication of the presence of clumping factor, protein A or MRSA capsular polysaccharides.

3.2.2.4 *Total coliforms*

Violet Red Bile agar (VRB) (Biolab CM107) was used for the enumeration of coliform organisms in the sample. The selectivity of the medium is due to the presence of bile salts and crystal violet. The presence of the coliform organisms is indicated by the red colour produced by the acid products of lactose fermentation. Lactose fermenters produce red or purple colonies often surrounded by a halo of the same colour.

The VRB agar was prepared as prescribed by Biolab (Code CM 107). VRB agar (40g) was suspended in 1 litre of water. It was then boiled until dissolved using a hot plate. The agar was then sterilised at 121^{0}C for 15 minutes in sealed Schott bottles. The sterilised agar was left to cool to 50^{0}C before using.

Duplicate pour plates were made by mixing the 1ml of 10^{-1} diluent sample with about 20 ml of the VRB agar in each sterile plastic petri dish. The plates were then incubated at 37^{0}C for 24hrs before evaluation.

3.2.2.5 *Escherichia coli*

Eosin Methylene Blue agar (Oxoid CM69) was used for the isolation of *Escherichia coli* in the samples.

The medium is made selective by the use of methylene blue and eosin, which in the presence of acid produced by *Escherichia coli* forms an amide bond giving the distinct metallic sheen. Other coliforms do not produce enough acid to cause this reaction. Eosin inhibits most gram-positive organisms.

The medium was prepared according to the method of Oxoid as follows: Eosin methylene blue agar (37.5g) was suspended in 1 litre of distilled water and brought to boil. This was done using a hot plate with a magnetic stirrer in place. The agar was then sterilised at 121°C for 15 minutes in sealed Schott bottles. The sterilised agar was left to cool to about 60°C. The media was then shaken in order to ensure uniform distribution of the flocculant, which is an essential part of this medium.

Duplicate pour plates were made by mixing the 1ml of 10^{-1} diluent sample with about 20ml of Eosin Methylene Blue in each sterile plastic petri dish. The plates were then incubated aerobically at 37^{0}C for 24hrs. Metallic green colonies were counted as positive *Escherichia coli* colonies.

3.2.2.6 *Clostridium perfringens,*

Perfringens, agar (Oxoid CM543) supplements type A (Oxoid SR076E) and type B (Oxoid SRO77E) was used for the cultivation and counting of *Clostridium perfringens.*

Being an anaerobic microorganism, the anaerobic conditions conducive for its growth was created by sealing the 90mm triple vented petri dishes with the activated Anaerocult A sachet (Merck 1.13827) moistened with water in an air tight jar. Anaerotest indicator (Merck 1.15112) was used to confirm anaerobic conditions. The blue reaction zone of the indicator was moistened, then introduced into the jar. Confirmation of the anaerobic environment was noted when the zone turned white. Anaerocult contains an oxygen-binding reagent mixture, which also produces carbondioxide.

The medium was prepared according to the instructions from Oxoid. Perfringens, agar (50g) was suspended in 500ml of distilled water and dissolved by bringing to boil on a hot plate. The agar was then sterilised at 121^{0}C for 15 minutes. It was then cooled to about 60^{0}C.

At this stage a vial of the following were added:

i. Perfringens (OPSP) Selective Supplement A (Oxoid SR076E)
ii. Perfringens (OPSP) Selective Supplement B (Oxoid SR077E)

A vial of Perfringens (OPSP) Selective Supplement A contains 50mg of sodium sulphadiazine, while a vial of Perfringens (OPSP) Selective Supplement B is composed of Oleondomycin Phosphate (0.25mg) and Polymyxin B sulphate (5000 I.U). To each of these vials 2 ml of sterile distilled water was aseptically added and mixed gently to dissolve. These were then added to 500ml of Perfringens agar base (OPSP) (CM543) mixed well and then let to cool to 50^{0}C. Duplicate pour plates were then made by mixing the 1ml of 10^{-1} diluent sample with about 20ml of the perfringens, agar in each 90ml sterile vented plastic petri dish. Once the petri dishes were dry they were overlayed with the agar. The plates were then incubated at 37^{0}C under anaerobic conditions (CO_2 atmosphere) for 18-24hrs.

3.2.2.7 *Salmonella*

Malthius analyzer (Malthius 2000 incubator) was used for the detection of *Salmonella*. The Malthius 2000 incubator is a bench mounted, water filled incubator

comprising control and scanning for the automatic electrical detection of microbial growth. The incubator is used in conjunction with a cooler unit, microcomputer and software, printer and Malthius cells. The full procedure is illustrated in Figure 3.6. The method uses a modification of Easter and Gibson's medium and Ogden's medium for the detection of *Salmonella* by conductance measurement (Easter & Gibson, 1985; Gibson, 1987; Ogden & Cann, 1987). It consists of the following stages:

- Pre-enrichment
- Growth and
- Confirmation

3.2.2.7.1 Pre-enrichment Broth (Glucose/Lysine Buffered Peptone Water)

Malthius *salmonella* Pre-enrichment Broth (30g; CM 509) was mixed well with 1 litre of distilled water according to the manufacturer's instruction. The broth (9ml quantities) was then dispensed into test tubes and then sterilised by autoclaving at 121°C for 15 minutes. Pre-enrichment broth is composed of:

Buffered peptone water		Biolab C134 Merck	20 g/l
L-Lysine	($C_6H_2ClN_2O_2$)	5700	5,0 g/l
D-glucose	($C_4H_{12}O_6$)	Univar 2676020	5,0 g/l

3.2.2.7.2 Growth medium

The detection mediums were prepared as follows:

Modified Easter and Gibson's method

Bacteriological peptone (Oxoid L37)	5.0g
Disodium hydrogen orthophosphate dihydrate ($Na_2HPO_4.2H_2O$) (Unilab 5043800)	10.0g
Dulcitol ($CH_2(OH).(CH.OH)_4.CH_2OH$) (Unilab 195600)	5.0g

Trimethylamine-N-oxide dihydrate ($C_3H_9NO.2H_2O$) (Sigma T-0514)	5.6g
Sodium Biselenite $C_6H_{12}N_2O_4S_2$ (Oxoid L121)	4.0g
L-cystine solution	10ml
Distilled water	1000ml
pH 7.2 ± 0.2	

Ogden's Lysine medium

Lactalbumin hydrolysate (Oxoid L48)	5.0g
D(+) glucose $C_6H_{12}0_6$ (BDH 10117)	10.0g
L-lysine monohydrochloride $C_6H_{15}ClN_2O_2$ (Sigma L5626)	10.0g
Sodium biselenite (Oxoid L121)	4.0g
L-cystine solution	10ml
Distilled water	1000ml
pH 7.0 ± 0.2	

All the ingredients of the specific media except the cystine solution were dissolved in water and heated. Overheating was avoided as this results in the production of an orange-red precipitate. The media is then cooled immediately and the cystine solution added to it. The cystine solution was prepared as follows: 0.1g L-cystine (Sigma C8755) was added to 15ml, 1 molar sodium hydroxide and diluted to 100ml with distilled water.

The Trimethylamine-N-oxide dihydrate (T.M.A.O) is important as the *Salmonella* reduces it to Trimethylamine (T.M.A) increasing the conductivity of the medium which can be detected and measured by the conductance/impedance Malthius equipment. During the incubation stage both positive and negative organism controls and negative media controls were included with each batch of sample.

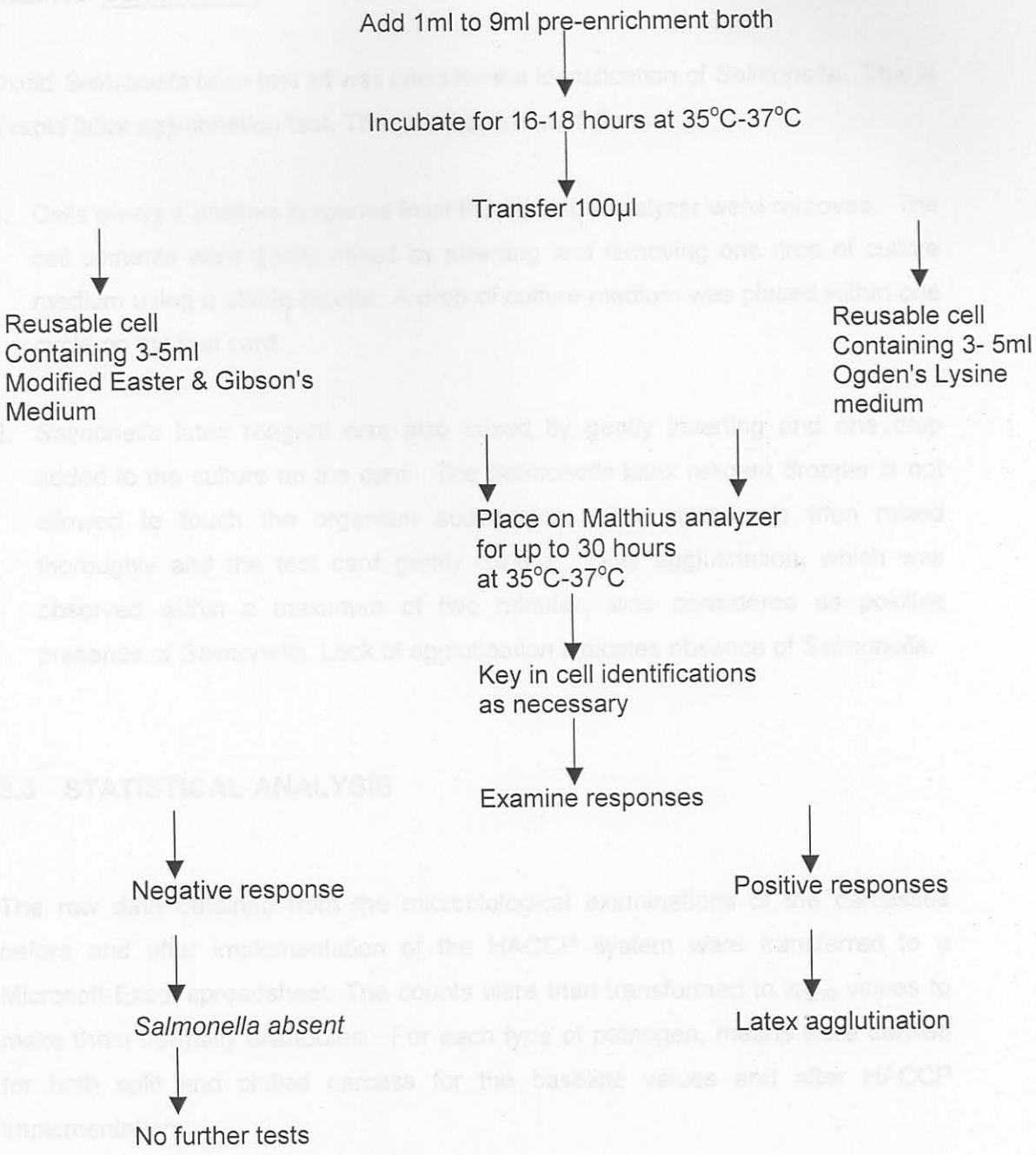


Figure 3.6: Malthius procedure for the detection of *Salmonella.*

3.2.2.7.3 Confirmation

Oxoid *Salmonella* latex test kit was used for the identification of *Salmonella*. This is a rapid latex agglutination test. The procedure is as follows:

1. Cells giving a positive response from the Malthius analyzer were removed. The cell contents were gently mixed by inverting and removing one drop of culture medium using a sterile pipette A drop of culture medium was placed within one circle on the test card.

