APPLICATION OF HAZARD ANALYSIS CRITICAL CONTROL POINT (HACCP) CONCEPT TO STUDY CATTLE SLAUGHTERHOUSE HYGIENE AND CARCASS CONTAMINATION IN ZAMBIA

BY

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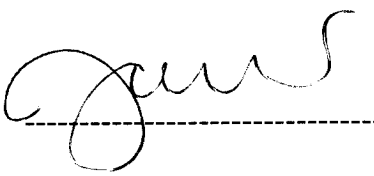

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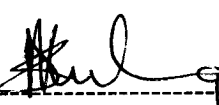

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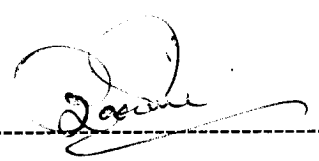

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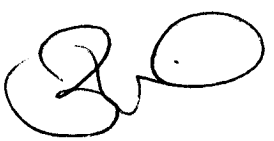
DECLARATION

I John Bwalya Muma do here by declare that this dissertation represents my own work and that it has not previously been submitted for a degree at this or any other university.

Signature:  ----------

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LIST OF ABBREVIATIONS AND ACRONYMS

APC	Aerobic Plate Count
APHA	American Public Health Association
CAP 535	Chapter 535, The Public Health Act of the Laws of Zambia
CCP	Critical Control Point
C.F.U	Colony Forming Unit
CAC	Codex Alimentarius Commission
CS	Commonwealth Secretariat
EHEC	Enterohaemorrhagic *E. coli*
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
EVEC	Enteroinvasive *E. coli*
FAO	Food and Agriculture Organisation of the United Nations
FCC	Faecal Coliform Count
GIT	Gastro-Intestinal Tract
GMP	Good Manufacturing Practice
GPP	Good Production Principles
GVP	Good Veterinary Practice
HACCP	Hazard Analysis Critical Control Point
HAS	Hygiene Assessment System
HUS	Human Uraemic Syndrome
ISO	International Standards Organisation

ITC	International Trade Centre
MAFF	Ministry of Agricultures Food and Fisheries
MHSS	Minimal Hygiene Satisfaction Score
MPN	Most Probable Number
PM	Post-mortem
SD	Standard Deviation
SEM	Standard Error of the Mean
SPS	Sanitary and Phytosanitary Agreement
SSC	Sum Square Count
TB	Tuberculosis
TCC	Total Coliform Count
VTEC	Verotoxigenic *E. coli*
WHO	World Health Organisation
WPCC	Whole Plate Colony Count
XLD	Xylose Lysine Desoxycholate Agar

ABSTRACT

The Hazard Analysis Critical Control Point (HACCP) concept was applied for the first time to four Zambian cattle slaughterhouses to study slaughterhouse hygiene and bacterial contamination of carcasses. Swabs were collected from carcass surfaces and also from the following environmental surfaces, which were likely to come in contact with carcasses: knives, cutting saws, tables, walls, floors, aprons and worker's hands. Samples were collected from animals at the following operational points: before skinning; after skinning; after evisceration; and after carcasses were chilled for 24 hours. At each operational point, four sites were swabbed except for point A were only two sites were swabbed. Similarly, four different sites were swabbed from each environmental surface per visit. A total of 2176 swabs were collected from 72 carcasses, and 96 swabs from the above mentioned environmental surfaces from slaughterhouses A and B. Observational results for various hygiene categories where scored for slaughterhouse A, B, C and D.

Slaughterhouses A and B, in which microbiological contamination analyses were done, had overall mean aerobic plate count, mean total coliform count, and mean coliform count ranges of $\log_{10}$ 3.67 - 4.14/cm^2; $\log_{10}$ 1.63 - 2.44/cm^2 and $\log_{10}$ 1.23 - 1.59/cm^2, respectively. Municipal slaughterhouses C and D, with no defined slaughter and dressing procedures, recorded lowest hygiene assessment scores (18.7% and 21.3% of hygiene satisfaction, respectively). Carcasses that recorded zero duplicate coliform count after skinning were in the range 25 - 30% where as zero duplicates after

evisceration ranged from 15 - 20.3%. Effect of chilling as a critical control point was better illustrated by changes in means of total coliform count (log_{10} 1.84 to 0.00) and faecal coliform count data (log_{10} 2.51 to 0.00/cm^2) than aerobic plate count data (log_{10} 4.10 to 3.82/cm^2). Salmonella was isolated from carcasses at all sampling points with skin samples recording high isolation frequencies.

The results of this study indicate that neither aerobic plate count nor total coliform counts alone is sufficient to analyse carcass contamination levels at various operational points but the two, however, are complimentary. This study has also demonstrated high levels of bacterial contamination on carcasses, which is known to be associated with poor hygiene. There appears a need to establish slaughter and dressing procedures, and quality assurance programmes based on risk assessment and maximum utilisation of resources. Meat standards also need to be set and this will need an active legislative support to improve both meat safety and quality, and hygiene standards in Zambian cattle slaughterhouses.

CHAPTER ONE: INTRODUCTION

Food contamination by biological agents is now recognised as a major health problem world-wide with bacterial agents contributing a large proportion to diseases in man etiologically traced to food (Anon., 1983; McCapes *et. al.*, 1991; and Danielsson-Tam, 1996). Presence of bacteria in and on meat constitutes a health hazard and affects keeping qualities. Different bacterial pathogens may cause illness in man ranging from mild chronic illness to acute fatal infections and intoxications. Bacteria in meat not only cause infections and intoxications to man, but can also deprive the population of the benefit of meat, which is a source of high valuable protein (Hathaway and Bullians, 1992).

Meat-borne pathogens also pose a serious health threat to workers in the meat industry. The problem has long been realised and the diseases are termed as occupational hazards, which include diseases such as are: anthrax, tuberculosis, brucellosis, rift valley, fever, dermatophylosis etc. The risk is not only limited to workers in meat establishments, but also to any other person involved in handling meat or meat products associated with these diseases. Strict ante-mortem and post-mortem inspections form a good preventive measure to infections in man (Andrew, 1985).

Foods of animal origin are important vehicles for transmission of several zoonoses including food-borne diseases. Conditions existing in slaughterhouses and the current meat handling practices in many developing countries have been found to contribute significantly to the spread of zoonotic diseases (Anon., 1983). These zoonoses transmitted in meat contribute significantly to human morbidity and mortality.

In the past, a number of methods have been used by meat hygienists to prevent supply of contaminated food to the consumers (Quevendo, 1992). In slaughterhouse operations, traditional inspection methods of meat for detection of organoleptic abnormalities was the method of assuring meat safety. However, current studies have shown that these methods are ineffective in hazard control and inefficient in resource utilisation (Baird, 1992; Quevendo, 1992; Berends *et., al.* 1993, Motarjemi *et., al.* 1996). Although much time and resources may be spent on these inspection methods, this does not match with the health risk posed by them as opposed to the risk posed by the presence of microbial pathogens (Hathaway and McKenzie, 1991; Bryan, 1992). The approach to meat quality assurance has, in recent years and especially in developed countries, shifted from detection of gross anatomical abnormalities, to a more preventive approach based on microbiological quality control (Hathaway and McKenzie, 1991; Murray, 1986)

Unfortunately in Zambia, like most developing nations, the approach to meat quality assurance is still traditional, with all sanitary requirements being vested in the inspector's knife and his senses (sight, touch and smell) (CAP 535 of the Laws of Zambia). Countries like Zambia, with a wide spectrum of food-borne zoonoses occurring in high prevalence rates, ought to have stringent quality assurance systems to ensure food safety (Motarjemi *et. al.,* 1996). In addition, the current socio-economic status of the people which denies most access to food preservation facilities, and the eating habits of most people in the country, demands that less contaminated meat be poured onto the market. Although the magnitude of public health and economic significance of meat-borne diseases are often under estimated in developing countries, application of meat quality assurance systems in production brings about positive economic changes through reduction in meat losses and reduced food-borne related illnesses (Anon., 1984; Bryan, 1992).

The HACCP system was introduced in order to assure food quality and safety. The HACCP system is a systematic approach to the identification, assessment, and control of hazards (Bryan, 1992). It offers a rational approach to the control of microbiological and chemical hazards in food (Bryan, 1992; Anon., 1993 b; Mortagemi, *et. al.*, 1996). The HACCP has been found to be the most cost-effective and secure means of assuring food safety of all types (Baird, 1992; Berends *et. al.*, 1993; Marton, 1993). It has proved to be very useful in assuring safety of all

food types in both the food manufacturing and service industries. The HACCP system is also an efficient hygiene audit tool which is now widely applied in hygiene monitoring and regulatory services (Anon, 1993b) . Its proof of quality performance, has lead to its being adopted by international trade organisations as a standard quality control programme in sanitary and phytosanitary requirements of international trade in animal and animal by-products (Anon., 1993a; and Anon., 1996). In veterinary public health, the HACCP system is being applied in meat and milk quality assurance at all levels of the transmission chain, from the farm to the consumer (Anon., 1993 b, Motarjemi *et. al.*, 1996).

The HACCP based system of controlling meat-borne pathogens has been recognised to enhance food safety, to better utilisation of inspection resources and provide timely response to problems (Bryan, 1992; Hathaway and Bullians, 1992). Although the HACCP system offers great opportunities in the control of food-borne diseases, it has not yet been applied in Zambia

In this study therefore, the HACCP concepts were to be applied to four slaughterhouses in Zambia to study microbiological quality of carcasses and hygiene practices in slaughterhouses. This was to be achieved by conducting microbiological investigations of critical control points, and the observation of operational procedures and hygiene standards. Slaughterhouse performance in various hygiene categories were assessed and scored based on the principals of Good Manufacturing Practice (GMP) and Hygiene Assessment System (HAS).

Study Objectives

This study describes the application of the HACCP concepts to bacterial contamination of beef carcasses and cattle slaughterhouse hygiene in Zambia with the following objectives:

1. To describe the slaughter and dressing procedures in Zambian cattle slaughterhouses and to identify bacterial contamination points.
2. To describe the general hygiene practices being applied in Zambian cattle slaughterhouses and assess their contribution to meat hygiene.
3. To determine levels of bacterial contamination of beef carcasses and cattle slaughterhouse environment using the HACCP concepts.

This study is centred on slaughterhouse proper, with a view of establishing a standard meat quality assurance programme which can be extended to other categories as well.

CHAPTER TWO: LITERATURE REVIEW

2.1 Meat Quality Control in Zambia

2.1.1 Meat Quality

Microbial, chemical and physical contamination of meat and meat products for human consumption has been and continue to be a problem of great concern to both meat hygienists and meat consumers (McCulloch and Whitehead, 1979; Gracey and Collins, 1992; Biss and Hathaway, 1996). These contaminations can have adverse effects on the consumers which may range from a mild illness to sudden death. Therefore, efforts have been made in devising methods of controlling such potential hazards in meat to achieve minimum contamination levels (Quevendo, 1992). The current meat inspection methods being applied in Zambia (CAP 535 of the laws of Zambia) have a traditional approach of following dogmatic inspection procedures which in most cases lack public health significance or have no scientific justification (Hathaway and McKenzie, 1991; Hathaway and Bullians, 1992 and Motarjemi *et. al.*, 1996).

2.1.2 Ante-mortem Inspection

Ante-mortem inspection is a critical and historical assessment of food animals of suitability for slaughter and acts as a primary safe guard for the final product (Murray, 1986). This consists of a balanced veterinary professional judgement involving welfare, handling practices, public and animal health in relation to regions and individual animals (Murray, 1986; Anon., 1993 a). Ante-mortem inspections removes from human food those animals with conditions undetectable on routine post-mortem inspections (Andrew, 1985; Berends, *et. al.*, 1993). Diseases such as anthrax and rabies, are diagnosed at this point and sick animals can then be prevented from entering the slaughter process. This step has beneficial effect in that, though preventive measures might have taken place at farm level, animals are presented to other hazards during transit to the slaughterhouse and during their brief stay in the lairage (Murray, 1986). Ante-mortem inspection becomes exceedingly important in developing countries like, Zambia, where a large population of livestock presented for slaughter comes from traditional farmers who hardly implement any good farming methods (Maff, 1994). In such countries, meat inspectors are urged to devote considerable time to ante-mortem inspection (Anon., 1983).

Ante-mortem inspection with the secondary objective of food safety assurance, that is detection and removal of hazards at the earliest possible point of occurrence (Craig, 1995). In addition,

ante-mortem inspection without farming history provides even a more incomplete assessment of animal health (Hathaway and McKenzie, 1991; and Berends *at. el.*, 1993).

2.1.3 Post-mortem Inspection

The degree to which PM inspection guarantees food safety is doubted by many authors (Christensen, 1981; Hathaway and Bullians, 1992; Berends *at. el.*, 1993). In most cases, PM inspections have often been found to be inappropriate to the spectrum and prevalence of defects present in a given class of livestock (Hathaway and McKenzie, 1991). While detailed inspection procedures may be required in some regions, such inspections may be a waste of resources if applied in other situations. (Hathaway and McKenzie, 1991; Berends *at. el.*, 1993). High risk class of animals may need additional attention while intense inspection may not be necessary in less risk animals. (Murray, 1986; Hathaway and McKenzie, 1991). For instance, in Zambia, old animals coming from Southern Province, where human and bovine TB is endemic, need a more intensive inspection approach than a steer coming from a commercial farm (Cook, *et. al.*, 1996). In most recent studies and reviews, these kinds of approach to meat quality assurance have been found not only to be expensive, but also ineffective in controlling meat-borne pathogens (Hathaway and McKenzie, 1991; Hathaway and Bullians, 1992; and Motarjemi *et. al.*, 1996). However, in countries where diseases such as TB and anthrax are endemic, PM inspections will continue to play a significant role not only in protecting human health from

zoonoses and reducing the aesthetic risk of meat, but also as an indispensable tool instituting an effective disease monitoring and surveillance system (Corner *et. al.*, 1990; Schwabe, 1984).

2.1.4. Legislation

Following Quevendo's (1992) legislative and regulation classification of countries with respect to meat quality regulation, Zambia can partially be classified under the second and third categories. It has a relatively good food legislation, though not up-to-date, but lacks the proper mechanisation and infrastructure, financial and technical support, and lacks enforcement. Whereas the national authority has legislative powers to control meat quality management, the local authorities also have both powers to make local regulations and enforce them (CAP 535 of the laws of Zambia). However, these local authorities lack financial and technical support to enforce food legislation.

2.2 Slaughterhouses

In Zambia, three animal slaughter premises can be distinguished. The term "abattoir" will be avoided throughout the discussions in this document because of its limited application use in defining the different animal slaughter premises. According to the Zambian set-up, the following classification will be applied so as to identify the various animal slaughter premises.

2.2.1 Slaughter Slabs

These are concrete slabs of any shape but usually rectangular, constructed in open air to which local people bring their animals for slaughter (Anon., 1983). Meat from slaughter slabs tends to be cheap and is usually consumed by people in the peri-urban areas. Recently, the trend is changing slightly as more and more urban dwellers are taking drives to these slaughter slabs to buy cheap meat. The same meat is also finding its way into the cities through street vendors.

Butchery back-yard slaughter slabs also constitute another category of slaughter slabs. Urban slaughterhouses are in strict competition with these slabs as most of these butcheries avoid taking their animals to local slaughterhouses for fear that their animals may not pass the test of the "strict" inspectors (Anon., 1983).

