PREREQUISITES FOR HACCP IN SMALL-SCALE POULTRY PRODUCTION

By

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DEDICATION

To my son Mauro, my husband Amadeu and all my parents for their encouragement, support and believing in me.

DECLARATION

I, Ana Bela M. V. Cambaza dos Muchangos, hereby declare that the work on which this dissertation is based is original and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree at this or any other University.

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SIGNATURE DATE

TABLE OF CONTENTS

CHAPTER 3 MATERIALS AND METHODS

CHAPTER 4 RESULTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

SUMMARY

Title:

PREREQUISITES FOR HACCP IN SMALL-SCALE POULTRY PRODUCTION

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Abstract:

Food borne diseases, and more especially diarrheal diseases, are an important cause of morbidity and mortality (WHO, 2007). Food borne diseases due to bacteria in the food usually manifest as episodes of gastro-intestinal disease (South African DVS, 2007). Most of food borne illnesses occurring annually are caused mainly by three bacteria; *Campylobacter* spp., nontyphoidal *Salmonella serovars*, and pathogenic *Escherichia coli*, including *E. coli* O157:H7 (Zhao *et al*, 2001; Mead, 2004).

The prevalence of food borne pathogens and epidemiological knowledge of the extent, sources, and causative factors that lead to food borne illness remain unknown in many parts of the world. In most developing countries data are not collected on such a basis that an assessment of the amount of illness or the causes can be made, but food borne illness is probably second only to malnutrition as the cause of death among children (Lund *et al*, 2000).

In Mozambique the situation is similar to other developing countries. The risk of eating poultry meat from formal and informal small scale producers processed in the formal abattoirs and at informal points of slaughter is unknown. However, the diarrheal diseases remain an important cause of mortalities according to data from the Mozambique National Ministry of Health.

The objective of the study were to investigate and describe the value chains for small scale poultry production in Maputo, indicating possible stages at which there was the risk of a hazard that would influence the final product and estimate the magnitude of this risk by using microbiological risk assessment in poultry meat. It included the informal and formal producers and processors. In the study area it was estimated that only 40% of total poultry production was processed in the formally abattoirs. The remaining 47% were sold live and 13% processed by informal processors at point of sale if the customer asked for the fowl to be slaughtered.

The method used was participatory risk analysis. The participants included state veterinary services, municipal health authorities, poultry farmers, poultry processors and vendors at live bird markets in Maputo. To quantify the magnitude and nature of the risks, microbiological risk assessment was used on water, equipment surfaces and hands of operatives (as a prerequisite to HACCP) and poultry carcases (at identified CCP's during the slaughter and dressing of fowls). Samples of poultry carcasses, water and swabs from surfaces and hands of operatives, were taken from poultry farms, live bird markets and poultry abattoirs. The samples were sent for laboratory examination where the tests included *E.coli* and Coliform Count and Aerobic Plate Count to verify if the carcasses were produced in a hygienic manner and if the poultry processing was controlled adequately.

The quality of the poultry carcasses collected from the three sectors was not satisfactory. Poultry meat from formal abattoirs was not found to be much safer than meat purchased at live bird markets and farms using informal slaughtering processes. To improve prerequisites, Hygiene Management Systems (HMS) and Hygiene Assessment Systems (HAS), using an appropriate audit system tailored to the type of processing (ie formal or informal) was proposed for all three value chains with a focus on critical control points.

CHAPTER 1

INTRODUCTION

1.1 Justification

1.1.1 Project description

“Safe Food, Fair Food” is a project designed by the International Livestock Research Institute (ILRI), to support intensified livestock production by improving the safety of livestock food products. This will ensure continued market access for the poor, who are often dependent on livestock, or animal derived proteins, as well as minimising the food-borne disease burden in sub-Saharan Africa. The project involving Ivory Coast, Ethiopia, Ghana, Kenya, Mali, Mozambique, South Africa and Tanzania, aims at adapting the risk-based approaches successfully used for food safety in developed countries and international trade, to domestic informal markets, which are readily accessible to the poor in sub–Saharan Africa. Formal top-down approaches will be complemented by the participation of stakeholders, including traders and consumers in informal markets, in the assessment and evaluation of food safety risks.

In the informal markets conventional regulation and inspection methods often fail and private or civil sector alternatives have not emerged, as a result most livestock-derived food products are potentially unsafe. Risk-based approaches for assessing and managing food safety offer a powerful new method for reducing the health burden imposed by food-borne disease (ILRI, 2009). This project will develop tools and evidence that allows for risk analysis to be applied in informal markets, so building capacity and demand among stakeholders and safeguarding both consumer health and farmer livelihoods.

According to ILRI (2009), previous research on food safety has identified participatory methodologies as effective in overcoming the problems of information collection in data-scarce contexts, of building stakeholder ownerships well as ensuring sustainable risk management. To consolidate training in Food Safety Participatory Risk Analysis, multi-sectoral country teams will undertake a proof-of-concept participatory risk analysis on a selected food safety problem. In Mozambique the poultry sector was selected for the study, as current evidence suggested that it is both a promising livelihood strategy for the poor and that food safety may compromise health of consumers. The number of poultry farmers and poultry vendors, most of them women, has grown significantly in recent years around the main urban and peri-urban centres and there is evidence that this target group is neither trained nor well-informed about food safety practices.