2. *Salmonella* latex reagent was also mixed by gently inverting and one drop added to the culture on the card. The *Salmonella* latex reagent dropper is not allowed to touch the organism suspension. The mixture is then mixed thoroughly and the test card gently rocked. Only agglutination, which was observed within a maximum of two minutes, was considered as positive presence of *Salmonella*. Lack of agglutination indicates absence of *Salmonella*.

3.3 STATISTICAL ANALYSIS

The raw data obtained from the microbiological examinations of the carcasses before and after implementation of the HACCP system were transferred to a Microsoft Excel spreadsheet. The counts were then transformed to $\log_{10}$ values to make them normally distributed. For each type of pathogen, means were derived for both split and chilled carcass for the baseline values and after HACCP implementation.

A comparison of means for microbial populations aerobic plate count, total coliform count, *Escherichia coli* and *Staphylococcus aureus* was then done as follows:

- Between splitting and overnight chilling at baseline and after HACCP
- Before HACCP and after HACCP.

All comparisons were made using the Tukey's Honestly Significant Difference (HSD) test option at 5% level of significance using the Statistica programme (StaSoft, Inc., 1995) for windows. Statistical analysis -0.5 $\log_{10}$cfu/cm^2 was entered for samples in which *Escherichia coli* and C*lostridium perfringens,* was not detected (Gill, Badoni, Macginns, 1999; Gill & Jones, 1998).

CHAPTER 4

RESULTS

4.1 EFFECT OF HACCP IMPLEMENTATION ON MICROBIAL LOADS OF CARCASSES

Baseline data were taken to build up a profile on the microbial status of the plant to assess the effectiveness of the HACCP implementation on the pathogen loads. The statistical evaluations of the data showed that all variables were significantly reduced ($p < 0.05$) after HACCP implementation except for the aerobic plate count data and *Escherichia coli* at splitting of carcass as illustrated in Tables 4.1 and 4.2. The summaries of the mean $\log_{10}$ counts reductions for each pathogen at the carcass splitting step and after 24hours of chilling after HACCP implementation are also indicated in Tables 4.1 and 4.2 respectively.

A consistent positive hygiene trend was achieved for most of the variables tested over the 5-week duration as illustrated in Figures 4.1 to 4.5. Minimal detections in as low as 40% of the carcasses at splitting and 2% after 24 hours chilling for *Escherichia coli* and 14% at splitting and 0% after 24 hours chilling for *Clostridium perfringens,* was recorded after HACCP implementation. *Staphylococcus aureus* was reduced significantly as illustrated in Figure 4.2. HACCP implementation resulted in a significant overall reduction of total coliforms both at the splitting step and after chilling as illustrated in Figure 4.3. In general total coliform reductions of approximately 0.52 and 1 log cycle were observed for the splitting step and 24 hours chilling, respectively.

The presence/absence test was used for *Salmonella* detection and results are therefore reported as % detection. *Salmonella* was isolated from only 2% of the carcasses during the baseline data collection at the splitting step. After HACCP implementation all the samples were negative both at the splitting of carcass and after 24 hours chilling.

Table 4.1: Mean[1] microbiological counts ($\log_{10}$ cm^{-2}) on bovine carcasses at baseline and after HACCP implementation taken after splitting of the carcass.

Microorganism	Baseline N = 50	HACCP N = 50	Reduction[2]	P - value
Aerobic plate counts	3.77[a]	3.74[a]	0.03	0.85
Staphylococcus aureus	2.12[a]	1.53[b]	0.59	0.0001
Total coliform	1.08[a]	0.56[b]	0.52	0.0004
Escherichia coli	-0.06[a] (64% of carcasses)	-0.20[a] (40% of carcasses)	0.14	0.214
Clostridium perfringens,	-0.37[a] (28% of carcasses)	-0.48[b] (4% of carcasses)	0.11	0.006
Salmonella	2% of carcasses	0% of carcasses		

[2]Reduction = $\log_{10}$cfu/cm^2 Baseline - $\log_{10}$cfu/cm^{2}HACCP.

[1]Means within rows with different superscripts are significantly different ($p<0.05$)

Table 4.2: Mean[1] microbiological counts ($\log_{10}$ cm^{-2}) on bovine carcasses at baseline and after HACCP implementation taken after 24 hours chilling of the carcass.

Microorganism	Baseline N = 50	HACCP N = 50	Reduction [2]	P - value
Aerobic plate counts	3.33[a]	3.52[b]	-0.19	0.04
Staphylococcus aureus	1.98[a]	0.64[b]	1.34	0.0001
Total coliform	0.77[a]	-0.30[b]	1.07	0.0001
Escherichia coli	0.01[a] (62% of carcasses)	-0.50[b] (2% of carcasses)	0.51	0.0001
Clostridium perfringens,	-0.44[a] (14% of carcasses)	-0.50[b] (0% of carcasses)	0.06	0.02
Salmonella	0% of carcasses	0% of carcasses		

[2]Reduction = $\log_{10}$cfu/cm^2 Baseline - $\log_{10}$cfu/cm^{2}HACCP.

[1]Means within rows with different superscripts are significantly different ($p<0.05$)

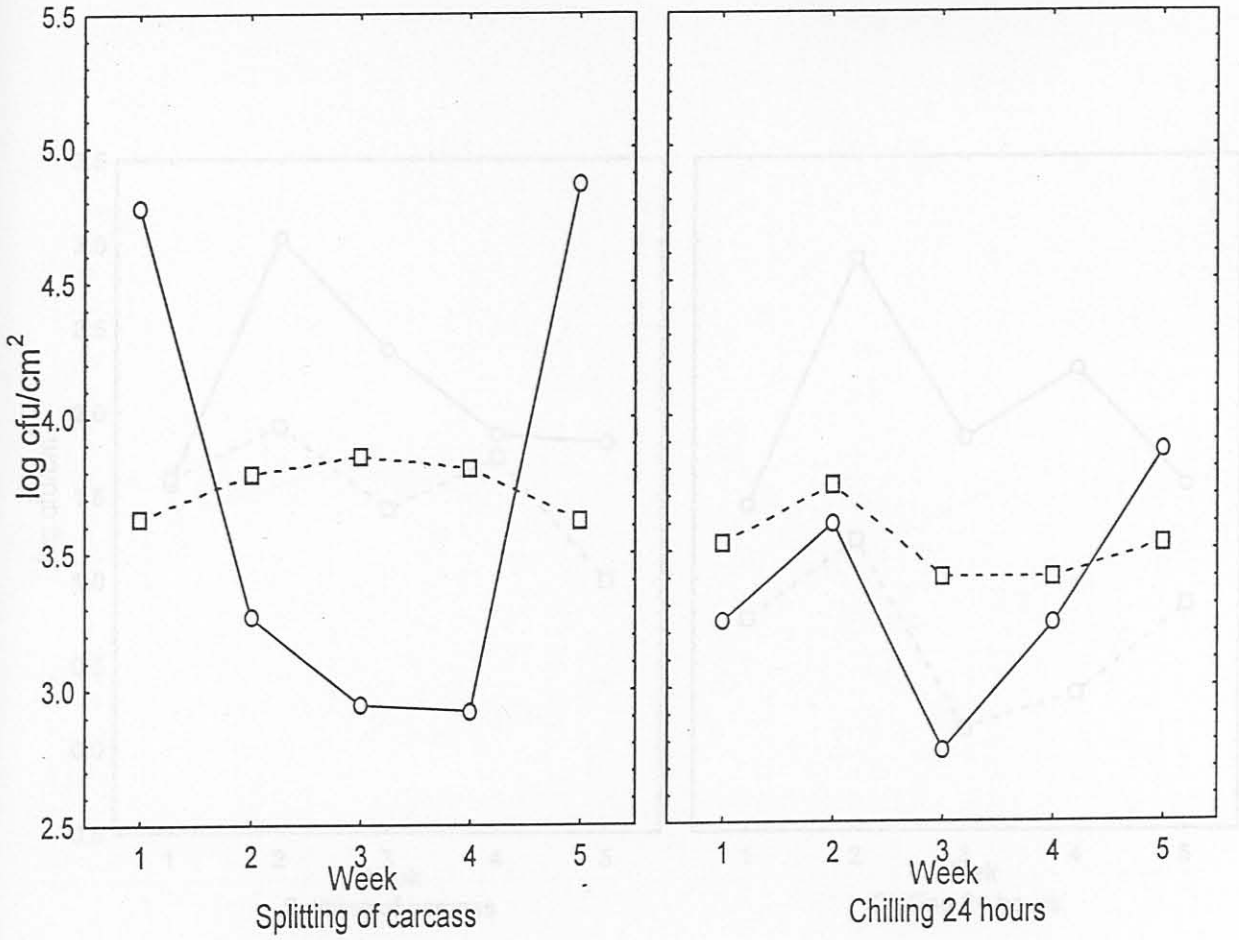


Figure 4.1: Effect of HACCP implementation (O = before training □ = after training) on aerobic plate counts ($log_{10}cfu/cm^2$) at the carcass splitting step (p = 0.85) and after 24 hours chilling (p = 0.04).