Slaughter slabs can be categorised into two, according to management: The first category comprise those recognised by the local authorities and have their health personnel conducting meat inspection, and the second category are those illegally operating without inspectors. These are the most common slaughter premises in Zambia.

2.2.2 Slaughter Shelters

These are improved forms of shelters and constitute a simple building construction with little to no machinery inside except for a few hooks for hanging carcasses and aiding evisceration and skinning. These are usually defined as "small slaughterhouse" (Anon., 1983). Usually there is free air circulation with the outside environment. They may or may not have lairage facilities but usually have a crush pen for handling the animals and in some places for euthanasia. In Zambia, these are the second commonly found and are located almost in all district centres. This category has both meat inspectors from the local authority and meat quality graders from the ministry of agriculture.

2.2.3 Slaughterhouse Proper

This is a building for the slaughter of animals for human food. It may vary in sophistication and size but usually has the following facilities: lairage, slaughter area, refrigeration facilities, offal, gut, tripe area, hide and skin area, cutting room, dispatch area and amenities for personnel (Blood and Studdert, 1988; Anon., 1983). In Zambia, this constitutes the smallest percentage of the slaughter premises and the majority are privately owned, with a few owned by municipal authorities.

2.3 Bacterial Meat-borne Pathogens and their Sources

Meat and meat products derived from animals suffering from zoonotic diseases or contaminated with meat-borne bacteria can cause both human infection and intoxication (Gracey and Collins, 1992; Schwabe, 1984). The end result of these infections and intoxications can be morbidity and /or mortality to both the food handler and the consumer. Meat-borne diseases not only cause disease in humans but also deprive the population of the benefit of meat, milk, poultry and fish which are important high protein sources (Schwabe, 1984; and Anon., 1983).

There is a large number of meat contaminants that can be found in and on meat (Gracey and Collins, 1992). These may affect safety, wholesomeness and/or keeping quality (Dainty *et. al.*, 1983). These meat contaminants can be classified into three broad categories namely: biological, chemical and physical contaminants (Gracey and Collins, 1992). It has been noted that, the largest source of harm to human health in food are, firstly, biological contaminants and secondly nutritional imbalances (McCapes *at. el.*, 1991). Since this study mainly focuses on bacterial meat contaminants, the discussion will be centred around these pathogens.

Mayr (1980) reviewed the origin of diseases traced to food, and noted their aetiology to be as follows: 70 per cent bacteriological, 21 per cent chemical, seven per cent parasitological and two per cent virological. MaCapes *et. al.*, (1991) reported investigations of food-borne diseases in the United States during the period 1973 - 1984. Taking the total episodes for which the aetiology was determined to be 100 per cent, bacterial agents were responsible for 68 per cent, chemical agents 22 per cent, viral agents five percent and parasitic agents five per cent of the episodes. These investigation results maintain the same trend as that reviewed by Mayr (1980).

Almost any agent capable of causing an infection can be transmitted in food as a contaminant (Gracey and Collins, 1992). High protein foods of animal origin serve as ready vehicles for transportation of bacterial disease agents to humans. In the same study, McCapes *et. al.*, (1991) noted the type of food involved to be according to the proportions illustrated in figure 2.1 below.

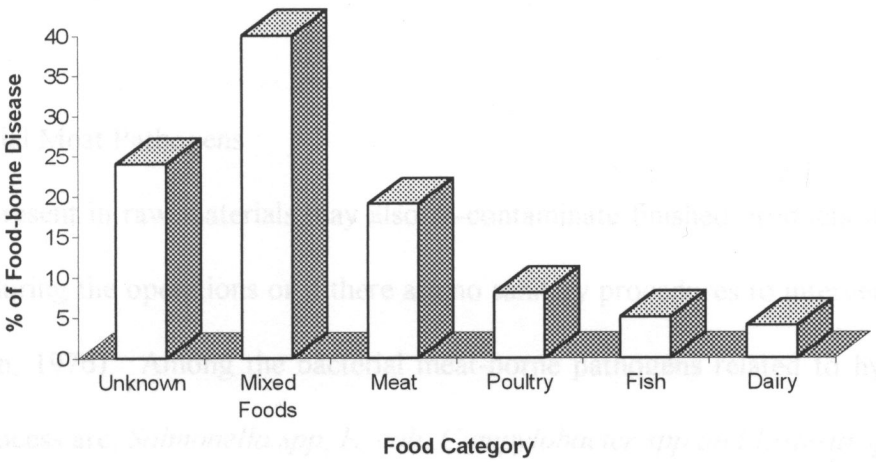


Fig. 2.1: Food vehicles in 1,591 bacterial disease episodes in USA (1973 - 1984) (Adapted from food-borne disease in USA, McCapes *et. al.*, 1991)

From the illustration in figure 2.1, it can be observed that meat was a single known food category among others which was responsible for a large proportion of episodes. This scenario is not unique only to United States, but also applies to other regions where these food types are consumed in such proportions. However, the importance of a particular type of food as medium for disease transmission will also depend on other factors rather than mere availability. Among them are: eating habits; socio-economic status of the community; and existence and prevalence levels of food-borne agents in the region (Motarjemi, *et. al.*, 1996; Bettelhem, 1997). In Zambia, beef is the major source of protein, then followed by poultry, and fish in that order, but since food-borne diseases are usually under reported, it might be difficult to quantify the significance of these foods as vehicles of disease transmission than simply depending on the

consumption amounts.

2.3.1 Bacterial Meat Pathogens

Pathogens present in raw materials may also re-contaminate finished products if hygiene is not adhered to during the operations or if there are no sanitary procedures to intervene (Constantine and Riemann, 1976). Among the bacterial meat-borne pathogens related to hygiene of cattle slaughter process are; *Salmonella spp, E. coli, Campylobacter spp and Listeria spp.*

Salmonella

The group *Salmonella* refers to a widely prevalent bacterial genus comprising over 2500 serovars which have closely related characteristics (Holt, 1994). *Salmonella typhimurium* and *S. enteritidis* are of particular interest in human salmonellosis. (Gerner-Smith *et,. al.* 1996, Wierup, 1996). Infection with *Salmonellae* may cause asymptomatic gastro-enteritis, meningitis, bacteraemia sepsis and death (Anon., 1988, Wierup, 1996). There are two important characteristics which has made *Salmonellae* to be widely distributed in nature and these are: the ability to infect a wide range of animals, both mammals and reptiles; and the ability to remain alive for a long period outside the host environment. *Salmonellas* have been reported to be the most important causal agents of food-borne diseases in many countries (Anon., 1989). The primary source of *Salmonellas* in food chain is from infected food animals (Wierup, 1996).

Hygiene during slaughter process, the second line of defence, is of critical importance to avoid contamination of carcasses with gastro-intestinal content and the skin (Anon., 1988; Wierup, 1996).

Escherichia coli

Escherichia coli is part of the microflora of the intestinal tract of human beings and most animals but not all serovars are harmless (Doyle, 1990). Pathogenic strains have been grouped into four categories based on distinct virulence properties; differences in interactions with the mucosa; distinct clinical syndromes; differences in epidemiology; and distinct O:H sero-groups (Doyle, 1990). These groups are: enteropathogenic *E. coli* (EPEC); enteroinvasive *E. coli* (EIEC); enterotoxigenic *E. coli* (ETEC); and enterohaemorrhagic/verotoxigenic *E. coli* (EHEC/VTEC) (Doyle, 1990). verotoxigenic *E. coli* and particularly 0157, are now recognised as important disease agent in human diarrhoea (Smith, 1996). *E. coli* 0157:H7 is one of the pathogenic members of the genus *Escherichia*, first identified as a pathogen during outbreaks in United States. *E. coli* is present in the intestinal tract, on the skin surfaces and udder of food animals. (Doyle, 1990; and Smith, 1996). During slaughter operations, contamination of meat with faecal, ingesta and milk poses a great danger of *E. coli* 0157:H7 contamination. verotoxigenic *E. coli* of specific types causes haemorrhagic colitis and uraemic syndrome (HUS) in humans (Vold *et. al.*, 1996). During the 1996 outbreak of EHEC 0157 which started among

elementary school children in Hiroshima, a total of 9578 cases were reported with 11 deaths and more than 90 diagnosed cases of HUS (Bettelhem, 1997). The risk is high in children and immuno-compromised people (Bettelhem, 1997). Therefore, during inspection, visible contaminations need to be trimmed prior to washing the carcasses. Washing of carcasses before chilling, drives the organisms into deeper tissues and makes it hard to remove them from carcasses.

Camplyobacter

Human camplyobacter enteritis are mainly caused by *C. jejuni* and *C. coli* (Berndtson, 1996). A large number of campylobacteriosis outbreaks in human results from consumption of raw and undercooked poultry products (Berndtson, 1996; De Jong and Andersson, 1996). Unpasteurised milk also causes sporadic cases as well as some large outbreaks (Berndtson, 1996, De Jong and Addersson, 1996). The disease is characterised by explosive watery foul-smelling diarrhoea sometimes containing blood. Infection can occur through contaminated meat and by drinking raw milk (De Jong and Andersson, 1996). Recently, campylobacteriosis has become a leading cause of bacterial gastro-intestinal disease (Wierup, 1996; and De Jong and Andersson, 1996). The increase in the incidence of campylobacteriosis reported cases has been attributed to the availability of new selective media and the increase in the number of immuno-compromised

people (Berndtson, 1996).

Listeria monocytogenes

Listeria monocytogenes is said to be the only human pathogenic species among the genus *Listeria* (Billie, 1996). In humans, as well as in ruminants, *L. monocytogenes* causes two main clinical pictures (Billie, 1996). In pregnant women it causes bacterimic episodes which if not treated may result in miscarriage, stillbirth, premature birth, or very ill new-born babies (Billie, 1996). In Australia, cases of listeriosis were reported in pregnant women causing stillbirths or mid-trimester miscarriage with a case fatality of 55 per cent (Watson, and Olt, 1990). In non-pregnant adult, infections have affinity towards the central nervous system and may result in meningitis or meningo-encephalitis. Listeria may contaminate carcasses during slaughter and dressing of carcasses (Nesbakken *et. al.*, 1996).

Table 2.1: Source categories of common meat-borne pathogens in the slaughterhouse

Meat-borne Bacteria Category		
Pathogens found in and on meat	Pathogens found in the environment	Pathogens from operators
Bacillus anthracis	*Clostridium*	*Staphylococcus*
Mycobacterium	*Bacillus cereus*	*Shigella*
Salmonella	*Staphylococcus*	*E. coli*
Camplyobacter	*Streptococcus*	*Streptococcus*
E. coli 0157:H	*E. coli*	*(Hepatitis A virus (HAV)*
Staphylococcus		*Norwalk virus)*
Listeria monocytogenes		
Brucella		
Yersinia enterocolitica		
Clostridium		

2.3.2 Sources of Meat-borne Pathogens

Meat quality assurance involves several aspects which includes: good farming practices; good slaughtering and dressing procedures of the carcasses in slaughterhouses; and good processing and distribution of finished products to the final consumers. In such a set-up, three production levels can be identified (Fig. 2.1). These components include: 1. Farming subsystem 2. Slaughtering subsystem 3. Processing and delivery subsystems. At each of these production

levels Critical Control Points (CCP_s)can be identified and preventive or control measures implemented to improve the quality of the ultimate product.

2.3.2.1 Farm Environment Subsystem

Conditions under which animals presented for slaughter have been reared contribute to quality of meat products (Gracey and Collins, 1992; Biss and Hathaway, 1996). Rearing conditions contributes significantly to the initial contamination on the animals which is of great significance to contamination on the slaughter floor. According to the Zambian livestock set-up, animal production can be categorised into two groups comprising commercial and traditional herds. The traditionally managed herd is estimated to comprise about 80 per cent of the national herd, and management of these animals is on natural pasture with little to no supplementation (Maff, 1994).

2.3.2.1.1 The Traditional Herd

Under traditional management, animals have poor shelter and in most cases are highly confined to small kraals at night, making transmission of diseases easier. Traditional cattle management has a problem of uncontrolled interaction of animals from different cattle herds. Animals are brought together, especially during dry seasons, to common grazing pastures, watering points, dipping facilities (where available) and in some communities circulation of bulls for breeding

purposes. All these factors contribute proportionally to disease transmission and maintenance in local herds. Strong beliefs embraced by some traditional cattle owners such as, vaccinating cattle causes abortion, illness or death; or that a vaccinated oxen is not going to plough in the next farming season, frustrate disease control efforts of ill equipped veterinary officials in traditional herds (Dietvorst, 1995). In Zambia, easily detectable and economically significant diseases like anthrax, tuberculosis are still a major problem in traditionally managed cattle and pose a great risk to human health.

2.3.2.1.2 The Commercial Herd

The commercial herd is, in many aspects, comparable to animal management in developed countries. In this herd most easily detectable infectious diseases have been eradicated or are at a minimal level. This is due to changes in the production patterns of animal husbandry, which affords production of "healthy" animals which are only carriers of pathogenic micro-organisms not usually detectable during inspection. Disease problems in this herd are termed "production diseases." These diseases are economically not disruptive but affect efficiency of production. Since these production diseases (e.g. *Salmonella*) do not always manifest clinical symptoms, farmers fail to appreciate the public health problems caused by these diseases (Simonsen *et. al.*, 1987). Commercial cattle management exposes animals to certain hazards which might not be so significant in traditionally managed cattle. The use of veterinary drugs (antimicrobials and

hormonal therapy), anabolics, acaricides and agrochemicals forms a potential chemical hazard in the commercial herd. Ngoma *et. al.,* (1993) noted that tetracyclines, streptomycin and sulphonamides were most widely used by commercial farmers in Zambia. He further observed that contamination of cattle and pig meat by antibiotic resistant *E. coli* and *Salmonella* was not a problem in Zambia. It should be pointed out, however, that the findings do not describe the level of antibiotic residual presence in Zambian meat. While this might give a clue to the use of certain antibiotics, residue presence of antibiotics needs independent data to argue this point. The commercial herd is often dynamic, and this allows animals to be sold at an early age as compared to the old and often unproductive animals coming from traditional farmers. Therefore, age related diseases can be divided in two categories, with old age related diseases like tuberculosis being a potential hazards in meat delivered from traditionally managed cattle and diseases of young animals being more frequently encountered in commercial herds.

Based on this categorisation, prediction of possible hazards from a given slaughter lot by understanding the kind of management animals have been reared under, can be made. This prediction can help in devoting inspection time and resources to possible hazards in a given category of livestock. In Zambia, it might be true to say, chemical hazards are a potential problem in commercially managed cattle whereas biological hazards are a potential hazard in traditional cattle. The two, however, are not mutually exclusive. As part of control efforts, the

principles of good production practice (GPP) and of good veterinary practice (GVP) should be exercised at farm level for better control of food-borne pathogens.

2.3.2.2 Slaughterhouse Environment

A slaughterhouse is a building for slaughter of animals for human food. This may widely vary in size, mechanisation, and other facilities depending on location and government ordinances, but usually has the following facilities or has them nearby: a slaughter area, refrigeration area, condemned meat area, tripe area, hide and skin area, cutting room, dispatch area, amenities for personnel, a veterinary office and the lairage (Blood and Studdert, 1988).

Slaughterhouse hygiene and ultimate meat contamination are a function of a complex set of events and interactions between the host cattle, disease agent and environmental factors (Habtemariam, 1983). Among the notable variables with potential influence on meat contamination are: design of the slaughterhouse, slaughter and dressing procedures, management practices, sanitary practice and carcass inspection procedures (Habtemariam, 1983). Contribution to meat contamination in slaughterhouses is from three aspects, namely: slaughter and dressing procedures, slaughterhouse personnel, and slaughterhouse environment (Table 2. 1) (Anon., 1984).

2.4. The Hazard Analysis Critical Control Point System

The HACCP system is a systematic approach to identification, assessment and control of food microbiological hazards (Bryan, 1992). Application of the HACCP system is based on the following seven principles: 1. Hazard analysis; 2. Determination of critical control points; 3. Establishment of preventive or control measures; 4. Establishment of monitoring procedures; 5. Establishment of corrective action procedures; 6. Establishment of verification procedures and; 7. Establishment of documentation procedures as appropriate (Bryan, 1992; Anon, 1993 b)

The HACCP system is a relatively recent quality assurance strategy that has been found to be the most cost effective and secure means of assuring food safety of all types (Baird, 1992; Berends *et. al.*, 1993; and Marton, 1993). The concept was developed in the U S A in the 1960_s jointly by the Pillsbury Company, the National Aeronautic and Space Administration (NASA) and the US Army Natick Laboratories. The reason for its development was the need to have a very safe supply of high quality food for the astronauts (Baird, 1992; and Quevendo, 1992). This followed the recognition that reliance on traditional inspection methods and testing of food for the absence of specific pathogenic micro-organisms did not provide a good degree of safety assurance needed for space missions (Baird, 1992). These findings were further supported by findings that, traditional emphasis on labour intensive organoleptic inspection procedures usually

conducted in slaughterhouses (e.g. routine meat inspection or microbiological sampling and testing), contributed far less to the safety and wholesomeness of food than does emphasis on production hygiene (Christensen, 1981; Anon., 1983; Dubbert, 1984; Hathaway and Pullen, 1990; and Berends *et. al.*, 1993).

2.4.1 Application of the HACCP system in Cattle Slaughterhouses

Application of the HACCP system in meat industry involves identification of actual hazards (infectious, toxic and physical) associated with production (hazard analysis), application of preventive or control measures to steps or processes in the production line so as to eliminate, prevent or minimise hazards associated with each control point (Critical Control Point), specifying critical limits to be met at each control point, establishing monitoring procedures and applying corrective action (Hathaway and McKenzie, 1991).

2.4.1.1 Hazard Analysis

A hazard is the unacceptable bacterial contamination in food or, chemicals or substances such as toxins, enzymes or products of microbial metabolism that may adversely affect food safety or quality and/or its keeping qualities (Bryan, 1992).

Meat hazards include such elements as: biological, chemical and physical contaminants (Gracey and Collins, 1992). Hazard analysis in reference to meat hygiene involves identification of hazards, which is a qualitative indication that a condition or substance in meat may severely affect human health, and a risk assessment, which is the likelihood of adverse effects from exposure to such a specific hazard or absence of beneficial influence (Hathaway *et. al.,* 1988). Hazard analysis thus involves developing a list of all possible conditions of public health, animal health, and of aesthetic importance that may cause changes in meat quality. CAP 535 of the laws of Zambia provides such a list of meat hazards which are checked for during ante-mortem and post-mortem inspections. However, developing an updated list of all potential hazards is hardly possible as new hazards continue to be discovered while old ones continue or are re-emerge. While hazard analysis may not identify all potential hazards, it may be useful in identifying critical control points where possible hazards of a particular type may be controlled (Bryan, 1992, Anon., 1993 b).

2.4.1.2 Critical Control Point

A critical control point (CCP) in an operation, is a step, procedure or process in which preventive or control measures could be exercised to eliminate, prevent or minimise a particular hazard (Bryan, 1992). Determination of CCP_s is based on preventing or controlling identified hazards. Contamination of meat and meat products resulting from either micro or macro

contaminants, can be controlled at various points on the beef production line (Fig 2.2). Meat production process can be divided into three main domains or sub-systems namely: farming, slaughtering and processing sub-systems (Anon., 1993 b). In the cattle slaughtering sub-system three critical points have often been identified namely; skinning, evisceration and chilling (Hathaway and Bullians, 1992).

2.4.1.2.1 Skinning

The skin and the gastro-intestinal tract are the principal sources of contamination, and activities on the slaughter floor, particularly those associated with removal of the pelt and gastro-intestinal tract, are the principal methods by which that contamination is transferred to the previously sterile carcass (Biss and Hathaway, 1996). The skin is also the principal source of contamination by *Salmonella* (Peel and Simmon, 1978). The skin is usually heavily loaded with different types of bacteria (Hadley *et. al.*, 1997). While pelting tend to prevent this bacteria from contaminating otherwise free carcasses, the physical process of removing a hide from the carcass poses biggest hazard to meat contamination (Hadley *et. al.*, 1997). Operators need to wash their hands and sterilise the equipment at all appropriate time to prevent cross contamination between the hide and the carcass (Hadley *et. al.*, 1997).

2.4.1.2 .2 Evisceration (Disembowelment)

Evisceration is the extrusion of viscera or internal organs. These internal organs include the gastro-intestinal tract. Just prior to death and shortly afterwards, a number of intestinal bacteria may invade the hosts tissues and some of these bacteria are potentially pathogenic (Carter and Cole, 1990). Therefore, a quick removal of the viscera soon after death is very important. Although evisceration is vital, it is the highest risk operation in the slaughtering procedure as regards contamination (Stolle, 1986). Rupture of the oesophagus, rumen or any other part of the gastro-intestinal tract (GIT) during evisceration may lead to meat contamination. Good evisceration techniques also reduce bacterial contamination on the slaughter floor (Hathaway and McKenzie, 1991).

2.4.1.2. 3 Chilling

Refrigeration of carcasses immediately after slaughter is recommended as this tends to inhibit bacterial growth (Dainty, *et, al.*, 1983). McCulloch and Whitehead (1979) conducted examinations of warm carcasses of cattle, sheep and pigs as they came off the line, and the same carcasses were examined after about 20 hours of chilling at a temperature of 9 ^{0}C. The results of direct contact colony counts of unclassified colonies per cm^2 of warm carcasses were estimated to assess the effect of chilling on bacterial growth. Chi-square test showed that after

chilling the carcasses, there was a rise in colony count ($0.001<p<0.05$) on cattle carcasses and a drop in colony count ($0.001<p<0.05$) on sheep; colony count on pig carcasses remained largely unchanged at all levels of data divisions (McCulloch and Whitehead 1979). Regression analysis studies of the results of indirect count, unclassified colony count of warmed and chilled carcasses, however, showed no significant relationship between warmed and chilled carcasses (McCulloch and Whitehead 1979). Reducing processing time and chilling of carcasses immediately after slaughter are important measures in maintaining minimal contamination levels (Hathaway and Bullians, 1992).

2.4.1.3 Establishment of Preventive Criteria

Establishment of preventive or control criteria that indicate whether an operation is under control at a particular CCP is a very vital procedure (Bryan, 1992). These criteria are specified limits or characteristics of a physical, chemical or biological nature which ensures that a product is safe (Hathaway and Bullians, 1992).

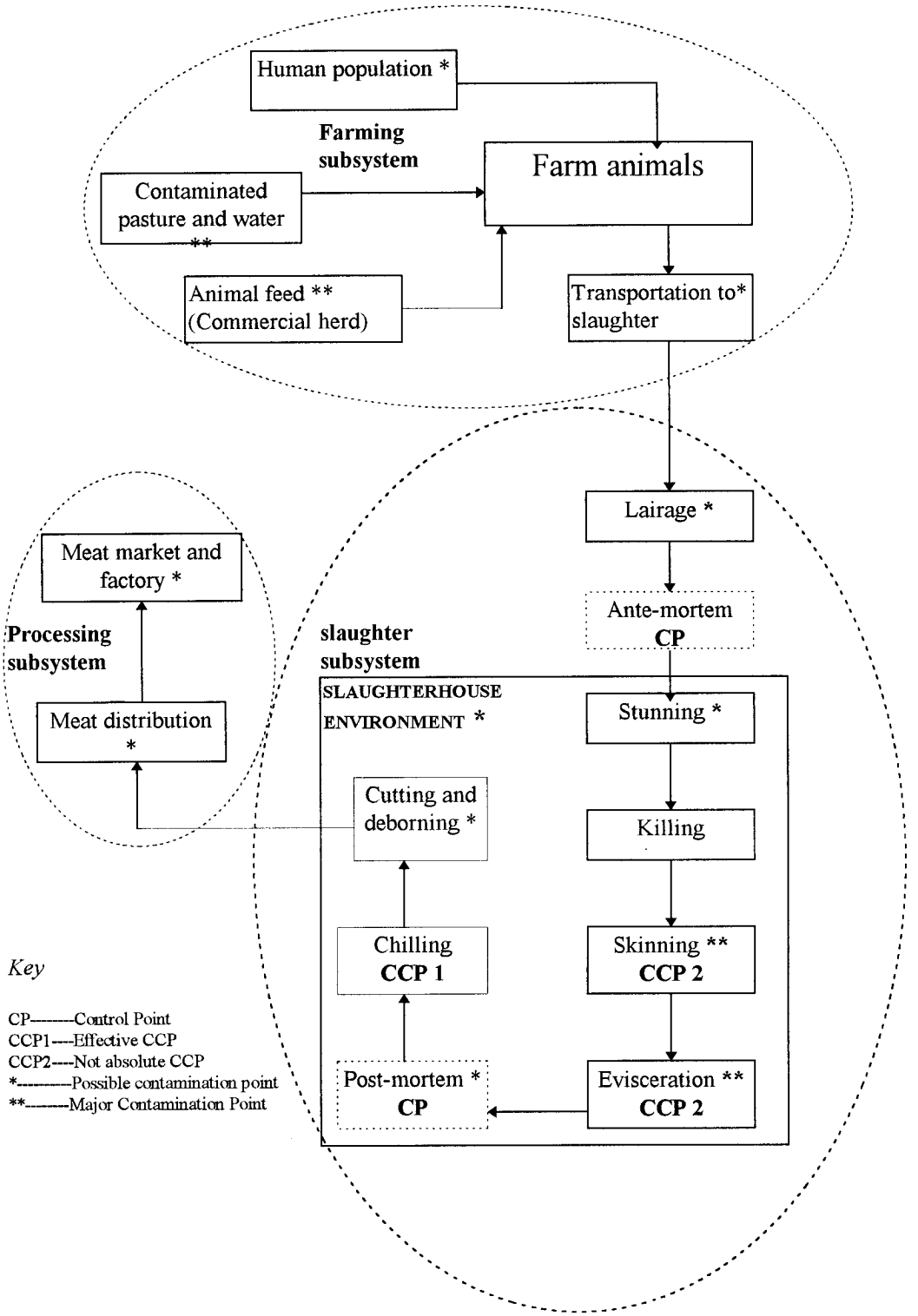
2.4.1.4 Monitoring

Monitoring of CCP_s involves systematic observation, measurement or recording of significant factors for control of hazards (Bryan, 1992; Anon., 1993 b). This ensures that all procedures are working according to the established criteria. This also enables corrective action to be taken

where the HACCP programme is found to be out of control (Bryan, 1992; Anon., 1993 b). Monitoring may be continuous or periodic as appropriate for a given process. This might involve making observations, taking physical and chemical parameters or carrying out analytical tests (Hathaway and Bullians, 1991). Hathaway and Bullians (1992) suggested monitoring of carcasses just before they are put in the chilling rooms as this approach appeared practical in terms of available resources.

2.4.2 Other Applications of the HACCP System

The HACCP system may be applied as an hygiene audit tool to regulate and monitor adherence to sanitary regulations (Anon., 1993 b). Application of the HACCP system does not only aid authorities with hygiene auditing but also promotes international trade by increasing the confidence of both the exporting and importing countries (Anon., 1993 b, Motarjemi *et. al.*, 1996). Some countries have been considering the requirement that imported food meet the same HACCP requirements as those stipulated for domestically produced products (Motarjemi *et. al.*, 1996). Recent discussions on Sanitary and Phytosanitary (SPS) agreement and the work of the Codex, Alimentarius Commission (CAC) has put the HACCP system as a reference international food safety requirement (Anon., 1993 a; and Anon., 1996).


Human population *
Farming subsystem
Farm animals
Contaminated pasture and water **
Animal feed ** (Commercial herd)
Transportation to* slaughter
Lairage *
Meat market and factory *
Ante-mortem CP
Processing subsystem
slaughter subsystem
SLAUGHTERHOUSE ENVIRONMENT *
Stunning *
Meat distribution *
Cutting and deborning *
Killing
Chilling CCP 1
Skinning ** CCP 2
Post-mortem * CP
Evisceration ** CCP 2
Key
CP-------Control Point
CCP1----Effective CCP
CCP2----Not absolute CCP
*--------Possible contamination point
**-------Major Contamination Point

CHAPTER THREE: MATERIALS AND METHODS

3.1 **Materials**

All media preparations were done according to manufacturer's instructions.

Media for Bateriological Enumeration and Isolation

1. Bismuth sulphite agar (Oxoid, CM201)	Highly selective for Salmonella
2. Brilliant green agar (Oxoid, CM263)	A selective media for Salmonella isolation
3. Brilliant green bile broth (Oxoid, CM31)	Detection of gas forming *E. coli*
4. Desoxycholate lactose agar (Nissui) and 5.Violet red bile agar (oxoid, CM107)	Selective, differentiation between lactose-positive and lactose negative Enterobacteriaceace
6. Lauryl trypotse broth (Difco)	Standard medium for detecting coliform bacteria in water and waste
7. MacConkey agar (Oxoid, CM7)	Differential media for isolation of coliforms
8. Peptone water (Oxoid, CM9)	Preliminary non-selective enrichment media
9. Rappaort-Vassiadis (RV) enrichment broth (Oxoid, CM669)	Selective enrichment media for Salmonella isolation
10. Selenite broth (Nissui)	Enrichment medium for *Salmonella* and *Shigella*

11. Standard plate count agar (Oxoid, CM)	General purpose medium for growth and enumeration of non-fastidious organisms
12. Tryptose phosphate broth (Difco)	For cultivation of fastidious micro-organisms
13. Xylose lysine desoxycholate (XLD) medium (Oxoid, CM469)	A weakly selective medium for differentiation of Enterobacteriaceae
Media for Biochemical	Tests
1. Triple sugar iron (TSI) agar, E-MA27 (Eikein)	• Hydrogen sulphide (H_2S) production • Metabolism of Sugars with acid production • Gas production
2. Lysine iron (LIA) agar, 05116 (Nissui)	• Decarboxylation of lysine to give amine cadaverine • H_2S production
3. Methyl-red/Voges-Proskauer (MR-VP) broth, 11383 (BBL)	• Utilisation of glucose for formation of large amounts of acid • Metabolism of glucose to produce acetoin (actylmethyl carbinol), 2,3-butanediol or diacetyl
4. SIM medium, 05106 (Nissui)	• H_2S production • Indole production • Motility
5. Urea agar, CM53 (Oxoid)	• Hydrolysis of urea
6. Simmons citrate agar, E-MA34 (Eikein)	• Metabolism of citrate as a sole carbohydrate source
7. Dorset egg slants (Denken Seiken Co. Ltd)	• Preservation of Salmonella

Other Reagents

Denken Seiken Co. Ltd, Salmonella O and H antiserum 12-Nihombashikabuto-cho, Chuku, Tokyo, Japan.