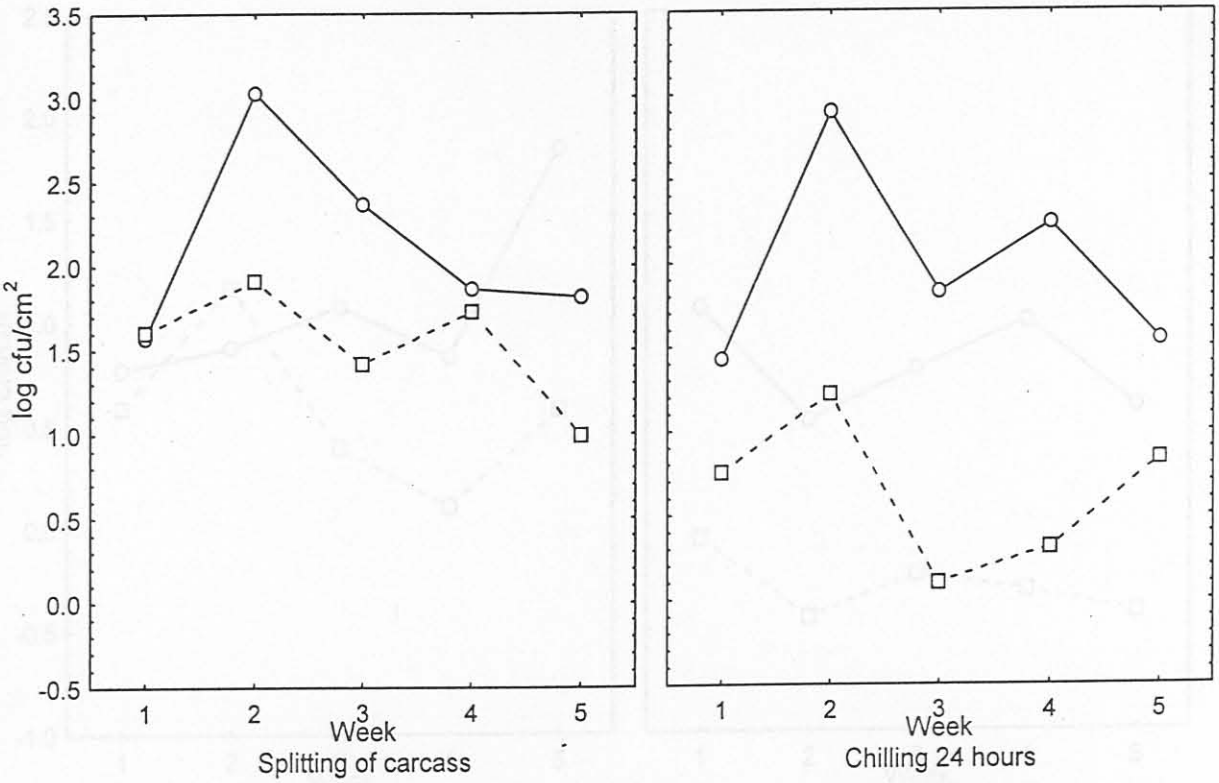


Figure 4.2: Effect of HACCP implementation (O = before training □ = after training) on *Staphylococcus aureus* counts ($log_{10}cfu/cm^2$) at the carcass splitting step (p = 0.0001) and after 24 hours chilling (p = 0.0001).

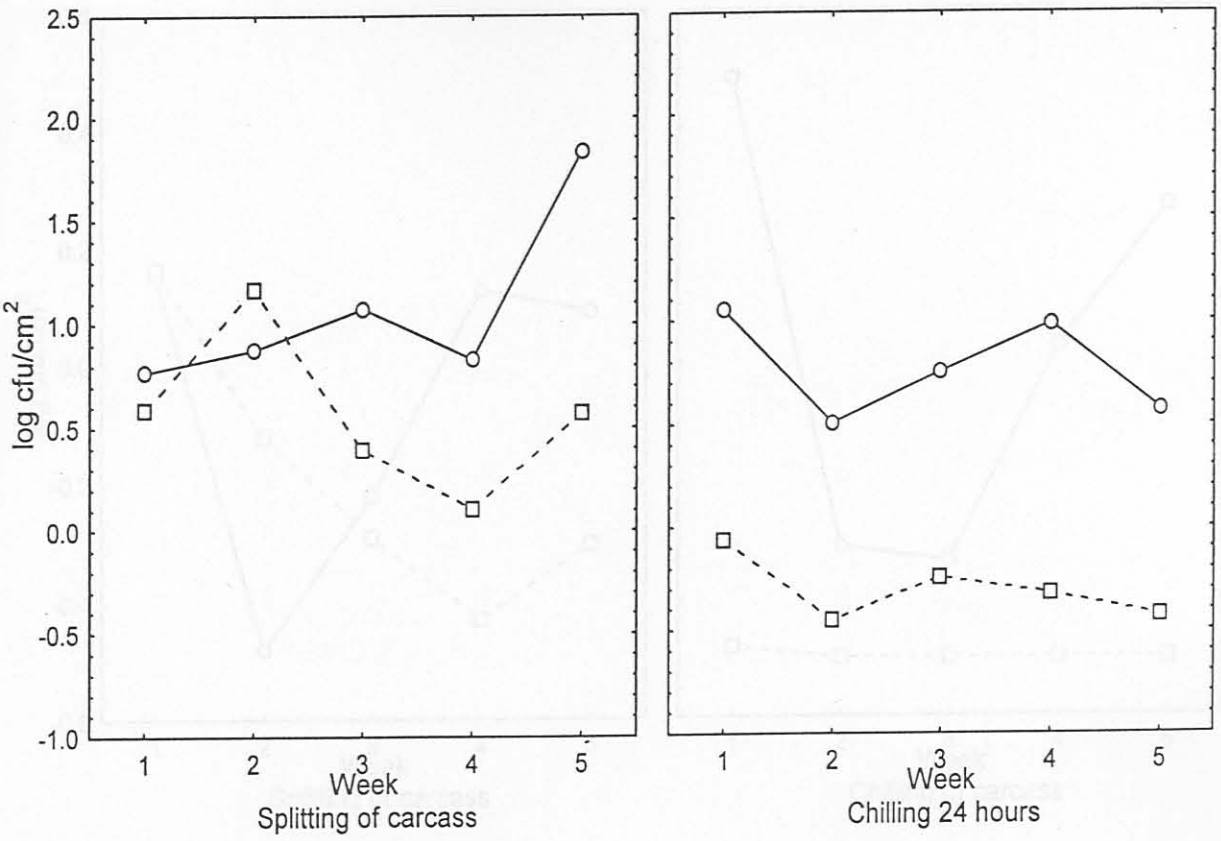


Figure 4.3: Effect of HACCP implementation (O = before training □ = after training) on total coliform counts ($log_{10}cfu/cm^2$) at the carcass splitting step (p = 0.0004) and after 24 hours chilling (p = 0.0001).

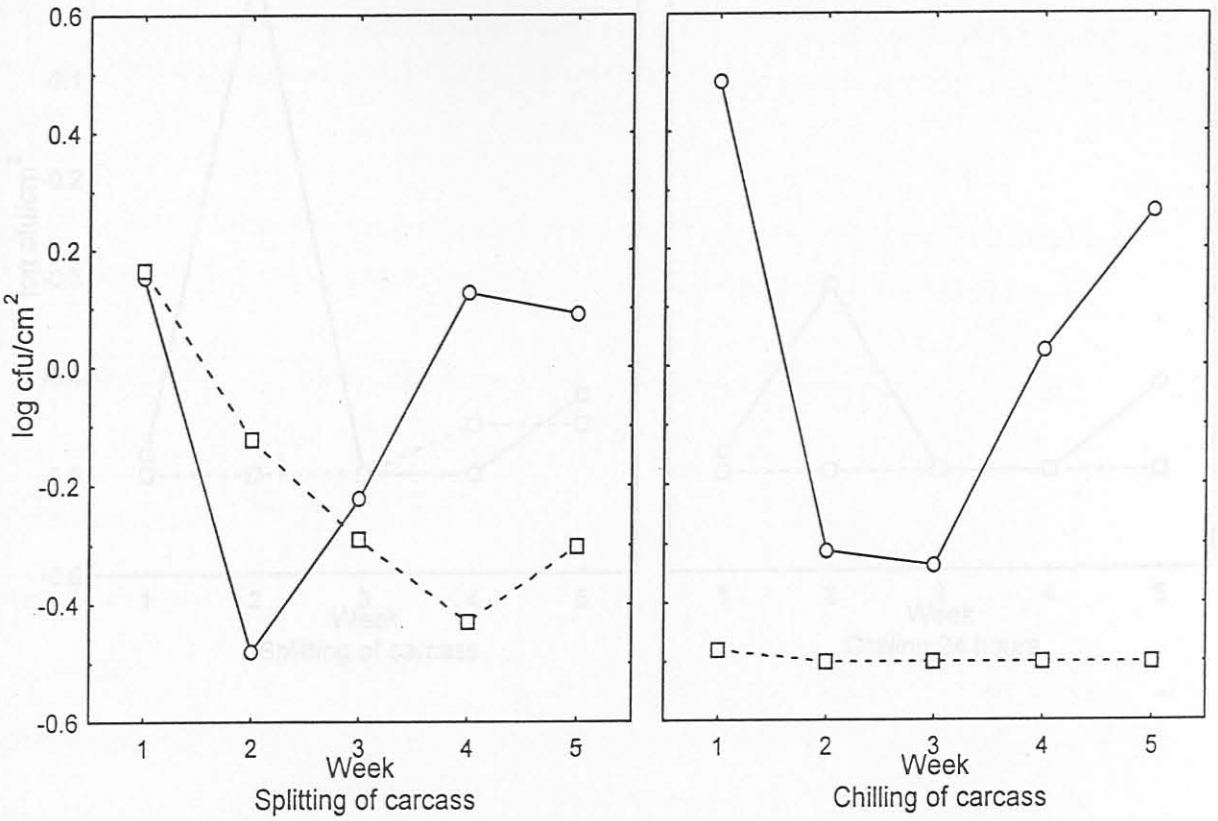


Figure 4.4: Effect of HACCP implementation (O = before training □ = after training) on *Escherichia coli* counts ($log_{10}cfu/cm^2$) at the carcass splitting step (p = 0.214) and after 24 hours chilling (p = 0.0001).

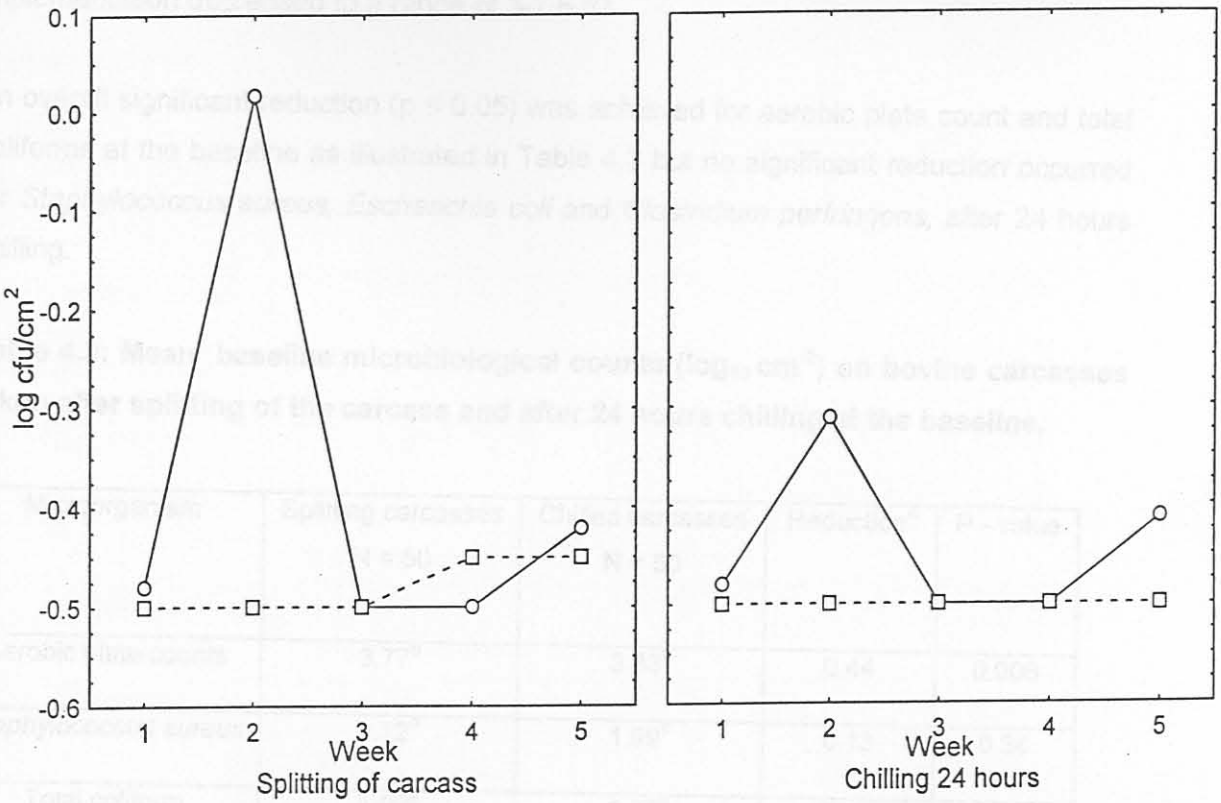


Figure 4.5: Effect of HACCP implementation (O = before training □ = after training) on *Clostridium perfrigens* counts ($log_{10}cfu/cm^2$) at the carcass splitting step (p = 0.006) and after 24 hours chilling (p = 0.02).

4.2 EFFECT OF CHILLING ON MICROBIAL LOADS OF CARCASSES

An assessment was also done to determine if chilling had any significant effect on the carcass microbial loads for the baseline and after HACCP implementation. Temperatures of the carcasses, which had been stored in the chill rooms over a period of 24 hours for the baseline were in the range of 8-14 °C whereas those after HACCP implementation decreased to a range of 3-7.5 °C.

An overall significant reduction ($p < 0.05$) was achieved for aerobic plate count and total coliforms at the baseline as illustrated in Table 4.3 but no significant reduction occurred for *Staphylococcus aureus, Escherichia coli* and *Clostridium perfringens,* after 24 hours chilling.

Table 4.3: Mean[1] baseline microbiological counts (log_{10} cm^{-2}) on bovine carcasses taken after splitting of the carcass and after 24 hours chilling at the baseline.

Microorganism	Splitting carcasses N = 50	Chilled carcasses N = 50	Reduction[2]	P - value
Aerobic plate counts	3.77^{a}	3.33^{b}	0.44	0.006
Staphylococcus aureus	2.12^{b}	1.99^{b}	0.13	0.34
Total coliform	1.08^{a}	0.77^{b}	0.31	0.01
Escherichia coli	-0.06^{a} (64% of carcasses)	0.01^{a} (62% of carcasses)	-0.07	0.47
Clostridium perfringens,	-0.43^{a} (28% of carcasses)	-0.44^{a} (14% of carcasses)	0.01	0.11
Salmonella	2% of carcasses	0% of carcasses		

[1]Means within rows with different superscripts are significantly different ($p<0.05$)
[2]Reduction = $log_{10}cfu/cm^2$ splitting step - $log_{10}cfu/cm^2$ 24 hours chilling.

However, after HACCP implementation an overall significant reduction ($p < 0.05$) was achieved for all the pathogens except for the *Clostridium perfringens.* It should be

noted, however, that no *Clostridium perfringens,* was found in any of the samples after HACCP implementation. The mean log results for each specific pathogen at the baseline and after HACCP implementation are indicated in Table 4.4.

Table 4.4: Mean[1] microbiological counts (log_{10} cm^{-2}) on bovine carcasses taken after splitting of the carcass and after 24 hours chilling after HACCP implementation.

Microorganism	Splitting carcasses N = 50	Chilled carcasses N = 50	Reduction[2]	P - value
Aerobic plate counts	3.77[a]	3.52[b]	0.25	0.003
Staphylococcus aureus	1.53[a]	0.64[b]	0.89	0.0001
Total coliform	0.56[a]	-0.30[b]	0.86	0.0001
Escherichia coli	-0.20[a] (40% of carcasses)	-0.50[b] (2% of carcasses)	0.7	0.0001
Clostridium perfringens,	-0.48[b] (4% of carcasses)	-0.50[b] (0% of carcasses)	0.02	0.16
Salmonella	0%	0%		

[1]Means within rows with different superscripts are significantly different ($p<0.05$)
[2]Reduction = $log_{10}cfu/cm^2$ splitting step - $log_{10}cfu/cm^2$ 24 hours chilling.

From the results obtained it is noted that chilling reduced the aerobic plate counts significantly both at the baseline and after HACCP as illustrated in Figure 4.6. *Staphylococcus aureus* was however, not reduced by chilling at baseline but a significant reduction was noted after HACCP implementation as illustrated in Figure 4.7. For total coliforms a significant reduction was achieved at both the baseline and after HACCP implementation. The levels at the baseline were reduced from 1.08 to 0.77 $log_{10}cfu/cm^2$ after 24 hours chilling at the splitting step, while those after HACCP implementation were reduced from 0.56 at the splitting step to a mean $log_{10}cfu/cm^2$ of -

0.30 after chilling as illustrated in Figure 4.8. A significant reduction of approximately 1 log cycle for *Escherichia coli* counts was achieved after HACCP implementation at the chilling phase whereas data at the baseline recorded an increase of 0.07 $log_{10}cm^2$ as illustrated in Figure 4.9. Generally, *Clostridium perfringens,* was detected in very few carcasses with 0% detections after HACCP implementation and 14% detection at the baseline in the chilling step (Figure 4.10). *Salmonella* was not isolated from any of the carcasses after chilling both at the baseline and after HACCP implementation.

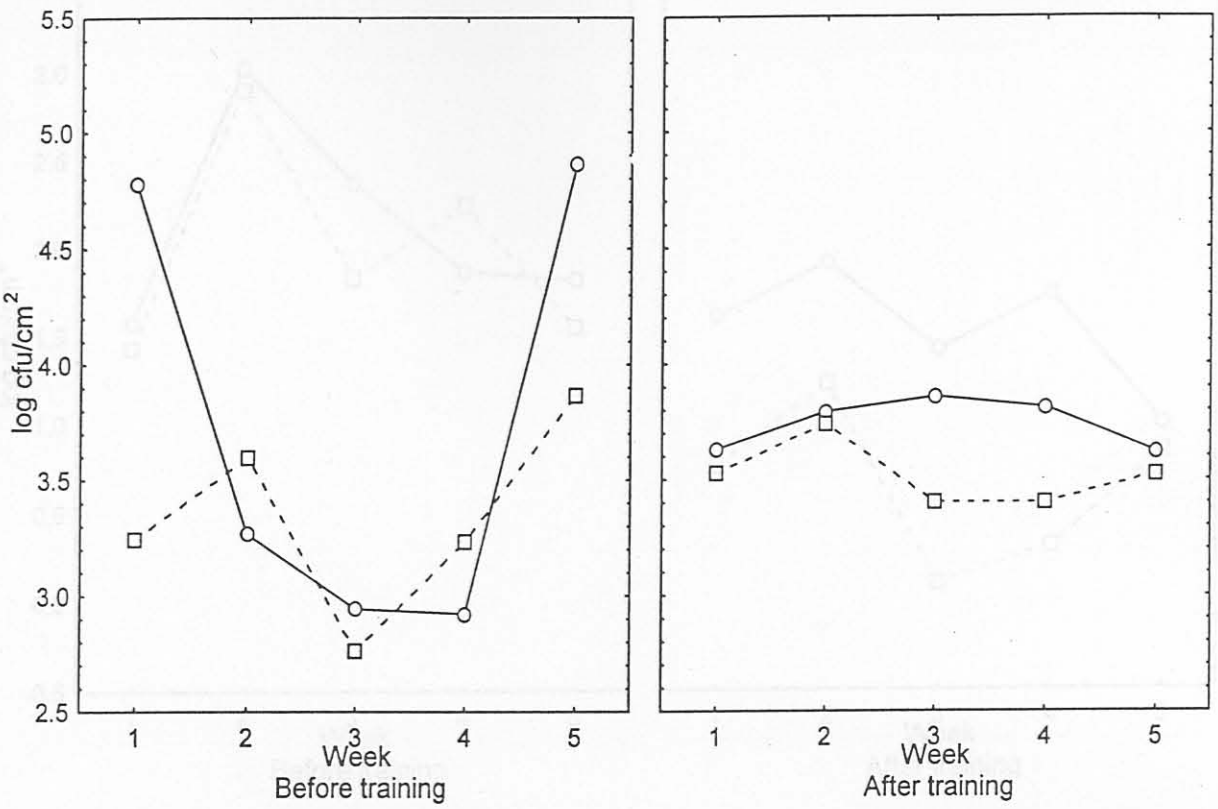


Figure 4.6: Effect of chilling (O = after splitting, □ = 24 hours chilling) on aerobic plate counts ($log_{10}cfu/cm^2$) before (p = 0.006) and after training (p = 0.003).