Sampling Materials

Metal templates

The templates were made of aluminium wire which formed a square loop of 5 x 5 cm^2 and a handle for holding. Metal templates were sterilised by first wrapping them in aluminium foil and then autoclaving for one hour at 180 ^{0}C using dry heat sterilisation

Wood applicators

Wood applicators were made of 20 cm long wood sticks with a diameter of 4 mm. These were packed in a plastic cylindrical container made of two jackets, with the top jacket having a bit smaller diameter than the bottom one so that the two made a slight overlap when fitted together. A small hole was drilled in the top jacket through which sticks were removed once sterilised. After filling the bottom jacket with sticks, the top part was fitted over, making a slight overlap, and the two were held in position by tapping with autoclave tape. The hole in the top jacket was also sealed with autoclave tape and sterilised for fifteen minutes at 121 ^{0}C.

Swabs

Swabs were made from a square piece of non-absorbent cotton gauze (10 x 10 cm^2) that was folded twice to form a swab with four layers. Several of such swabs were made and packed in piles of eight in glass petri-dishes (70 x 25 mm). To increase the adsorbing effect of the swabs, 10 ml of 0.1 per cent peptone water was added to each petri-dish. The petri-dishes were then sealed with autoclave tape and sterilised at 121^0C for 15 minutes.

Sample containers

Sample containers used were 200 ml wide mouthed glass bottles filled with 100 ml sterile 0.1 per cent peptone water (Difco).

Spirit lamp

For samples taken during operations, previously used templates were sterilised by dipping in 70 per cent alcohol and flaming in spirit lamp for five minutes.

3.2 Cattle Slaughterhouses

Four slaughterhouses A and B; from Lusaka Province and C and D from Copperbelt Province were randomly selected. Sampling for bacteriological examinations was, however, only done for Lusaka slaughterhouses. This was because of availability of proper laboratory facilities needed for the study, and the existence of an established slaughter process which facilitated systematic sampling. At slaughterhouses C and D, only observational data, based on hygiene assessment questionnaire, was collected. Eight visits were made to slaughterhouse A, four to slaughterhouse B and two to slaughterhouse C and D.

3.2.1 Slaughterhouse A

Slaughterhouse A was situated in Lusaka Province and was located right in the city centre. The plant had lairage facility, slaughter hall, offal room, refrigeration facilities and workers amenities. The plant also had a processing section in which beef steak, sausages and minced meat was processed and packed. The plant mainly slaughtered cattle originating from Southern and Western Provinces with some coming from commercial farms in the vicinity. Very few sheep and goats were slaughtered at this slaughterhouse.

This slaughterhouse had a powered overhead dressing system and used a mechanical hide stripping which applied an upward-pulling (head to tail). It's slaughter capacity was about 300

animals per day and had a full storage capacity of over 2000 carcasses. But at the time of the study, only two chilling rooms were operational with capacities of about 800 and 1000 carcasses for the big and small chilling rooms, respectively.

Slaughter and dressing operations

Once animals were off-loaded, they were kept in the lairage for 24 hours. The lairage was steel built with a concrete floor. On the day of slaughter, animals were led through a crush pen leading to the stunning box. During this process, animals passed through dirt stagnant water which filled a trough with an approximated size of 10 m long, 1m wide, and 1m deep. Animals bathed in this water up to the level of the elbows in case of big size cattle (400-500 kg) and shoulder high in case of small size cattle (250-300 kg). Animals which fell down in the process got a complete body wash. This bath left a visible dirty coating on the skin resulting from a mixture of faecal matter, mud and water. During rainy season, water naturally accumulated, but this did not result into increased water level since at about 80 cm, water overflowed into a drainage outlet.

Ante-mortem inspections was not performed. During stunning, a captive bolt pistol was used after which carcasses were hauled and hoisted up on the hoist and bled. Bleeding was done by making bilateral incisions of the jugular veins and carotid arteries through the throat. The

carcasses were then conveyed to the slaughter hall through an elevated climb of about 20 m at an angle of about 30°. On the slaughter floor, the head was removed first and hang on the conveyor which conveyed them for inspection. The hooves were then removed and put in a trolley.

Skinning and Evisceration

Skinning was done using a mechanical puller during which skin sleeves of the fore-limbs were attached to the puller using two iron chains, each of about 30 cm length. After skinning, the breast bone was cut by either using a cutting saw or a hand-held axe and the carcass was ready for evisceration. Evisceration was done with the operative standing on a conveyor which was meant to convey offals to an aperture of the tripe room. Firstly, the colon was cut, followed by opening of the gall bladder (search for gall stones). This was followed by puncturing two holes in the rumen to "facilitate" handling. The oesophagus was then cut at the level of the diaphragm and the rectum was also cut to make a final release of the abdominal viscera Then with both hands inserted in the rumen puncture holes, abdominal viscera was dislodged. This was followed by cutting the diaphragm and incising the trachea to remove the thoracic viscera. The lungs and heart were removed and put on a conveyor to be inspected later and put in trolleys. After carcass bisection, the kidneys were removed and carcasses with dirt were washed with tap water and sometimes using a rag of polythene bag. The carcasses were then rolled down to the

chilling rooms, down through an equivalent depression as the climb from stunning box to the slaughter floor. Carcasses were stored at a temperature of about 4 ^{0}C.

3.2.2 Slaughterhouse B

Slaughterhouse B was constructed in 1990 and had a building capacity of about 300m^2 comprising a slaughter and processing plant and a dry rendering plant. The plant was privately owned and was situated in Lusaka rural, about 30 km outside the city. Much of its surroundings comprised grazing pasture. Adjoining the slaughter and processing plant was a lairage, constructed with steel metal bars and elevated on a concrete slab at about one meter high from the ground. The lairage which measured about 10 x 50 m^2 was well partitioned and had an iron roof raised to about five meters from the floor. The slaughter hall measured about 10 x 25 m.^{2} The plant also had two chilling rooms, with a capacity of 75 carcasses each and two rooms for frozen products with capacities of about 100 carcasses each.

This slaughterhouse had a manually operated overhead rail dressing system which used gravitational force to operate it's system. It had a slaughter capacity of 120 carcasses per day. During the time of study, the slaughter size ranged from 10 to 60 carcasses per day and the average carcass speed-line was 15 carcasses per hour. The slaughterhouse received animals from both commercial and traditional farmers.

Slaughter and Dressing procedures

Among the differences in the operational procedures between slaughterhouses A and B were as follows: Where as slaughterhouse A had a mechanical puller, hide stripping in slaughterhouse B was done manually with a downward pulling procedure (tail to head). Unlike Slaughterhouse A, in this plant thoracic viscera were hooked onto the racks which made them slide into the red offal-cubical were inspection was done. Slaughterhouse B also had no physical separation between clean and dirt operations. This plant also had a form of carcass and visceral identification. Carcasses in slaughterhouse B were chilled at an average temperature between 6 ^{0}C and 7 ^{0}C.

3.2.3 Slaughterhouse C and D

These two slaughterhouses differed a lot with the above mentioned plants in design and processing procedures. These would better be described as slaughters, rather than slaughterhouses.

Slaughterhouse C

Slaughterhouse C was built in 1931 and was situated approximately three kilometres east of Ndola city centre in an industrial area. The slaughterhouse had no boundary fence. The lairage was an open court measuring about 64 m^2. It was located about 50 m from the slaughterhouse.

There was no off-loading bay but instead an ant-hill was used for this purpose. The building was about 100 m^2 in area and had very basic facilities for hanging and dressing carcasses.

The slaughter hall was divided into five partitions comprising: the main slaughter hall, two small slaughter rooms, the trippery and guttery room, and a small holding room. All the above mentioned rooms except for the trippery and guttery rooms were fitted with overhead rails. All operations were manual except for a an electric splitting saw in the main slaughter hall. There were three pulleys which were used to raise and lower the carcasses. Because of poor water supply, two drums with capacities of 200 litres each, were kept in the main slaughter hall as storage tanks and from these, water was drawn with buckets for washing carcasses. Only natural light was utilised in the slaughterhouse. The trippery and guttery room was fitted with a round metal drum with a diameter of about 1.5 m and was about 0.8 m deep. The drum was filled with water which was used for washing white offals.

Most of the animals slaughtered at this slaughterhouse were cattle (average per year 5,929) and goats (average per year 496) as shown in the records for the past 10 years. At this slaughterhouse operations were manual and the slaughter capacity undefined because of lack of defined dressing and slaughter procedures.

Animals were often slaughtered after more than 24 hours. Animals slaughtered at this slaughterhouse came both by rail and by road from Southern, Western and Central Provinces, with Southern Province supplying the largest number. Upon arrival, animals were grazed within the slaughterhouse premises until they were sold out. Time between arrival and slaughter varied considerably as slaughter depended upon availability of meat buyers

After stunning, skinning and dressing was done in groups, with the number of groups present being dependent upon the number of clients whose animals were being slaughtered on that particular day. Except for meat processing companies that rarely brought their animals to this slaughterhouse, butchers, restaurant and canteens brought their own people to "help" and "monitor" dressing of their animals. On average, four working groups, each comprising four persons including one council employee, were often seen during the operations.

With the animal in ventral recumbence, the exposed ventral half of the carcass was skinned leaving the dorsal aspect, on which the animal was lying unskinned. Abdominal viscera were removed and dragged to the trippery and guttery room. With the carcass still lying on the floor, it was split in the mid-line leaving the two halves still attached by the muscles and the skin. The carcass was then rolled so as to lie in lateral recumbence. The top half-segment of the carcass was skinned up to the level of the back mid-line and the resultant loose skin flipped-over and spread on the floor to lie opposite the carcass. This was followed by division of the skinned

half-segment into two portions. The skinned hind quarter was first cut off and hang on a hook and like-wise the thoracic segment was also cut off and hang on a hook, separately. Assuming the carcass was in left lateral recumbence, the carcass was then flipped over to lie in the right lateral recumbence on the spread skin of the left segment. Skinning then continued on the right side of the carcass and once completed, the skin from the second half-segment was stretched and spread on the floor. The right half-carcass was also divided into two pieces and hang on hooks. The head was finally cut off the skin and placed on a concrete bench while the skin was dragged outside by the client's representative

After the white offals were cleaned, they were collected by the client's representative who used the rumen as a carrier bag for other offals and feet (Plate 2). This was dragged outside the slaughterhouse or was put adjacent to the red offals.

Slaughterhouse D

The slaughterhouse was situated in Kitwe and was located in the industrial area north of the city. The facility was owned by the city council. The slaughterhouse was mainly used for cattle slaughter but there was a weekly provision to slaughter pigs. Slaughterhouse D was built in the late 1940s as a local authority service to the local butcheries and other meat traders within the locality of the city council. It had an incomplete wall boundary fence which enclosed all the

slaughter facilities including the manure disposal pits. The slaughterhouse was sited on a three hectare plot in the industrial area north of the city. Facilities on the site included a lairage of about 25 x 15 m made of steel pipes and concrete floor. There was a small off-loading bay adjoining the lairage.

The slaughterhouse measured about 8 x12 m and had two partitions: a slaughter hall and the holding section. The main slaughter hall was conveniently divided into the slaughter section and the trippery and guttery section without any physical separation. Both the slaughter hall and the holding room were fitted with over-head rails. All round the walls of the slaughter hall were fitted several hooks at about a meter from the ground. Red offals were hang here after evisceration for inspection. All operations were manually done and the average daily kill was about 15 animals.

The slaughter and dressing procedures in this slaughterhouse were similar to those of slaughterhouse C. Differences included the stunning methods in which case this slaughterhouse use a chisel-shaped metal rod (about 10 cm long and weighing 50 grams) to stun the animals by piecing the brain through *cisterna magna* instead of the captive bolt pistol. In this slaughterhouse unlike A, B, and C, viscera were hang on hooks fitted all round the walls of the slaughterhouse at about a meter height.

3.3 Sampling Procedures

3.3.1 Environmental Surfaces

Environmental surfaces which were sampled for bacteriological examination included: working tables, knives, cutting saws, workers protective clothing, floors and walls.

Samples were collected by swabbing a 100 cm^2 area of each of the articles mentioned above during each visit. Swabbing was done with the aid of a sterile metal template which out-lined a square area of 25 cm^2. A swab was picked from the petri-dish using a sterile wood applicator. During sampling, the autoclave tape was removed from the exit hole in the top jacket of the applicator's container, and the container was turned upside down. A gentle shake allowed one of the sticks to pip and this was removed and held in one hand. The petri-dish containing the swabs was also opened by removing the autoclave tape. Using the wood applicator, a swab was picked-up by first placing the sterile tip on top of the first swab in the petri-dish and then rolling it over the surface of the swab. In this way, a swab was firmly twisted to the wood applicator. With the other hand holding the metal template to isolate the required surface area, the swab was slowly and thoroughly rubbed over the 25 cm^2 area, reversing direction between successive strokes (Plate 4). After swabbing, the top of a sample bottle was opened and the swab lowered into it by gently shaking the swab off the wood applicator. The bottle top was then placed back and the bottle was quickly placed in the cool box containing melting ice where it was maintained and transported. For each article four different sites were swabbed and the four swabs where

pooled in one sample bottle, except for floor samples where only two swabs were collected. All surface swabs were collected from the equipment after it had been used.

3.3.2 Carcass Surfaces

A total of 74 carcasses were sampled out of which 54 were from slaughterhouse A and 20 from slaughterhouse B. On-line systematic sampling of carcasses was applied to collect samples at the following points: point A, before pelting; point B, after pelting; point C, after evisceration; and point D, post-chilling (Plate 3). At each sampling point, the carcasses were swabbed at four sites (100 cm^2), except for point A (skin) at which only two sites (50 cm^2) were swabbed. This was because of a high bacterial count expected at this point. All swabs from a particular sampling point were pooled in one sample bottle. On a particular sampling day, an average of five carcasses and five environmental surfaces were sampled.

Point A (skin)

Two skin swabs were collected using the same technique described above for environmental surfaces. One swab was collected from the inguinal region and the other in the region of the brisket. The two swabs were pooled into 100 ml of sterile 0.1% peptone water and kept on melting ice.

Point B (after skinning)

The four sites sampled were; two swabs on the lateral aspects of the elbow region one on each side and the other two on the lateral thoracic region. These sites were chosen because they were the most likely areas to be contaminated as observed during the preliminary visits.

Point C (after evisceration)

Two swabs were collected from the medial aspect of the elbow region, one on each side, and two from inside of the thoracic region, also one from each side.

Point D (after chilling)

This was done only for slaughterhouse A because of its proximity to the laboratory facilities. Swab samples were collected just before carcass were put in chilling rooms. Procedure of sample collection was as described for point C above. The two sides of the carcasses were identified by fixing a tag on the sides of a particular carcass. Sample treatment also followed the same procedure as outlined below. After chilling the carcasses for 24 hours, samples were again taken from the same marked carcasses. After processing the samples, the same were incubated for 24 hours at 37 ^{0}C and again processed as mentioned in laboratory procedures below.