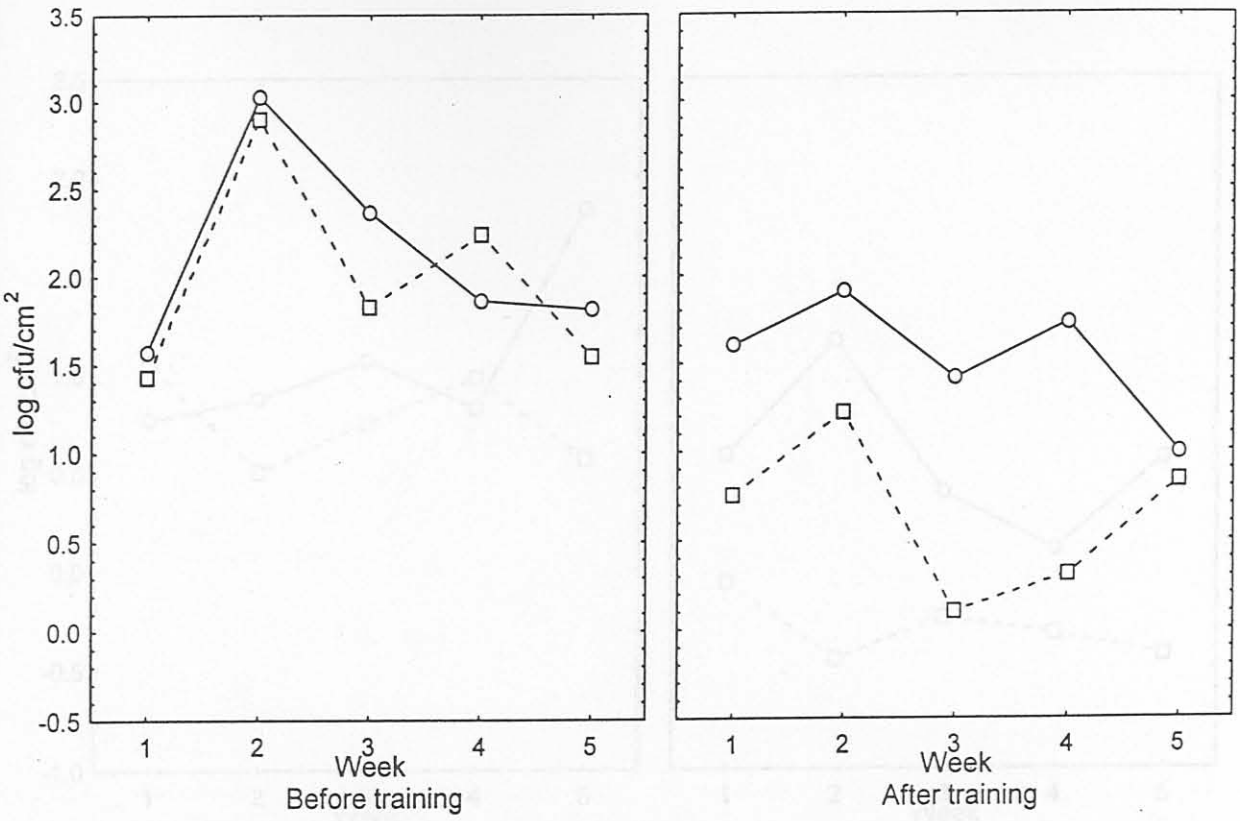


Figure 4.7: Effect of chilling (O = after splitting, □ = 24 hours chilling) on *Staphylococcus* counts ($log_{10}cfu/cm^2$) before (p = 0.34) and after training (p = 0.0001).

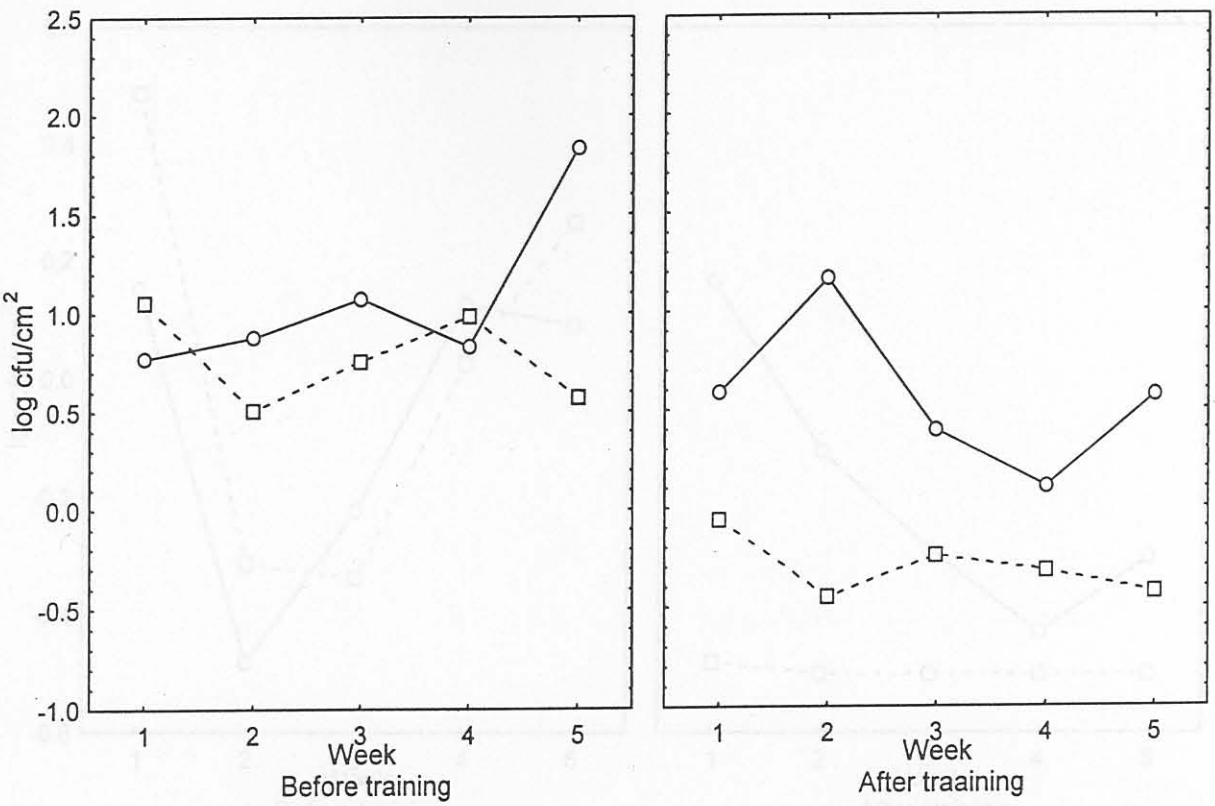


Figure 4.8: Effect of chilling (O = after splitting, □ = 24 hours chilling) on total coliform counts ($log_{10}cfu/cm^2$) before (p = 0.01) and after training (p = 0.0001).

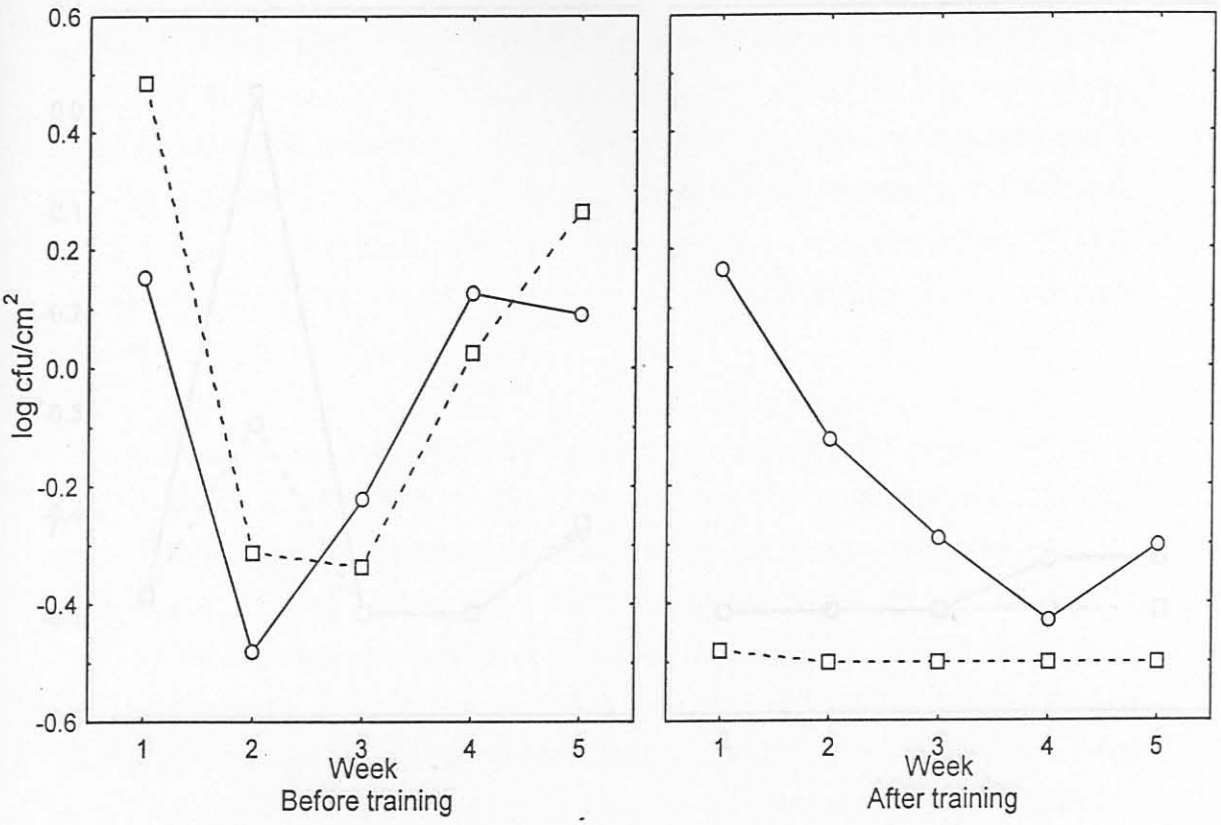


Figure 4.9: Effect of chilling (O = after splitting, □ = 24 hours chilling) on *Escherichia coli* counts ($log_{10}cfu/cm^2$) before (p = 0.47) and after training (p = 0.0001).

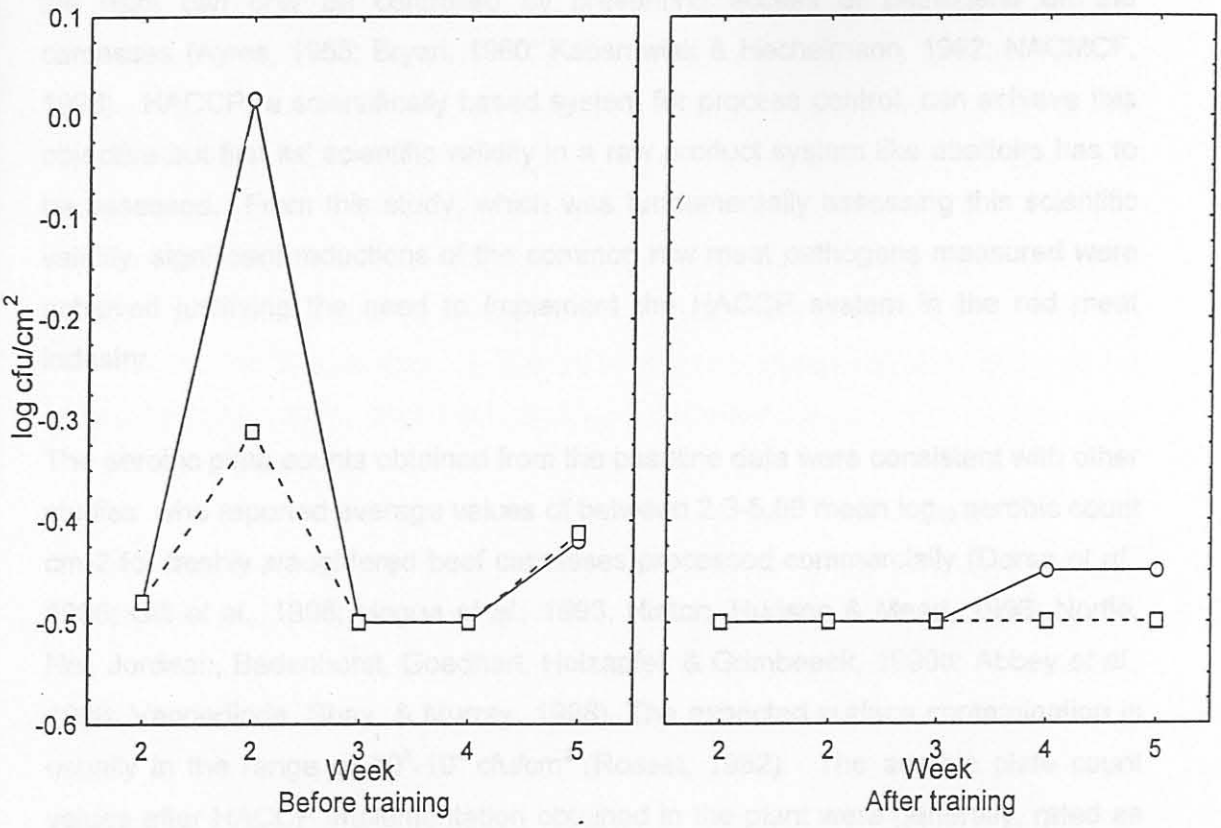


Figure 4.10: Effect of chilling (O = after splitting, □ = 24 hours chilling) on *Clostridium perfrigens* counts ($log_{10}cfu/cm^2$) before (p = 0.11) and after training (p =0.16).

CHAPTER 5

DISCUSSION

There is a general consensus that microbiological contamination during slaughter and dressing is the most important source of meat-borne public health hazards and the risks can only be controlled by preventing access of pathogens on the carcasses (Ayres, 1955; Bryan, 1980; Kapsrowiak & Hechelmann, 1992; NACMCF, 1993). HACCP, a scientifically based system for process control, can achieve this objective but first its' scientific validity in a raw product system like abattoirs has to be assessed. From this study, which was fundamentally assessing this scientific validity, significant reductions of the common raw meat pathogens measured were achieved justifying the need to implement the HACCP system in the red meat industry.

The aerobic plate counts obtained from the baseline data were consistent with other studies who reported average values of between 2.3-5.89 mean $\log_{10}$ aerobic count cm-2 for freshly slaughtered beef carcasses processed commercially (Dorsa *et al.,* 1996; Gill *et al.,* 1998; Hogue *et al.,* 1993; Hinton, Hudson & Mead, 1998; Nortjé, Nel, Jordaan, Badenhorst, Goedhart, Holzapfel, & Grimbeeek, 1990a; Abbey *et al.,* 1998; Vanderlinde, Shay, & Murray, 1998). The expected surface contamination is usually in the range of 10^3-10^4 cfu/cm^2 (Rosset, 1982). The aerobic plate count values after HACCP implementation obtained in the plant were generally, rated as fair according to the rating scale from the ICMCF (1986) in which the maximum acceptable microbiological limit for carcass meat before chilling is 10^6 cfu/cm^2 and chilled carcass meat is 10^7 cfu/cm^2. The non-significant reduction of the values after splitting of the carcasses could be attributed to higher handling of the carcasses as this abattoir was manually operated without basic equipments like hooks for pulling carcasses. There was also a constant movement of personnel from one station to the next to clear backlogs and this can easily compromise hygienic principles. There is therefore, also a need to train the personnel for a longer duration on basic hygiene. Hogue *et al.* (1993) reported that controlling aerobic plate counts in small volume slaughter houses tend to be a lot more difficult

due to non specialization of labour and less uniformity of cattle slaughtered. This seems to be in agreement with the bovine source for slaughter in this particular abattoir as it catered for a heterogeneous group of farmers. The reductions in the aerobic plate count values after HACCP implementation at the chilling stage was a pointer at proper time/temperature maintenance. Usually within the first 24hours a reduction of total counts is expected in the chill room if running temperatures are less than 10 °C but with subsequent days the log counts will increase. Any analysis done within this period will record lower counts as reported in this study. However, if the chill temperatures exceed 10 °C a consistent growth of pathogens will be reported (Nottingham, 1982). The reduction in counts in the chill room could be due to a loss in viability and injury of the surface bacteria. Rosset (1982) indicated that if warm carcasses (38-40 °C) are placed in a chill room at -1 to +5 °C, its surface cools rapidly down to 0 to +5 after 2.5-3 hours. The loss in energy due to the rapid chilling has a great influence in reducing the growth rate of the spoilage flora. In addition, the carcass surface is also dried with resultant reduction of its water activity (a_w) leading to increased stress of the microbes and sometimes death (Sofos *et al.,* 1999b).

Staphylococcus aureus was the most prevalent pathogen in the meat abattoir under investigation before and after HACCP implementation. This is in agreement with other researchers who also found a high prevalence of *Staphylococcus aureus* in raw meats (Brown, 1982). This is especially a significant observation as it points mainly to the hygiene of the personnel who are the main source of cross contamination of *Staphylococcus aureus* and temperature abuse within the abattoir (Troller, 1976; Patterson & Jackson, 1979) and monitoring their performance is essential (De Wit & Kampelmacher, 1982). The significantly reduced values (Tables 4.1 and 4.2) therefore imply that appropriate monitoring of the critical control points, skinning, evisceration and chilling and GMPs were effective. However, the significant reduction in the counts after the HACCP implementation at the chilling step (Table 4.4) could have been due to a combination of GMP's on the slaughter line and effective monitoring of time/temperature of the chill room. Temperature is described as one of the most effective means of killing *Staphylococcus aureus* (Troller, 1976) and the effect of the low temperatures is shown by studies carried out by Patterson & Jackson (1979). They found that at

chill temperatures of less than 7 °C *Staphylococcus aureus* undergoes an unbalanced metabolism resulting in injury and loss of viability.

The effect of HACCP on the faecal coliforms was also investigated since faecal organisms in beef carcass dressing processes are considered a major source of carcass contamination. Their reduction is important as it is viewed not only from a safety aspect but also from a hygiene point of view. Because of this the assessment of the effectiveness of a quality system against hygienic risks in an abattoir therefore always involves enumeration of the coliforms or *Escherichia coli* (Gill *et al.,* 1996). Faecal contamination of dressed carcasses occurs mainly as a result of direct contact with the faeces and contact with surfaces which have been in contact with the faeces, e.g. hides or from punctured gut (Bell, 1997). In this study significant reductions of total coliforms was achieved at both the control points but *Escherichia coli* was reduced significantly only after 24 hours chilling. Inspite of this *Escherichia coli* levels were generally, low in the abattoir (Tables 4.1 and 4.2). It should be noted that in cases where the detections are few, high values from even one carcass would result in great differences in means (Gill *et al.,* 1999). This could therefore explain the non-significant values for *Escherichia coli* at the splitting step in which a slight increase was recorded in the 5th week yet overall detections were low (40%) and were reducing consistently compared to the baseline values which were 60%. This reduction could be attributed to good slaughtering techniques and monitoring at the skinning and evisceration steps during carcass dressing. The baseline values for the total coliforms were comparative to previous studies in which values in the range of 0.9 - 2 $\log_{10}$cfu/cm^2 was recorded (Gill *et al.,* 1996; Gill *et al.,* 1998; Sofos *et al.,* 1999a). HACCP implementation Significantly reduced the total coliform populations to below the detection limit for some carcasses at the chilled stage. Once the carcass temperature falls below 10 °C growth of most mesophiles like coliforms are suppressed and further temperature reductions lead to loss in viability and injury (Brown, 1982). The baseline values for *Escherichia coli* were also comparative to those of other studies who reported general mean values of 0.71-2 $\log_{10}$cfu/cm^2 (Abbey *et al.,* 1998; Gill *et al.,* 1998; Sofos *et al.,* 1999a). The slight decrease in the *Escherichia coli* values after 24 hours chilling could also be attributed to improved slaughtering techniques and general improvement in the GMPs (proper washing of workers hands). Workers hands are the main source of

cross contamination of *Escherichia coli* as they rarely wash their hands effectively unless monitored (De Wit & Kampelmacher, 1982). The slightly higher *Escherichia coli* levels before HACCP implementation at chilling could have been due to high line speeds resulting in poor slaughtering, continual puncturing of the viscera combined with high chances of cross contamination as personnel rarely stopped to wash their hands. All these factors in combination with poor monitoring of temperature/time at the chilling stage could have provided an appropriate niche for the *Escherichia coli* growth. In general growth of coliforms depends on many other factors such as plant operation, geographical location and season (Sofos *et al.,* 1999a). He indicated that lower values were recorded during the dry season. This observation correlates well with those from this study, which was also carried out in the winter which is the dry season in the Hammanskraal region.