3.4 Laboratory Procedures for Aerobic Plate Count

3.4.1 Aerobic Plate Count (APC) (Viable Plate Count/ Heterotrophic Plate count)

This method provided an approximate enumeration of the total number of bacteria multiplying at 37 ^{0}C (Anon, 985) yields useful information about meat quality and provide supporting data on the coliform test results. In the laboratory, a particular sample was removed from the ice box and kept at room temperature for about 10 minutes before processing. Sample were swirled for two minutes by making back and forth movements and then allowed to stand for two minutes then swirled again for another minute on the mechanical shaker. From each sample bottle, 1.0 ml aliquots of the homogenate was pipetted into 9 ml of sterile 0.1% peptone water and serially diluted to make the following dilutions: 10^{-1}, 10^{-2} and 10^{-3}. Then duplicate 0.1 ml aliquots from the second dilutions (10^{-2}) were inoculated onto the surface of pre-dried nutrient agar plate marked with sample number, dilution factor, and source of the swab material, and incubated at 37 ^{0}C for 24 hours. Glass petri-dishes (90 x 15 mm) were used. Spreading of the inoculum on the media surface was done with a bent sterile glass rod (Messer, *et. al.*, 1985).

Counting and Spread Recording

Colony counting was done promptly after 24 hours of incubation. For each particular sample, colonies from the duplicate plates were counted and the average calculated. Results of sterility control for the media and diluting solution (peptone water) were also included for each lot of samples. Manual counting was done with the aid of the Erma counter which helped to magnify and illuminate colonies. During the trial runs, dilutions giving countable colonies per plate from a 0.1 ml inoculum were adopted in the final runs. Total bacteria in a millilitre of the rinsing solution (0.1 per cent peptone) was computed by multiplying the average number of colonies from the two plates by the reciprocal of the dilution used. Bacterial counts per cm^2 of carcass surface area were calculated and was equivalent to counts per ml (Appendix 2). On plates with colonies too dense to count, the four highest square counting method was applied (McCulloch and Whitehead, 1979; McCulloch and Whitehead 1981).

3.4.2 Total Coliform Count (TCC)

Plate counting using violet red agar (Oxoid CM107) was used in enumerating the coliforms. But before violet red agar became available, desoxycholate lactose agar (Nissui) was used. Duplicate 0.1 ml aliquots of the original sample solution were inoculated on pre-dried surfaces of agar and incubated at 37 ^{0}C for 24 hours. Spreading of the sample liquid on agar surfaces was done with a sterile glass rod. Colonies on duplicate plates were counted and the average

calculated. Three of the suspected coliform colonies per plate were taken from 10 per cent of the plates and inoculated in tubes containing brilliant green bile broth (Nissui) fitted with Durham tubes. These were incubated for 48 hours at 37 ^{0}C to test for gas production as a coliform presence confirmation test.

3.4.3 Faecal Coliform Count. (FCC)

For determination of faecal coliform densities, the multiple tube technique was used as a criterion of estimation of the degree of contamination and assessment of sanitary quality of the slaughterhouse operations (Anon, 1985; Messer, *et. al.*, 1985). The three dilutions used were: 10^{-1}, 10^{-2} and 10^{-3}. Five 1.0 ml replicate samples for each of the three dilutions were added to five test tubes, each containing 10 ml of lauryl tryptose broth. The tubes were incubated at 44.5 ^{0}C for 24 hours. After 24 hours, sample tubes were examined by gently shaking the tubes and examining them for gas production. If no gas had been formed and trapped in the Durham tube the test was recorded negative. Positive samples were recorded in tubes showing gas formation regardless of the amount. Positive results were indicated by formation of gas and production of acid as indicated by the indole test in replicates with highest sample dilution. Test results were recorded as Most Probable Number (MPN) index (Appendix 3).

3.4.4 Isolation of *Salmonella*

After processing for APC, TCC and MPN, the samples were incubated at 37 ^{0}C for 24 hours. From incubated samples, 5 ml aliquots were withdrawn and added to equal volumes of double-strength selenite broth and incubated at 37 ^{0}C for 18 hours. Rappaport enrichment broth was later used when stocks became available, in this case, 1 ml of the sample solution was added to 9 ml of the broth. From enriched samples, a loop-full of the sample was streaked on bismuth sulphite agar (modified), xylose lysine decarboxylate medium, and brilliant green agar. After 24 hours of incubation at 37 ^{0}C, suspicious *Salmonella* colonies were sub-cultured on the same media to get pure cultures. From these pure cultures, colonies were tested with biochemical test and also inoculated on tryptocase soy agar for serotyping. Serotyping was attempted on *Salmonella* isolates which conformed to characteristic biochemical reactions of the genus. After identification, the cultures were preserved on Dorset's egg slants and typtocase soy agar slants at 4 ^{0}C and sub-cultured every three months. Bacteria cultures were stored in screw-capped bottles and each was stored in two tubes.

Identification of Salmonella colonies

Salmonella colonies were suspected as colonies with black centres, light edges surrounded by a metallic lustre on bismuth sulphate agar; pink colonies surrounded by a red zone on brilliant

green agar; and on X L D agar the colonies appeared the same as the medium, translucent, sometimes with a black centre.

3.5 Hygiene Audit Surveillance

A questionnaire (Appendix 4) designed to assess the following: hygiene practices in slaughterhouses; monitoring and inspection services; and hygiene of slaughter and dressing, was applied to the four slaughterhouses. The questionnaire was designed on the principles of GMP and HAS.

3.6 Analysis of Results

Colony counting

Whole plate colony count (WPCC) on duplicate plates was used to enumerate bacteria. In situations of very dense colony growth, the Square Sum Count (SSC), which involved adding the number of colonies in four, one cm^2 squares (McCulloch and Whitehead, 1979), was used to estimate the total number of colonies on a plate (90 x 15 mm petri dish). Colonies growing too dense and too numerous to count were allocated the highest count classification at the time of the assessment (McCulloch and Whitehead, 1979).

Handling count data

Averages of duplicate plate count for each sample were calculated for each data set. The number of colony forming units (c.f.u) in 1 ml of sample solution (equivalent to 1 cm^2 of the swabbed surface) were calculated by multiplying the average count by the reciprocal of the dilution factor; which was 1000 for APC data and 10 for TTC data (Appendix 2). For point A count data, these were further multiplied by the factor two to make up the difference, for a 25 cm^2 sampling surface instead of 50 cm^2. All figures were rounded off to two significant digits and logarithmic transformation was applied by replacing X with log (X + 1) (Kirkwood, 1988 and Fowler and Cohen, 1990). Spearman's rank correlation was used to assess the degree of association between different bacteria categories while differences between sampling points were assessed with Man-Whitney U test. The Wilcoxon rank sum test was used to analyse the effect of chilling on bacteria multiplication. Variation in daily averages between days were analysed using Krusk-wallis test. Statistical calculations were done using C-Stat for Windows version 1.0 (Holman and Kennedy, 1993).

PLATE 1. Operations in slaughterhouse D showing two working groups with spectators who included clients, meat buyers, and scavengers. Note that all operations took place on the floor.

PLATE 2. Clients with "offal bags" and cattle heads which have been dragged outside waiting for transport. Offal bags were rumens which were packed with other GIT contents and red offals.

PLATE 3. One of the sampling points on the slaughter line of slaughterhouse B (point B, after skinning) illustrating the sampling method and site.

PLATE 4. Illustrates the sampling technique as at point B; also can see the metal template and the cotton swab wound on a wood applicator.

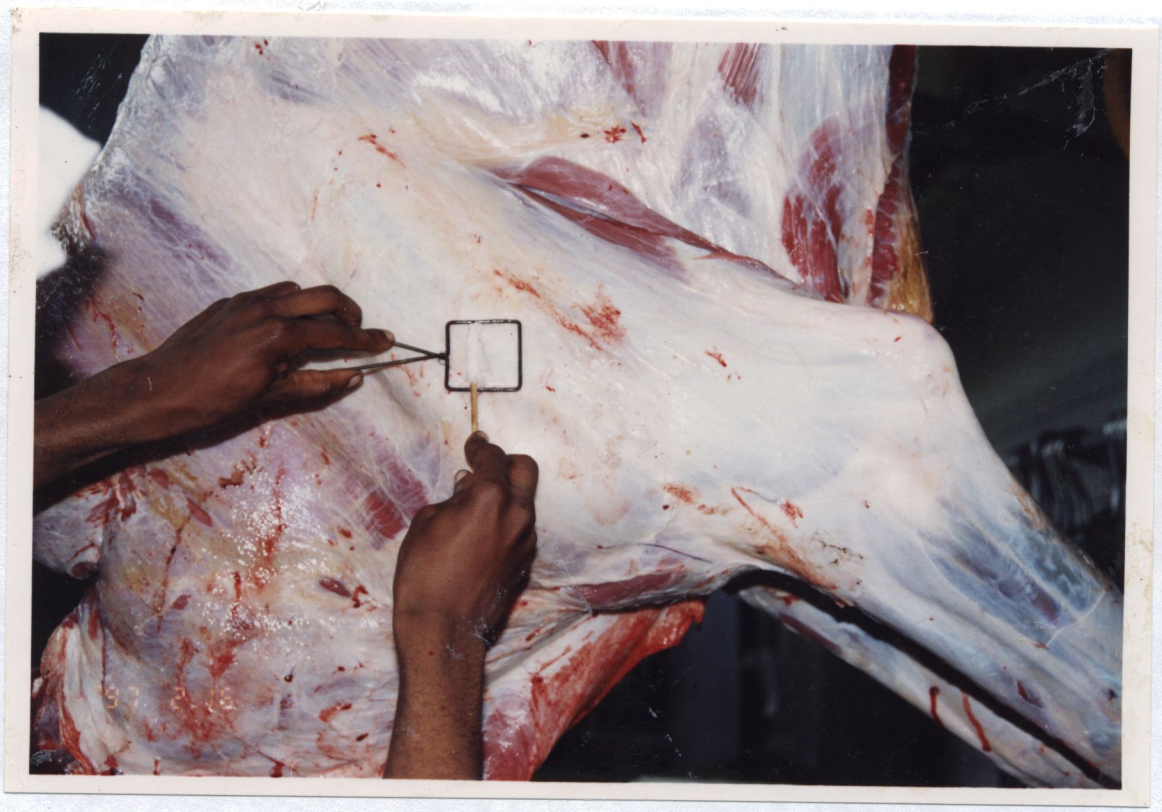


PLATE 5. Congestion in slaughterhouse C with all categories of people.

PLATE 6. Illustrates unhygienic slaughter with some operatives working with unprotected feet.

4.1.2 Slaughterhouse B

The mean APC on the skin was $\log_{10}$ 5.78; /cm^2; after skinning was $\log_{10}$ 4.14/cm^2; and after evisceration was $\log_{10}$ 3.67/cm^2 (Table 4.1). High count classification for APC was recorded under $\log_0$5.245/cm^2 for skin count (40%); $\log_{10}$ 4.245/cm^2 for counts after skinning (50%); and $\log_{10}$ 3.245/cm^2 for counts after evisceration (40%) (Fig. 4.2). In this slaughterhouse, mean TCC of the skin was $\log_{10}$ 2.68/cm^2; after skinning was $\log_{10}$ 1.66/cm^2; and after evisceration was $\log_{10}$ 1.63/cm^2 (Table 4.2). High counts for carcasses were recorded under the following classification: $\log_{10}$ 3.245/cm^2 for the skin (35%); $\log_1$01.245/cm^2 for counts after skinning (45%) and $\log_{10}$ 1.745/cm^2 for counts after evisceration (40%) (Fig 4.2).

The bacterial population at point A, was skewed to the left; at point C was skewed to the right; and at point B was symmetrical for slaughterhouse A and B (Figs 4.1, 4.2, 4.3, 4.4, 4.5, 4.6 and 4.7).

In slaughterhouse A and B, *Salmonella* on carcasses was recovered at points A, B, C and D. Out of the 21 *Salmonella* isolates, eight isolates were from point A, two from point B, five from point C and two from D. Five of the isolates were from environmental surfaces which included the worker's aprons, knives and the floor.

4.3 Environmental Surfaces

The Mean AP and TCC for environmental surfaces have been summarised in Table 4.5. Salmonella was isolated from the knives, floors and aprons.

4.4 Hygiene Audit

Based on the hygiene categories that were audited (Table 4.6), the hygiene satisfaction percentage for slaughterhouses A, B, C and D were: 45.3; 72.0; 18.7, and 21.3 per cent, respectively.

TABLE 4.1: Mean aerobic plate count (APC) at various sampling points at slaughterhouse A and B.

	Sampling Point		
	Skin	After Skinning	After Evisceration
Slaughterhouse A			
*Mean	5.10	4.10	3.97
95% C I of the mean	4.96 - 5.24	3.93 - 4.29	3.49 - 4.15
SEM	0.07	0.09	0.11
SD	0.37	0.50	0.61
Proportion duplicate zero counts (Total count 42)	0.00%	0.00%	15.00%
Slaughterhouse B			
*Mean	5.78	4.14	3.67
95% C I of the mean	5.42 - 6.14	3.88 - 4.40	3.37 - 3.97
SEM	0.17	0.12	0.14
SD	0.75	0.54	0.60
Proportion duplicate zero counts (Total count 20)	0.00%	0.00%	0.00%

*$Log_{10}APC/cm^2$

Mean excludes all duplicate zero counts and estimated values.

TABLE 4.2: Mean total coliform count (TCC) at various sampling points for slaughterhouse A and B.

	Sampling Point		
	Skin	After Skinning	After Evisceration
Slaughterhouse A			
*Mean	3.29	1.92	2.44
95% C I of the mean	3.12 - 3.47	1.60 - 2.25	2.12 - 2.75
SEM	0.09	0.16	0.15
SD	0.47	0.76	0.80
Proportion duplicate zero counts (Total count 34)	0.00%	25.00%	20.83%
Slaughterhouse B			
*Mean	2.68	1.66	1.63
95% C I of the mean	2.30 - 3.06	1.27 - 2.05	1.41 - 1.85
SEM	0.18	0.18	0.10
SD	0.78	0.67	0.43
Proportion duplicate zero counts (Total count 20)	0.00%	30.00%	15.00%

*$Log_{10}TCC/cm^2$

Mean excludes all duplicate zero counts and estimated values.

Table: 4.3 Mean MPN values at various sampling points at slaughterhouse A

	Sampling Point		
	Skin	After Skinning	After Evisceration
Slaughterhouse A			
*Mean	2.25	1.23	1.59
95% C I of the mean	2.00 - 2.50	0.96 - 1.49	1.23 - 1.95
SEM	0.12	0.13	0.17
SD	0.65	0.64	0.79
Proportion duplicate zero counts (Total count 20)	0.00%	13.79%	22.22%

*$Log_{10}MPN/cm^2$

Mean excludes all duplicate zero counts and estimated values.

Table 4.4 Comparison of the effect of chilling on APC and TCC count for slaughterhouse A.

	Pre-chilling			Post-chilling		
	APC	TCC	MPN	APC	TCC	MPN
!Mean	4.10	1.84	2.51	3.82	0.00	0.00
95% C I of the mean	3.71 - 4.49	1.01 - 2.67	1.82 - 3.20	1.23 - 1.95	0.00 - 0.00	0.00 - 0.00
SEM	0.17	0.37	0.83	0.15	0.00	0.00
SD	0.58	1.30	0.29	0.52	0.00	0.00
Proportion duplicate zero counts (No. of carcasses 12)	8.22%	0.00%	0.00%	0.00%	0.00%	0.00%

! $Log_{10}cm^2$

Mean excludes all duplicate zero counts and estimated values.

TABLE 4.5 Mean aerobic plate count (APC) and total coliform count (TCC) for environmental surfaces

	Slaughterhouse A		Slaughterhouse B	
Environmental Surface	APC*	#TCC	APC*	#TCC
Aprons	4.82	2.39	5.04	2.02
Floor	5.20	3.15	5.11	2.60
Hands	4.10	1.61	Not done	Not done
Knives	3.86	3.22	3.74	2.18
Saw-1 (opening brisket)	4.08	1.33	4.81	3.20
Saw-2 (bisecting carcass)	4.14	1.70	4.78	2.19
Tables	4.58	3.19	-	-
Walls	2.46	1.46	4.08	1.30

#$Log_{10}TCC/cm^2$

*$Log_{10}APC/cm^2$

Duplicate zero counts included

Table 4.6: Scores for Slaughterhouse Hygiene Audit

Hygiene Factor	Slaughterhouse Code			
	A (8)	B (4)	C (2)	D (2)
Lairage condition	4	5	2	3
Ante-mortem inspection	0	6	0	0
Skinning	8	6	2	2
Evisceration	4	8	2	2
Separation between clean and dirty operation	2	6	0	0
Personnel hygiene and practice	4	6	2	2
Maintenance and hygiene of premises	3	4	2	2
Water supply	3	4	2	2
Access by general public	3	5	1	1
General conditions and management	3	4	1	2
Total score	34	54	14	16
Hygiene Satisfaction %	45.3	72.0	18.7	21.3

Key: 0 = Not available; 1 = Bad; 2 = Very poor; 3 = Fair; 4 = Good; 5 = Excellent
( )= Number of visits made to the slaughterhouse

NB. Ante-mortem inspection, skinning, evisceration, separation of operations and hygiene of personnel were regarded to have significant contribution to meat contamination hence were weighted by doubling their scores.

Figures 4.1, 4.2, 4.3, 4.4, 4.5, 4.6 and 4.7 below illustrate the distributions of bacterial contamination at various operational points for slaughterhouses A and B. All distributions exclude zero duplicate counts.

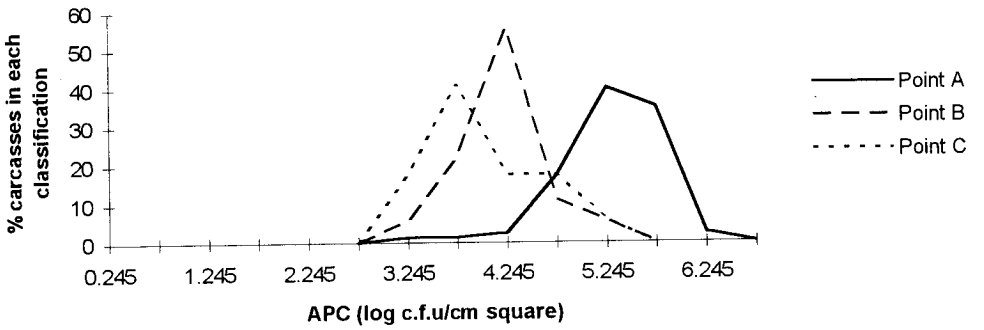


Fig 4.1: Frequency polygons of carcass APC levels showing distributions of aerobic bacteria (based on plate count) at various points of slaughter and dressing operations of slaughterhouse A namely: the skin (Point A), after skinning (Point B) and after evisceration (Point C).

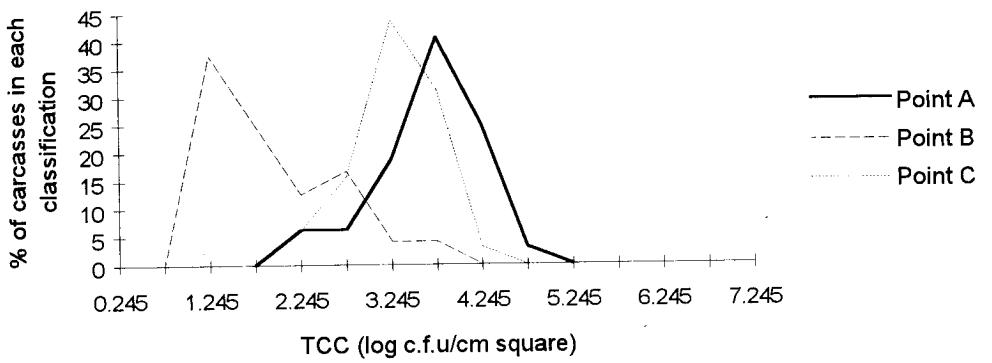


Fig 4.2: Frequency polygons of carcass TCC levels showing distributions of coliforms (based on plate count) at various points of slaughter and dressing operations of slaughterhouse A namely: the skin (Point A), after skinning (Point B) and after evisceration (Point C).

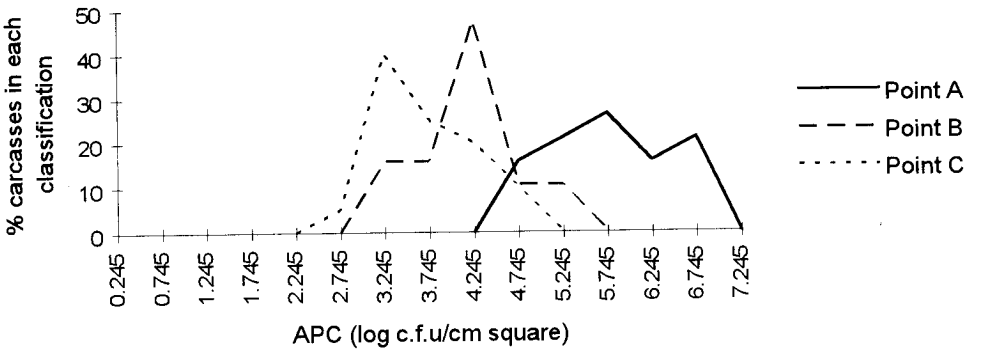


Fig 4:3 Frequency polygons of carcass APC levels showing distributions of aerobic bacteria (based on plate count) at various points of slaughter and dressing operations of slaughterhouse B namely: the skin (Point A), after skinning (Point B) and after evisceration (Point C).

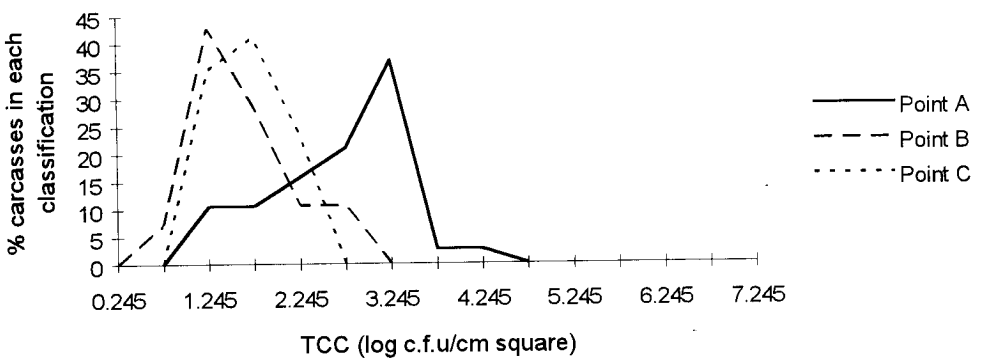


Fig 4.4: Frequency polygons of carcass TCC levels showing distributions of coliforms (based on plate count) at various points of slaughter and dressing operations of slaughterhouse B namely: the skin (Point A), after skinning (Point B) and after evisceration (Point C).

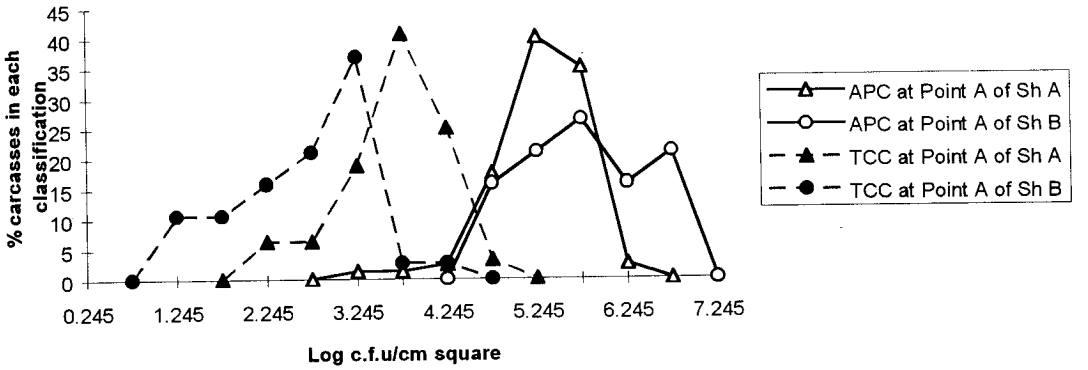


FIG 4.5: Frequency polygons showing distributions of coliforms (TCC) and aerobic bacteria (APC) at point A (skin) of slaughterhouse A and B.

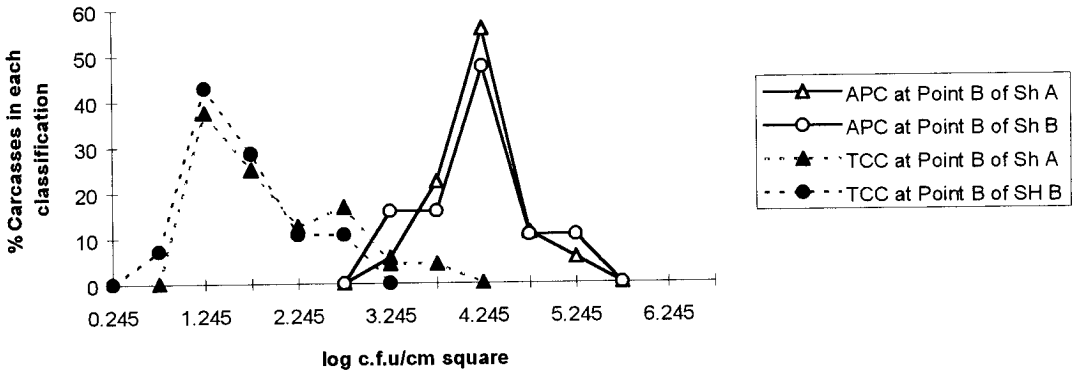


FIG 4.6: Frequency polygons showing distributions of coliforms (TCC) and aerobic bacteria (APC) at point B (after skinning) of slaughterhouse A and B.

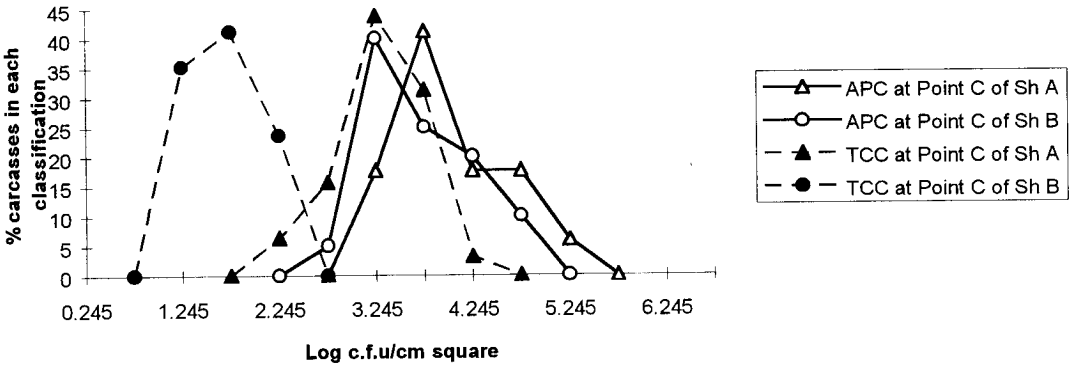


FIG 4.7: Frequency polygons showing distributions of coliforms (TCC) and aerobic bacteria (APC) at point C (after evisceration) of slaughterhouse A and B.

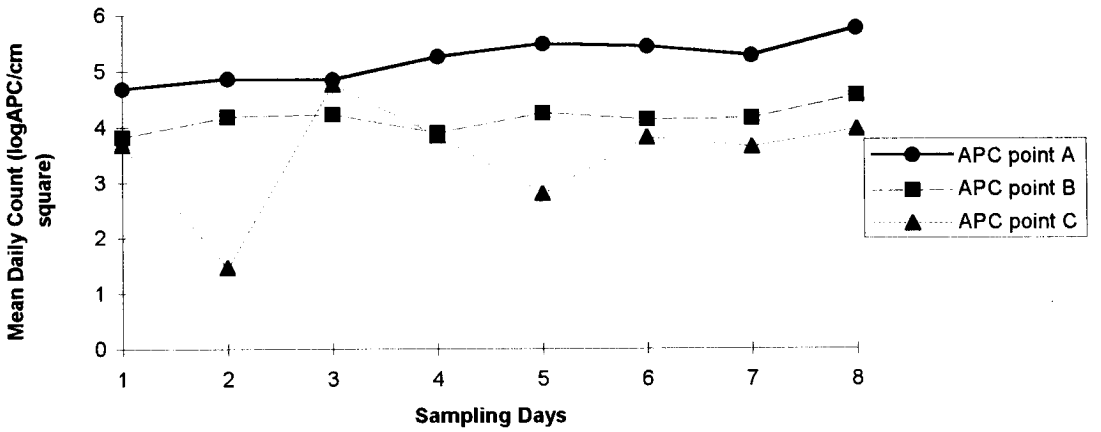


FIG 4.8: Mean aerobic plate count (APC) at various operational points (point A = skin; point B = after skinning; point C = after evisceration) indicating daily aerobic bacteria carcass contamination levels for slaughterhouse A.