Very few samples were positive for *Clostridium perfringens,*. Low incidences of the *Clostridium perfringens,* are usually expected in fresh meats with higher levels recorded only from offals and cooked meat products, which are kept at warm temperatures (Smart *et al.,* 1979; Craven, 1980). The pathogen is easily controlled by adherence to proper time/temperature as illustrated in Figure 4.10 in which 0% detections are recorded after HACCP implementation. Rosset (1982) showed that the lowest growth temperature for this pathogen is 20°C, which could therefore explain the reductions, which were achieved during the baseline evaluation despite the temperature abuse at which other pathogens grew. *Clostridium perfringens,* is also sensitive to low water activity, which inhibits its growth. Reduction in water activity is usually attained at the chilling stage as a result of evaporation (Rosset, 1982).

Salmonella was detected in very few of the carcasses. The few *Salmonella* incidences on the carcasses might be a reflection on the source of the livestock, which was extensive (open pastures and the short transportation distances), more than on existing conditions within the plant. Sofos *et al.* (1999a) showed that pasture animals tend to have a cleaner hide with less dung locks resulting in lower levels of pathogens. Such animals would also have more bacteria of soil origin unlike their counterparts finished in feedlots that might have more microorganisms of intestinal origin. The low levels could also be an indication of the advantages of

short travelling distances as the animals for slaughter all came from the farms in the same region in which the abattoir was located. The advantage of travelling distances on *Salmonella* levels has been explained by Kapsrowiak & Hechelmann (1992) who indicated that cross contamination of this pathogen depended on the stress levels of the animal which might increase during transportation. The animals might also become dirty during transportation and the degree of cleanliness during slaughter depends a lot on the dirtiness of the animals. Comparative work done by other researchers also recorded similar results in which little or nil detection of *Salmonella* in carcasses was recorded. For instance Sofos *et al.* (1999a) reported that of 30 carcasses sampled and analysed for *Salmonella* an average of 2.6% detection was recorded while Korsak *et al.* (1998) did not recover any *Salmonella* from 310 carcasses. Detection of *Salmonella* is usually prevalent after the break down of the carcasses, for instance in a bowl chopper during sausage production, as the meat is usually more evenly mixed hence a much higher chance of detecting *Salmonella* (Kilsby & Pugh, 1981). Therefore the best examination point for this pathogen might be at the subsequent processing stages but the control point is usually at the abattoir. The lack of *Salmonella* on the chilled carcasses could be explained by redistribution of the pathogens hence not necessarily sampled again (Gill *et al.,* 1996).

Overall for most of the pathogens, reductions were achieved after HACCP implementation due to proper time/temperature management and monitoring of the skinning and evisceration controlling points.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

The main aim of the study was to determine the effect of HACCP implementation on the microbiological status of carcasses in a specific C-class abattoir. This was done by statistically analysing the decrease of common meat pathogens. The usefulness of HACCP is apparent from the results of this study in that the implementation had an overall effect of reducing microbial levels on the carcasses. The *Staphylococcus aureus,* total coliforms and *Clostridium perfringens,* counts were significantly reduced ($p < 0.05$) whereas aerobic plate count and *Escherichia coli* counts were not significantly reduced at the splitting step. However, after 24 hours chilling Escherichia *coli* was also reduced significantly ($p < 0.05$). In addition, a consistent positive hygiene trend was achieved for all the microbes after HACCP implementation. Comparisons were also made on the carcasses between the skinning, evisceration and chilling before and after HACCP implementation. At the baseline a significant reduction ($p < 0.05$) was only attained for the aerobic plate counts and total coliforms. However, significant reductions were achieved for all the pathogens after HACCP implementation except for *Clostridium perfringens,* which was not reduced but inspite of this all the carcasses had nil detections after HACCP implementation. *Salmonella* was not detected from any of the samples after HACCP implementation at both the control points.

The reduction and consistency in the pathogen and hygiene levels is important not only from a consumer's safety aspect but also the quality impact of processors' raw material. This is because the carcass is a raw material for further processing and it should therefore have as low microbial loads as possible as the meat passes through many stages of handling with various hygiene strains before ultimately reaching the consumers. Consistent quality ensures supplier reliability. Pathogens even in low numbers are a direct hazard because they have the potential to cross contaminate other meat and to grow to high numbers in the event of poor storage posing a risk of food poisoning to the consumers. From this study it is obvious that total elimination of pathogens from beef products is not possible but it is important

to note that a significant reduction of the microbial levels are achieved by HACCP implementation. It is recommended that to be able to achieve total microbial destruction, decontamination methods, which can be used in conjunction with HACCP, should be explored.

The need for reducing pathogens on carcasses has always been a concern to the meat processing industry but recent increases in cases of food poisoning diseases, some fatal by the food-borne pathogens like *Escherichia coli*, *Clostridium perfringens, Staphylococcus aureus* and *Salmonella* has catalysed this concern. This is further worsened by the fact that a lot of emphasis of carcass microbial control is usually placed in the secondary processing stages while the actual deposition and distribution of pathogens occurs in the slaughter stage. For instance the current study might have reported nil detection of pathogens like *Salmonella* yet this organism might be picked up when analysing minced meat from a bowl chopper due to mixing or redistribution of the pathogens from the carcasses. Unfortunately this has led to less emphasis being placed on the abattoir steps as critical control points yet, the state of the carcass where the introduction occurred, is ignored (Kilsby & Pugh, 1981).

The main limitation with this study is that most of the personnel in the abattoir were illiterate and needed constant training for a longer duration. Achieving immediate positive results were therefore, not easy. The high employee turn -over also calls for constant training and can lead to hygiene fluctuations within the line. The workers remuneration also depended on the number of carcasses they processed hence chain speeds tended to be fairly high and these could compromise the hygiene of the product. There is also still a lack of agreed minimum microbial levels for the meat industry which implies that there is still a lack of yardstick for measuring whether the reductions achieved are significant for a HACCP system. There are also general limitations with the use of minimum microbial limits from other countries due to the conditions e.g. facilities, storage and distribution, which vary tremendously internationally and one standard may not be good enough

It is recommended that for a more comprehensive picture of the performance of a HACCP system in the South African abattoir industry a study on multiple abattoirs

with different conditions should be done concurrently over a longer duration. This will enable conclusive results on the value of HACCP implementation. The results achieved so far can form a basis for implementation of a HACCP system in small red meat abattoirs in South Africa.

CHAPTER 7

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APPENDIX 1

TARGET AREAS OF INSPECTION

i. Plan of the plant lay out
ii. Flow of materials
iii. Movement of staff, equipment and materials from dirty areas to clean areas
iv. Uninterrupted flow of raw materials to final product
v. Storage of products and temperature control
vi. Critical control points

MANAGEMENT

i. Technical staff structure and responsibilities, qualifications/training/experience and hours of work
ii. Company training policy for all staff grade
iii. Availability of written product and operational specifications
iv. Availability of maintenance and sanitation manuals
v. Availability of test methods manuals
vi. Availability of written quality control (QC) organization and specifications
vii. Recording, presentation and checking of QC data
viii. Procedures for dealing with out of specification situations
ix. Product security, recall procedures and other emergency procedures
x. Familiarity of staff with appropriate codes of GMP
xi. Steps taken to ensure visitors, maintenance staff and outside contractors do not affect product quality

GENERAL AREAS

Factory environment

Premise should be in a location free from undue risk of contamination from outside sources and should be accessible and free from flooding risks.

i. Is the area exposed to potentially harmful substances, which could lead to unacceptable level of this substance in processed food?
ii. Is the factory in an area free from airborne contaminants?
iii. Is there a danger of flooding from poor drainage or effluent storage tanks?
iv. Are roadways to and in the vicinity of the plant suitable for wheeled traffic and do they have adequate draining?

Hygiene

The premise should be constructed so that contaminants brought into the factory can be maintained. And contaminants from the factory can be removed without danger to product contamination and pest infestation.

i. Are pest control measures carried out and documented evidence available?
ii. Do personnel who handle pest control understand the potential hazards associated with control procedures?
iii. How often are pest control inspections done?
iv. Can access to the processing areas by animals and man be controlled?
v. Are effluent and waste disposal systems capable of coping with peak loads and are they maintained in a sanitary condition?