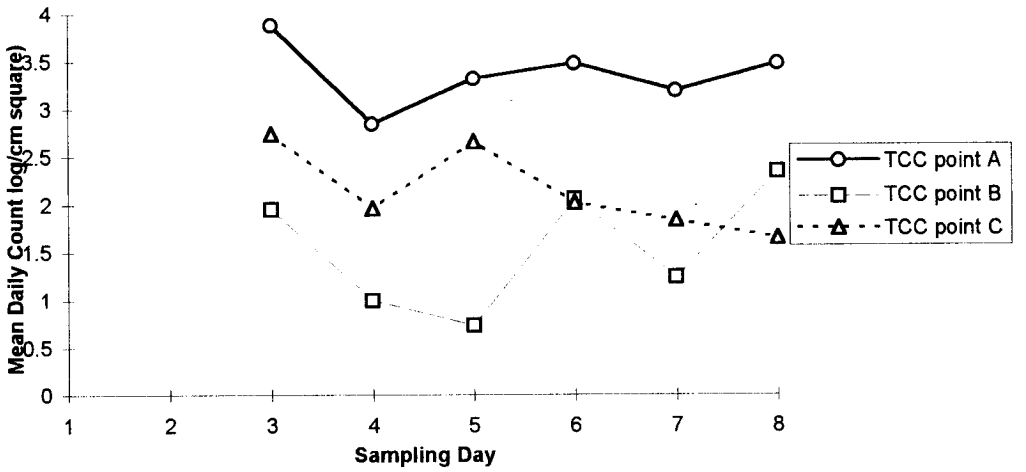


FIG 4.9: Mean total coliform count (TCC) at various operational points (point A = skin; point B = after skinning; point C = after evisceration) indicating daily coliform carcass contamination levels for slaughterhouse A

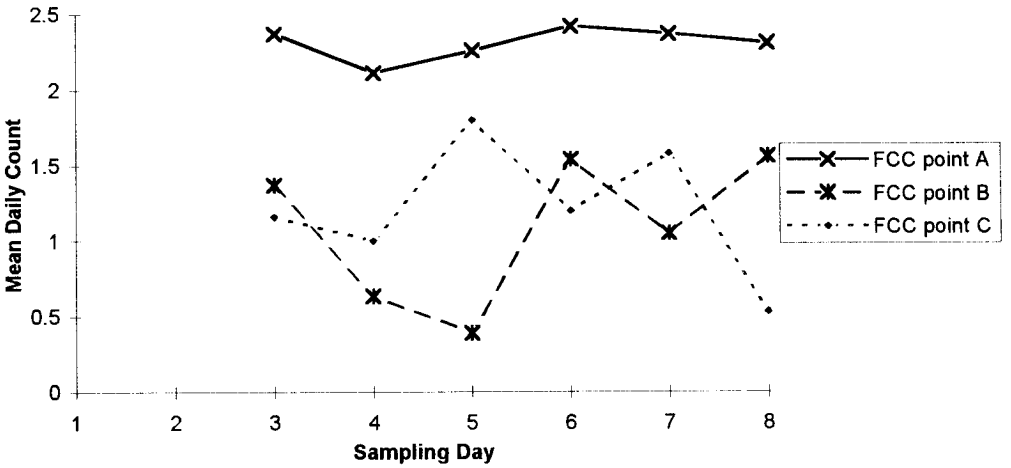


FIG 4.10: Mean Faecal coliform count (FCC) at various operational points (point A = skin; point B= after skinning; point C = after evisceration) indicating daily faecal coliform carcass contamination levels for slaughterhouse A

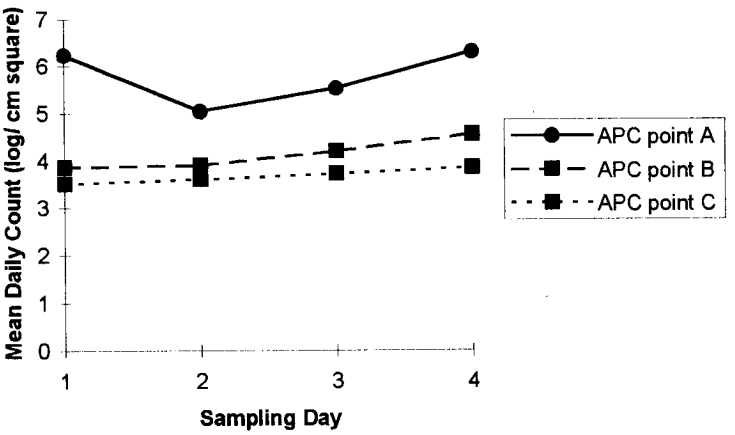


FIG 4.11: Mean aerobic plate count (APC) at various operational points (point A = skin; point B = after skinning; point C = after evisceration) indicating daily aerobic bacteria carcass contamination levels for slaughterhouse B

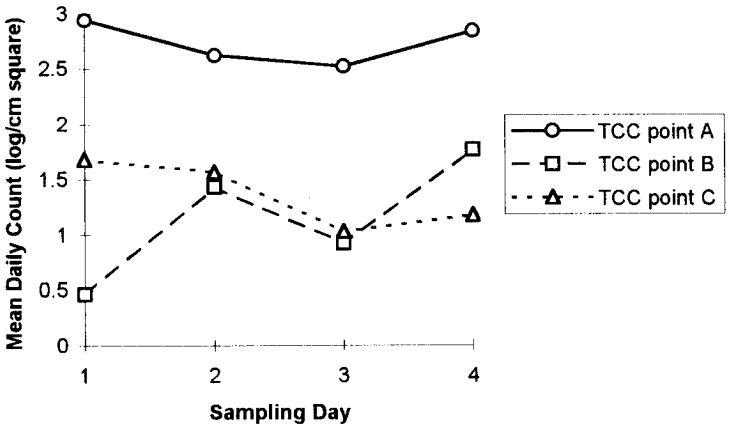


FIG 4.12: Mean coliform count (TCC) at various operational points (point A = skin; point B = after skinning; point C = after evisceration) indicating daily coliform carcass contamination levels for slaughterhouse A

CHAPTER FIVE: DISCUSSION

The HACCP system was applied to slaughterhouses in Zambia to describe microbiological quality of carcasses and hygiene practices in slaughterhouses. This kind of study was applied for the first time. Using the HACCP concepts, it was possible to systematically study the slaughter and dressing procedures and to point out CCPs on the processing line with significant contribution to meat quality. Microbiological studies showed that animals presented for slaughter at slaughterhouse A and B had high skin contamination. The mean APC at this point (point A) for both slaughterhouse A and B and for all bacterial categories was high. The poor hygienic state of animals presented for slaughter and further skin contamination obtained in the lairages, contributed to high count at point A. Under conditions of inappropriate handling techniques, the heavy contamination on the skin made transfer of dirt to sterile carcasses easier. The HACCP system demands use of contamination-free raw materials into a production process to reduce the ultimate contamination of the end products (Bryan, 1992; Anon., 1993 a). Similary, the skinning procedures at point B were not hygienically done in slaughterhouses A and B as reviewed by the microbiological analysis. Skinning procedures were also found to be inappropriate in slaughterhouses C and D. Slaughterhouse A used mechanical hide stripping while the rest skinned the animals manually. Despite slaughterhouse A using a mechanical hide puller, carcass contamination levels recorded during skinning did not demonstrate the expected benefit of mechanical hide stripping. This was due to inappropriate handling of carcasses by the

operators who seemed ignorant of basic hygiene operations. For slaughterhouses which did manual skinning, contamination during skinning mainly resulted from carcass manipulations during skinning and when carcasses were being pushed through the processing line.

Contamination due to evisceration at point C showed the smallest overall APC mean for both slaughterhouses A and B but count variation was high at this point for all bacterial categories. In case of slaughterhouse A, variation in carcass contamination levels observed during evisceration was possibly due to lack of a standardised procedure for evisceration and changes in the operators doing this operation. These contaminations depended much on two operations at which points the gut was likely to rapture. The first point was during opening of the brisket when the brisket saw was used. The extent to which the saw went inside the animal, determined whether the abomasum would be punctured or not. The second point was during evisceration, where no precaution was under-taken to prevent spillage of ruminal contents. This was further exacerbated by voluntary perforation of the rumen to "facilitate" handling and dislodging of abdominal viscera. Slaughterhouse B had a relatively better evisceration techniques, but faecal contamination might have resulted mainly from periodic puncture of the reticulum when opening the brisket.

Chilling of carcasses at point D was found to be effective in holding multiplication of both coliforms and mesophilic aerobic bacteria. However, spoilage aerobic bacteria which continue to grow even at 0^0C, (Dainty, *et., al.* 1983) must have proliferated at this point. As a result, no significant statistical difference was observed in APC before and after chilling. If carcasses have been unhygienically dressed and inspected, chilling might not accomplish its task as CCP as far as pathogens are concerned. Despite the level of contamination, its role as CCP is manifested in prolonging the shelf life of the product. The increased growth of coliforms observed after incubating samples for 24 hours, demonstrated that chilling only inhibited growth of mesophilic aerobic bacteria and almost all coliforms. Chilling temperatures maintained these categories of bacteria dormant but could be resuscitated at favourable conditions. McCulloch and Whitehead (1979) conducted a similar study in which the effect of chilling on the estimation of undifferentiated colony numbers was assessed. Regression analysis showed no significant relationship between warm and chilled carcasses. Their results are comparable to the APC data in this study.

Using the observational results, it was also possible to investigate slaughterhouse C and D which had no defined operational procedures. If hazards were to be identified in the slaughter and dressing activities of slaughterhouse C and D, without a defined processing procedure, it would be difficult to find CCP_s where control measures could be applied. Implementing quality

assurance programmes like the HACCP system would be very difficult because the HACCP system not only needs well instructed operators, but also well defined operational procedures in which identified potential hazards can be prevented or controlled at identified CCP_s (Bryan, 1992; and Anon., 1993 a). When observational results were applied to predictive microbiological contamination of slaughterhouses C and D, carcass contamination in these slaughterhouses was likely to occur during skinning, evisceration and from human contacts during processing. Based on hygiene audit assessment, slaughterhouse A had a relatively better hygiene practice as indicated by a relatively high hygiene satisfaction percentage (72 %) and slaughterhouses C and D recorded very poor hygiene standards (Table 4.6). Hudson, Mead and Hinton (1996), while comparing the mean total viable count for different slaughterhouses to the mean HAS score, observed that there was a significant correlation ($p<0.001$) between the meat quality and the mean score for selected categories of hygiene audit performance. It was further noted that slaughter and dressing practices; and the number of people in physical contact with the carcass between slaughter and chilling were of most value in predicting the tendency for a given slaughterhouse to produce relatively "clean" or "dirty" carcasses (in relation to microbial contamination). Since correlation exists between the overall hygiene assessment score and the tendency for slaughterhouses to produce meat of relatively "clean" or "dirty" microbiological status, (Hudson *et. al.*, 1996), it is important for slaughterhouses to aim for high hygiene scores. A slaughterhouse with a Minimum Hygiene Satisfaction Score (MHSS) of about 70

percent (Table 4.6) was bound to produce meat for local consumption whereas that for export plants needed to be about 80 percent MHSS. Under this classification, only slaughterhouse B would qualify to produce meat fit for local consumption.

In similar studies done in United Kingdom, carcass contamination levels were assessed just before chilling (Hudson *at. el.*, 1996) and results of total viable count ranged from $\log_{10}$ 2.79 to $\log_{10}$ 3.79 c. f. u/cm^2. In another study conducted in Tunisia (Fliss *et. al.*, 1991), Municipal slaughterhouses, which recorded highest contamination levels, had total aerobic mesophilic count of 106 c.f.u/cm^2 ($\log_{10}$ 2.02/ cm^2). In yet another study conducted in Australia, Widders *et. al.*, (1995) observed maximum carcass contamination levels of 108 c. f. u./cm^2 ($\log_{10}$ 2.03/ cm^2). When results in this study ($\log_{10}$ 3.67 - 4.14/ cm^2) are compared to what has already been done by other researchers, carcass contamination levels observed appeared to be too high. While differences in sampling sites and techniques existed, these cannot fully account for the observed differences. Other reasons attributed to the differences in contamination levels could be: design of slaughterhouses (Anon., 1993 b); and hygiene of the processing procedures (Hudson *et. al.*, 1996). Microbial contaminations in slaughterhouse A and B appeared to have been influenced by slaughter and dressing procedures, while in slaughterhouse C and D contamination levels might have been influenced both by slaughterhouse design and slaughter and dressing procedures.

In addition to understanding the significance of CCPs, the study also helped in understanding the characteristics of bacterial populations at various CCPs. At point A, more carcasses had APCs greater than the mean while at point C more carcasses had APCs less than the mean. At point B, however, the number of carcasses with APCs lesser or greater than the mean appeared to be almost equal. The same trend was observed for both slaughterhouses A and B with only a slight difference appearing at point A in which case the distribution appeared to be a bit symmetrical for slaughterhouse B. For figures 4.2 and 4.4, showing TCC distributions for slaughterhouse A and B, all points, A, B, and C depicted a similar trend on both figures, showing a left skew, a right skew and a symmetrical distribution for points A, B and C, respectively. Distribution figures (Figs. 4.1; 4.2; 4.3; and 4.4) show that there was consistence in the characteristics of bacteria distribution for all slaughterhouses. When modal classes of APC distributions are compared (Figs. 4.1 and 4.3), it was observed that there were relatively more carcasses getting contaminated with aerobic bacteria during skinning (point B) than was occurring during evisceration. Further, levels of contamination with aerobic bacteria was high during skinning than it was during evisceration. The opposite was true with coliform distributions, in which case more carcasses were becoming contaminated with coliforms during evisceration and less so during skinning. Likewise, the levels of coliform contamination were high during evisceration than they were during skinning (Figs. 4.2 and 4.4). These distributions

of bacteria categories have a bearing on the source of contamination. Contamination of carcasses during skinning had its source mainly from the animal's skin, operative's hands, equipment and environment. A large proportion of bacteria likely to survive longer on these surfaces are the gram positive bacteria (e. g staphylococci and streptococci). Contamination during evisceration mainly comes from ingesta and faecal contents, in which the main flora are coliforms (e.g. *E. coli, Klebsiella, Enterobacter, Citrobacter*). This origin is further supported by a strong correlation between total coliform count and faecal coliform count ($p < 0.01$).

A significant difference in the median APC count was observed between points A and B and between points A and C ($P< 0.02$) for slaughterhouses A and B.. There was no significant difference in the median APC count between points B and C ($p< 0.05$). No significant difference was recorded in the median APC before chilling and that obtained after chilling the carcasses for 24 hours ($p< 0.05$). However, the difference in the mean coliform count before and after chilling was very significant for matched pairs. There was statistically significant difference between the median count of points A and B ($P < 0.05$). Results for slaughterhouse B showed APC and TCC to be weakly correlated ($P<0.05$) at points A and B. However, no correlation between aerobic bacterial count and coliforms count was observed at point C ($P<0.05$).

Results for general coliform contamination showed high coliform contamination on the skin for both slaughterhouses and least coliform contamination occurring during skinning. There were more carcasses which recorded zero duplicate coliform count after skinning (25-30 per cent) than after evisceration (15-20.8 per cent). Faecal coliform contamination for slaughterhouse A (Table 4.3) also showed a similar pattern as that for general coliform (Table 4.2).

There was no coliform growth recorded after chilling the carcasses for 24 hours (Table 4.2 and 4.3). However, after incubating the samples for 24 hours at 37 ^{0}C and treating them similarly, massive growths were observed on both APC cultures and coliform culture media. The Mann-Whitney U-test showed that there was a significant difference in total coliform contamination levels between any given two sampling points except between point B and C.

On a daily basis, slaughterhouse A had generally little fluctuations in the average APC, TCC, and MPN values except for point C which showed a significant difference in daily average APC and TCC values ($p<0.01$) (Figures 4.8 and 4.9). There was also a statistically significant difference in the daily average TCC at point B ($p<0.05$). No statistically significant difference was recorded on the daily average MPN values (Figure 4.10). In case of slaughterhouse B, daily averages for all categories of results showed no statistically significant differences between days except at point A for the APC values where significant variation ($p<0.05$) in daily counts was recorded (Figure 4.11).

Serological examinations attempted on 20 isolates showed that, there were more *Salmonella* isolates recovered at point A for slaughterhouses A and B. This showed that *Salmonella* carcass contamination was likely to take place at this point. In a study conducted in slaughterhouses by Peel and Simmon (1978), they observed the hide to be a principle source of *Salmonella*. The findings in the present study agrees with that of by Peel and Simmon (1978). Under the current standards of hygiene, low-grade meat, which is being poured onto the market, might pose hazard to human health both in public eating places and homes. While infections might not come from direct consumption of contaminated meat but problems arise from cross-contamination, in which case contamination of ready-to-eat foods and foods eaten raw or without sufficient boiling (e.g. salads) poses a great risk. The risk is often high in children and immuno-compromised people.