Construction

The ability to maintain hygiene in a premise depends a lot on the structure of the premise. This relates to the actual material of construction and design, as well as the plant layout and separation of areas presenting different levels of hazards.

i. Are all buildings of sound construction and suitable for the intended purpose, well maintained (from reception to the distribution)
ii. Does design of building allow for easy cleaning and prevent ingress and harboring of pests?
iii. Is their adequate space for production, maintenance and storage?

iv. Is the building design and lay out such that the flow from raw material to finished product can take place with minimum chances of cross contamination?
v. Are floors of suitable material, washable, non-slip and well drained?
vi. Are walls of appropriate construction and design, no crevices and unsealed joints, light colour and cleanable?
vii. Does the ceiling design minimize the chances of accumulation of dirt and condensation?
viii. Are windows designed to prevent entrance of pests, e.g. birds?
ix. Are doors of appropriate construction and close fitting?
x. Is there adequate lighting, and food protected from any breakage's?
xi. Adequate ventilation and temperature control?
xii. Are auxiliary structures including overhead pipes and surfaces constructed so that they can be cleaned do not collect dirt and do not encourage malpractice's that can lead to product contamination?
xiii. Is there a staff canteen?
xiv. Are toilets, canteen and rest areas adequately separated from the processing hall?
xv. Are adequate storage areas, changing facilities and lockers available for the staff?
xvi. Are adequate hand washing facilities provided in the factory?
xvii. Is there an adequate facility for cleaning small items of the plant?
xviii. Are there adequate waste disposal facilities for waste product and inedible material before removal from the site?
xix. Is waste removed on a regular basis?

Toilets

i. Are there sufficient toilets for staff members?
ii. Are they cleaned and maintained regularly?

Changing rooms

i. Are lockers available and general changing room kept clean?

Personnel

One of the major sources of potential contaminants of all types is from people and their working practices. Hence, they must be adequately controlled. All personnel concerned with production should ideally receive medical screening before handling food. The screening should be directed to the absence of nose, ears and throat infection; Skin infections such as boils, sores, etc, gastrointestinal illness or contact with known cases of food borne illness. Training of personnel should embody the importance personnel hygiene and the legal requirements of current food hygiene regulations.

i. Have personnel received health certificate by medical screening?
ii. Do personnel conform to the requirements of the food hygiene (SABS 049 1989)?
iii. Do personnel wear adequate protective clothing and avoid taking personnel effects like jewelry to the plant?
iv. Do personnel wash their hands adequately after being away from the processing hall?
v. Do personnel eat, expectorate, smoke, pick ears and or nose in the plant?
vi. Are visitors prevented from entering the process hall unless they take adequate hygienic precautions?
vii. Is training adequate?

Quality control systems

i. Determine CCPs for the operation
ii. Examine QC manuals- are they available, can available copies be revised, do they contain details of procedures and standards, can they be used for training as well as reference purposes?
iii. Examine spot check data
iv. Assess whether staff are corresponding to QC data; what actions are taken?
v. Collect blank QC forms are tests listed carried out?

Cleaning and sanitation

In order to achieve safety and quality it is imperative to have adequate cleaning and sanitation systems operating within the factory. They should be written down and demonstrated to be effective.

i. Are materials of construction compatible with the cleaning and disinfecting agents used?
ii. Is the system of cleaning up used appropriate with the equipment? Is dirt sprayed on to clean equipment?
iii. Where adequate is equipment dismantled for cleaning?
iv. Are the chemicals clearly labelled and the hazards and remedial actions associated with their use clearly stated?
v. Are only approved cleaning agents used and instructions for use followed?
vi. Are documented cleaning procedures available and are followed?
vii. Are work surfaces properly rinsed?
viii. Is frequency and time of cleaning adequate to ensure a good hygiene standard?
ix. Are their adequate protective clothing?
x. Is protective clothing properly washed?
xi. When not in use are cleaning materials and equipment kept separate in designated areas?
xii. Are cleaning equipment clearly marked to designate those of the processing hall from those of other facilities like toilets?

ABATTOIR

The abattoir includes handling of livestock, slaughtering and dressing, and dealing with offals, blood, and glands and condemned material. The following facilities are required for slaughterhouses:

i. Adequate lairage for holding animals
ii. Slaughter premises large enough for work to be carried out satisfactorily. Where both pigs and other animals are slaughtered, a special place must be provided for slaughter pigs unless the species are slaughtered at different times; in such cases scalding, depilation, scraping, and singeing must be clearly separated from the slaughter line either an open space of at least 5 m or by a partition of at least 3 m high
iii. A room for emptying and cleansing intestines
iv. Rooms for dressing gut and tripe
v. Separate rooms for the storage of fat and hides, horns and hooves that are not removed on the day of slaughter

vi. Premises reserved solely for the sick and suspect animals, slaughter of such animals, detained meat
vii. Adequate chilling rooms to cope with target throughput
viii. Adequate equipped, lockable room for veterinary use
ix. Changing rooms, wash basins, showers and flush lavatories which do not open directly into the workrooms. Wash basins must have hot and cold water, materials for cleansing and disinfecting hands and hand dryers
x. Capability for veterinary inspection to be carried out at any time
xi. Means of controlling access to and exit from the slaughter house
xii. An adequate separation between clean and dirty sections
xiii. In the workrooms adequate equipment for cleansing and disinfecting hands as near as possible to the workstation. Taps must not be hand operable; there must be hot and cold running water. For cleaning instruments water must not be less than 82°C
xiv. An overhead system of rails for handling of meat
xv. Instruments and equipment of non-corrosion and easily cleaned material
xvi. A special section for manure
xvii. Laboratory

Chilling

i. Are chilled products held at between 0-4°C not over 7°C
i. Are temperature records available
ii. Are temperature monitoring facilities calibrated regularly
iii. What is the maximum number of carcass for each chill room to operate efficiently?
iv. Are carcasses allowed to touch walls, floors or other carcasses? Does water condensation drip on the carcass
v. Are hot carcasses mixed with chilled carcasses?
vi. Are there adequate drainage in the chill rooms and are floors cleaned regularly?
vii. Is there a sign outside giving maximum and minimum loading and approximate chilling times?

How were the chilling times established is there a first in / first out control system?

Slaughtering

i. What method of stunning is used and is it used correctly? Are animals stunned properly the first time?
ii. Are sterilizes available for sticking knives and steels?
iii. Are sticking techniques and bleeding times satisfactory?
iv. How is the blood handled?
v. What is the condition of offal conveyors, trolleys, inspection platforms, tables chutes, bins and covers, saws, blades and knives?
vi. Are condemned meat containers clearly labelled and only used for condemned meat?
vii. What cleaning procedure is used for the slaughter hall?
viii. Does each carcass go through inspection and identification stamp?

Condemned meat

i. Are there proper facilities for holding materials unfit for human consumption prior to dispatch?
ii. Is the facility lockable?
iii. Are staining facilities available for unfit meat?

Lairage

i. Is the number of pens adequate for the number of animals held?
ii. Are pen design and construction acceptable (e.g. height and rail gaps)
iii. What is the state of lairage floors?
iv. Are pens cleaned upon arrival of new animals?
v. How are pens cleaned?
vi. What is the general condition of the animals? (fighting, bruising, stress)
vii. How long are the animals held before slaughter? (are they fed if held for longer periods)
viii. Is there a supply of fresh water for animals? Are the heights of troughs satisfactory?
ix. Does the lairage have adequate ventilation and lighting?
x. Are animal's species placed into separate pens if mixed species are slaughtered?

xi. How are the animals handled by the lairage staff? Are there hose pipes, sticks, drain rods or other items, which can be used to drive animals inhumanely near the lairage?

Animal isolation block

i. Is the position suitable in relation to that of the lairage?

ii. Is the block lockable?

iii. Are boot and hand washing facilities available?

iv. How often is the isolation block cleaned?

Manure bay

i. Is the manure bay sited in a suitable position (i.e. dirty side of the plant)?

ii. Is the bay kept tidy?

iii. Is the manure covered? Are there signs of fluid leakage?

iv. How often is the manure collected

v. Have steps been taken to reduce the presence of insects near the bay?

Animal feed store

i. Is the feed store sited in a suitable position?

ii. Is the store kept tidy?

iii. Is the feed rotated regularly?

iv. Have steps been taken to eliminate the presence of rodents, birds and insects in the store?

Animal unloading

i. What distance do the animals travel from far to the factory?

ii. Is their adequate vehicle docking and turning space?

iii. What times are the animals delivered?

iv. Are there facilities to clean vehicles after unloading?

v. What is the general condition of vehicles? Are there signs of mould growth, algae or dried faeces? Is there adequate ventilation?

vi. Is the correct number of animals loaded into each vehicle? Is adequate documentation available?

vii. How are the animals treated during offloading from vehicles?

Are there signs of bruises, abscess or stick marks on the animals?

ABATTOIR INSPECTION FORM

Factory type and class -		
Age of building –	Size of factory -	number of employees -
code: s: satisfactory, f: fair, u: unsatisfactory		

Animal unloading and lairage		
1		Weeds
2		Litter
3		Waste disposal
4		Accessory buildings
5		Manure
6		Lairage
7		Other waste material
8		Toilets
9		Maintenance programme
Environmental waste programme		
1		Surface water drainage
2		Waste solids disposal
3		Waste liquids disposal
Environmental infestation		
1		Rodents
2		Insects
3		Birds
4		Domestic animals
Factory general		
1		Rodent proofing
2		Insect proofing
3		Adequate for its purpose
4		Maintanance
5		General orderliness
6		Screening

Factory processing rooms		
1		Floor cleanliness
2		Floor construction
3		Walls clealiness
4		Wall construction
5		Ceiling cleanliness
6		Ceiling construction

Factory facilities		
1		washing facilities
2		Toilets
3		Lighting
4		Ventilation
5		first aid
Factory waste control		
1		waste solids removal
2		waste liquids removal
3		waste containers
Factory cleanup		
1		Frequency
2		effectiveness checks
3		detergents and disinfectants
4		cleaning facilities
5		Documentation
Pest prevention and control		
1		Rodents
2		Flies
3		domestic pets
4		Birds
5		pest control procedures
Plant and equipment		
1		Sanitation
2		Procedures
3		Design
4		material storage
5		Maintance
Potable water supply		
1		Source
2		Treatment
3		Testing
Personnel hygiene		
1		hygiene training/knowledge
2		hand washing/expectoration
3		Clothing/hair
4		food contact
5		medical certificates

Dry storage		
1		Cleanliness and orderliness
2		Rodent infestation
3		Insect infestation
Chill storage		
1		Cleanliness and orderliness
2		Rodent infestation
3		Insect infestation
4		Adequacy of equipment
5		Time delay before refrigeration
Control action		
1		Raw material sources
2		Handling
3		Production process
4		Final product
5		Transportation
Raw material final		
1		Spoilage
2		Infestation
3		Handling
Bacterial swab results		
1		Total count
2		Staphylococci
3		Coliforms
4		*Escherichia coli*