Most environmental surfaces sampled showed high contamination levels (Table 4.5). Slaughterhouse A recorded highest APC contamination levels on the floor and aprons and least on the walls whereas slaughterhouse B showed high contamination levels on the floor and the splitting saw. Coliform contaminations were high on knives, tables and floors for slaughterhouse A and on the floor and saw for slaughterhouse B. High bacterial contamination levels were observed on environmental surfaces sampled, mainly because there were no sterilising facilities in slaughterhouses A, C, and D. Slaughterhouse B which only sterilised

knives using hot water also recorded high count on other surfaces. No slaughterhouse used disinfectants in the operations at the time of study. Surfaces that showed high contamination levels acted as possible sources of microbial spread to sterile carcasses.

Conclusion

Generally, both analytical and observational results in this study demonstrated low hygiene standards in the investigated slaughterhouses and significant bacterial carcass contamination levels. Among the factors which contributed most to carcass contamination were: the design of the slaughterhouses and mechanisation; the slaughter and dressing procedures; and slaughterhouse hygiene. All bacterial categories used to monitor contamination and hygiene standards showed certain trends vital in understanding population characteristics of contaminating bacteria in relation to various CPP_s on the slaughter line. These population trends were found to be similar for the investigated slaughterhouses, though hygiene standards in these slaughterhouses were different. However, general coliform and faecal coliform bacteria populations showed closely related population characteristics, and using either of them would be sufficient. The MPN index method was found to be labour intensive. The APC and TCC test results also showed that neither APC nor TCC alone would be sufficient to assess carcass contamination levels and environmental hygiene. The two, however, were found to be complimentary and should be applied together when monitoring hygiene and carcass

contamination levels. The APC was found to be more appropriate in investigating efficiency of skinning while TCC and FCC results were more useful in assessing evisceration and chilling effects.

Recommendations

1. Slaughterhouses need to develop specific slaughter and dressing procedures with well defined CCPs. This will guard against random performance of operations and inappropriate handling of procedures.

2. There is need to involve meat inspectors with veterinary science back-ground. This will guard against non-performance of ante-mortem inspections due to incompetence, and improve on the quality of PM inspections.

4. Slaughter and dressing skills for carrying out hygienic slaughter and dressing need to be instilled in the minds of the operators. This should be complemented with basic knowledge in personal hygiene necessary for carrying out hygienic operations.

5. There is also need for strict enforcement of the legislation so that only slaughterhouses complying to hygiene regulation are allowed to operate.

6. There is also need to institute quality assurance programmes in meat slaughterhouses to guarantee production of quality meat and meat products. Implementing quality assurance programmes such as the HACCP system will need: legislative reforms; active legislative support; review of current meat inspection procedures; establishment of meat standards; and training of personnel. The HACCP system provides the basis for prioritising inspection resources based on risk assessment and also forms a powerful management tool in food quality assurance for efficient utilisation of inspection resources, and guides against bad product retains.

5.2 **Research Prospects**

The findings in this study showed bacterial carcass contamination levels in the investigated slaughterhouses to be high. How this meat is transported from the slaughterhouse and later processed, may also affect the quality of meat that finally reaches the consumer. Therefore, further work needs to be done to assess meat quality after it has left the slaughterhouse to gain insight in the quality of meat that finally reaches the kitchen.

This study was focused on cattle slaughter shelters and cattle slaughterhouse proper and did not cover slaughter slabs. However, there is an increase in the quantity of meat being consumed in the cities that is coming from slaughter slabs. Therefore similar work based on HACCP concepts needs to be extended to assess operations of slaughter slabs in this country.

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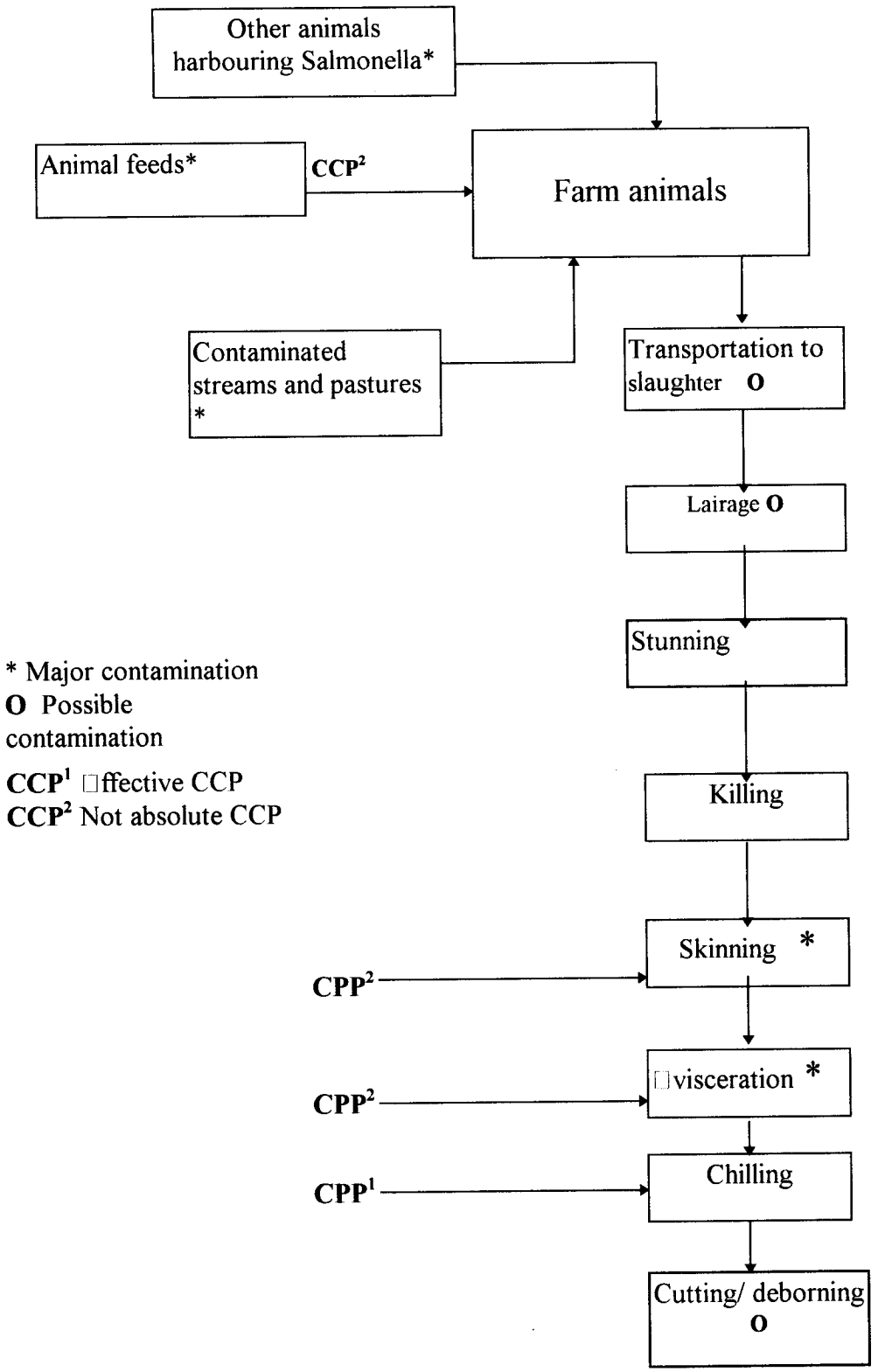
WIDDERS, D. R.; COATES, K. J.; WARNER, S.; BEATTIE, J. C.; MORGAN, I. R.; and

Appendix 1

Sources of contamination and critical control points before and during the slaughter of sheep and cattle (Figures redrawn from Salmonellosis control: the role of animal product and hygiene; WHO □xpert committee, Technical Report series 774



Appendix 2

CALCULATIONS ON DILUTIONS

a. Bacteria Concentration in the Sample Bottle

Four swabs each with 25 cm^2 area ($100cm^2$) were added to 100 ml of 0.1% peptone water

= $100cm^2$ carcass surface/ 100ml of peptone water

=1 cm^2 carcass surface/1ml of peptone water
(original sample solution)

---

b. General Coliforms

From 100 ml original solution, 0.1 ml aliquot was inoculated on violet red bile agar for enumeration of coliforms

Assuming Y was the average coliform count from a 0.1ml inoculation; the number of coliforms in 1ml of the original solution would be:

Y x 10 = Coliform count/ml

= Coliform count/ cm^2 of carcass surface area

=10Y c.f.u/cm^2

---

c. Aerobic Bacteria

From 100 ml of the original solution, 1ml aliquot was drawn and serially diluted to get the following concentrations: 10^{-1}, 10^{-2} and 10^{-3}. From the second dilution (10^{-2}), 0.1ml was inoculated onto standard plate agar for APC enumeration.

Suppose the average APC count from the 0.1ml inoculum was Q, then total APC count in 1ml (1 cm^2) of the original solution would be:

Q x dilution factor

Q x 1000 (APC/ml or cm^2)

= 1000Q c. f. u/cm^2

---

d. Faecal Coliform

Three dilutions, 10^{-1}, 10^{-2} and 10^{-3} were used. With this combination, all MPN indices were multiplied by 100 (Note that table indices in appendix three were calculated from a combination of 10 ml, 1.0 ml and 0.1 ml to give MPN index/100ml)

Therefore, under this combination, MPN indices in appendix three were multiplied by 100 to give:

$$\frac{\text{MPN index}}{100\text{ml}} \times 100$$

$$= \frac{\text{MPN index}}{\text{ml}} \quad \text{or} \quad \frac{\text{MPN index}}{\text{cm}^2}$$

$$= \text{Estimated c. f.u/cm}^2$$

Appendix 3

MPN INDEX AND 95 % CONFIDENCE LIMITS FOR VARIOUS COMBINATION OF POSITIVE RESULTS WHEN FIVE TUBES ARE USED PER DILUTION (10 ml, 1.0 ml, 0.1ml)

Combination of Positives	MPN Index/ 100ml	95% Confidence Limits		Combination of Positives	MPN Index/ 100ml	95% Confidence Limits	
		Lower	Upper			Lower	Upper
0-0-0	<2	-	-	4-3-0	27	12	67
0-0-1	2	1.0	10	4-3-1	33	15	77
0-1-0	2	1.0	10	4-4-0	34	16	80
0-2-0	4	1.0	13	5-0-0	23	9.0	86
1-0-0	2	1.0	11	5-0-1	30	10	110
1-0-1	4	1.0	15	5-0-2	40	20	140
1-1-0	4	1.0	15	5-1-0	30	10	120
1-1-1	6	2.0	18	5-1-1	50	20	150
1-2-0	6	2.0	18	5-1-2	60	30	180
2-0-0	4	1.0	17	5-2-0	50	20	170
2-0-1	7	2.0	20	5-2-1	70	30	210
2-1-0	7	2.0	21	5-2-2	90	40	250
2-1-1	9	3.0	24	5-3-0	80	30	250
2-2-0	9	3.0	25	5-3-1	110	40	300
2-3-0	12	5.0	29	5-3-2	140	60	360
3-0-0	8	3.0	24	5-3-3	170	80	410
3-0-1	11	4.0	29	5-4-0	130	50	390
3-1-0	11	4.0	29	5-4-1	170	70	480
3-1-1	14	6.0	35	5-4-2	220	100	580
3-2-0	14	6.0	35	5-4-3	280	120	690
3-2-1	17	7.0	40	5-4-4	350	160	820
4-0-0	13	5.0	38	5-5-0	240	100	940
4-0-1	17	7.0	45	5-5-1	300	100	1300
4-1-0	17	7.0	46	5-5-2	500	200	2000
4-1-1	21	9.0	55	5-5-3	900	300	2900
4-1-2	26	12	63	5-5-4	1600	600	5300
4-2-0	22	9.0	56	5-5-5	>1600	-	-
4-2-1	26	12	65				

Adapted from APHA (1985) Standard Methods for Examination of Waste Waters, 16 Ed.

SLAUGHTERHOUSE OPERATIONS

CHECKLIST

Name and Address:----------

Location: Province----------

Town/City:----------

Date:----------

SH/Code:----------

INSTRUCTIONS: Tick the appropriate number and were possible enter specific details in the space provided.

SECTION 1

Site: 1. Urban, 2. Peri-urban, 3. Rural.

Killing capacity: (a)Per day; 1.<30carcases, 2. <60 carcass, 3. >100 carcass or specify-------

---

(b) Per week;----------

Operation facilities 1. Slaughter, 2. Slaughter and Processing

Lairage facility: (a) Floor; 1. Concrete, 2. Muddy.

(b) Size in meters----------

Drainage facilities: 1. Fully operational, 2. Semi-blocked, 3. Blocked

Operating hall: (a) Floor 1. Smooth 2. Rough 3. Sloppy 4 Flat

(b)Walls 1. Adsorbent , 2. Non-adsorbent, 3. Smooth, 4 Rough,

5 Light, 6. Dark.

Chilling and refrigeration capacity:1<30 carcasses, 2.<60 carcasses 3.>100 carcasses or

specify----------

Water (a) Source:1. River/stream, 2. Bole hole, 3. Well, 4. Lake, 5. Dam.

(b) Quantity used per day or per month----------

Partition between slaughter hall and offal cleaning room 1. Complete, 2. Semi, 3 Non.

Slaughterhouse premises :1. Fenced, 2. Not fenced.

SECTION 2

Ownership:1. Private, 2. Municipal, 3. GRZ.

Workers health check:1. Present, 2. Not present. If present how often?----------

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Workers instruction on hygiene:: 1. Present, 2. Not present. If present how often?----------

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Plant maintenance:1. Present, 2. Not present. If present how often?----------

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SECTION 3

Operatives protective clothing's:1. Water resistant, 2. Non water resistance, 3. Not supplied.

Facilities for personnel: (a) 1. Hand washing 2. Showers 3. Lavatories

(b) Hand washing habits

(c) Changing rooms.

Pest control program:1. Rats, 2. Insects, 3. Any other----------

Fry screening:1. Present, 2. Not present.

Disinfectants used in operations, Specify, 1.----------2.----------

----------3.----------4.----------

- 5.----------

Sanitizing water temperatures 1. <70°C 2. <80°C 3. <80°C 4. specify----------

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SECTION 4

Animal source (a) Type of husbandry: 1. Commercial farms 2 Local farms 3. Both 1. and 2.

(b) Province of production: 1. Central, 2 .Copperbel,; 3. Easter, 4. Luapula, 5. Lusaka, 6. Northern, 7. N/Western, 8. Southern, 9. Western.

Sex of animal slaughter: 1. Mainly males, 2. Mainly females, 3. Both males and females.

Animal breeds mainly slaughtered: 1.----------2.----------3.----------

SECTION 5

Average kill: (a) Per day ----------(b) Per week----------

-

Active operation: (a) Days per week----------(b) Hours per day----------

-

State if any seasonal variations exists----------

Carcass speed line: (Number of carcasses passing a particular point per minute)----------

Number of meat inspectors per session----------

Inspection procedures (a) Ante-mortem:1. Performed, 2. Not Performed.

(b) Postmortem:1. Performed, 2. Not Performed.

(c) Laboratory checks 1. Bacteriology, 2. Antibiotics and Chemical residues, 3. Not performed.

SECTION 6

Market for the products: 1. Local, 2. Export, 3. Both 1 and 2.

Major reasons for condemnation -in the order of frequency (a) Whole carcass: 1.----------

----------2.----------3.----------

4.----------5----------

(b) Organ condemnations: 1.----------2.----------

----------3----------4----------

----5----------

Any emergencies during slaughter 1. Yes 2. No If yes specify----------

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Any other comments----------