1.2 Background

1.2.1 World poultry production

There are many species and breeds of poultry used for production of animal derived products. The domestic chicken (*Gallus domesticus*) has assumed world-wide importance and accounts for more than 90% of the world's poultry flocks (Silverside & Jones, 1992). Poultry accounts for some 27% of the world's meat consumption and chicken is the principal type of poultry meat (Lund et al, 2000).

Poultry production is a big business throughout the world. It varies from free-range village chickens in developing countries to highly intensive systems, mostly used in developed countries, where it is consideredone of the most technologically advanced sectors in agriculture (Lund et al, 2000). This is a highly competitive industry where feed suppliers, producers, processors and manufacturers of equipment have all appreciated the economies of scale continue to develop bigger and more automated systems of operation to supply an insatiable market (Silverside & Jones, 1992).

Poultry products are universally popular, the meat is perceived as wholesome, healthy and nutritious, relatively low in fat and with more desirable unsaturated fatty-acid content than other meats (Lund et al, 2000; Mead, 2004). Poultry meat is easy to prepare and is widely utilized in fast-food establishments. It has become a standard form of affordable protein (Silverside & Jones, 1992). The relative cost of poultry meat has over the last 20 years fallen markedly in comparison with other meats internationally and it remains a cheap staple food (FAO, 2009; Sutherland et al, 1986). Broiler production represents one of the most efficient means to convert vegetable protein to animal protein, while providing an affordable source of animal protein. Contributing to the popularity of poultry meat are its affordability, nutritional value, universal acceptability, year-round availability, convenience, and consistency in quality and health aspects (Bilgili, 2010).

The world poultry industry has experienced a period of slowing growth as a reaction to generally low returns to chicken production, caused by the global economic downturn, trade disruptions, avian influenza and most recently, sharply higher grain prices. The demand for chicken is normally correlated with the world economy, when the world economy improves demand usually accelerates (Aho, 2006, Sluis, 2010). Due to the economic crisis, consumers worldwide reconsidered their spending and overall decided to buy less meat; poultry meat suffered the least compared to most other meats, but still received a fair share of the drop. In the EU the future of the poultry industry is very uncertain, the price of broilers dropped 3% in 2009, and fortunately the price of feed also decreased improving overall returns compared to the loss experienced in 2008 (Sluis, 2010).

1.2.2 Small scale poultry production

Small scale poultry production is very important in developing countries, particularly far from the main commercial and industrial centers. Throughout the world there are those who, produce small numbers of poultry carcasses or small amounts of poultry meat or poultry meat products for home consumption and sale. They present a particular problem to the food hygienist or legislator as they carry out their activities in small premises which frequently do not have standards or facilities (Bremner & Johnston, 1996).

There continues to be a need for small scale processing facilities. For economic survival, small producers of poultry meat have to find a niche market which does not bring them into direct competition with the large producers (Bremner & Johnston, 1996). They can do this by supplying small numbers of carcasses or live birds directly to the consumer or to a local vender who wants a small amount of poultry.

The small scale producer may rely on consumers who buy from a farm shop and are aware that the product has been produced on that farm which they can see and by the farmer they can meet, rather than in a large factory (Bremner & Johnston, 1996). Mass produced poultry is generally considered inferior to the traditional reared bird, but to a large extent consumer taste is dictated by economics (Sutherland et al, 1986). It is clear that price is the key factor in the successful marking of poultry products, and this is inextricably linked to quality, in fact, affordability can be considered a measure of quality (Mead, 2004).

1.2.3 Poultry processing

The internationally accepted norm for food safety, especially during slaughter and processing of meat, is the Hazard Analysis, Critical Control Point (HACCP) system (FAO & WHO, 1999). When it is applied to food safety management, it uses the approach of controlling critical points in food handling to prevent food safety problems. However, HACCP cannot be applied if prerequisites are not in place (FAO&WHO 2006). At the level of poultry farming and processing in Mozambique at the beginning of this study, it was decided to first get the prerequisites right in all levels of poultry production, including informal markets and abattoirs, in order to facilitate introduction of HACCP at a later stage.

Processing in developing countries tends to be more labour intensive. The primary market of broilers is often confined to whole carcasses and cut portions (Bilgili, 2010). Access to processing is a critical issue for small producers. In poor regions, poultry are often sold live or are slaughtered at the point of sale (Mead, 2004). At live markets in larger cities, live birds are sold to consumers, who are expected to slaughter them at home. Often, once a customer chooses a bird, it is processed at an on-site facility, usually state-licensed in developed countries (Fanatico, 2003).

Internationally, some small producers are raising poultry outdoors on pasture, processing the bird's on-farm, and selling the meat directly to customers with no inspection. The small plants or on-farm processors use a single tank of hot water. It can take a while to heat the water in a small scalder to the right temperature, and it can be tricky to maintain that temperature, especially when fresh water is added. Most on-farm processors do not replace the water during processing – and the water gets very dirty (Fanatico, 2003).

Mobile slaughter facilities designed for on-farm use would be more appropriate for countries lacking infrastructure or centralized slaughter and processing facilities. These could minimize health risks to consumers and increase product quality through providing processing equipment infrastructure to small scale produces. Successful use could improve bird welfare, the quality of carcasses and meat, and also reduce labour costs and waste (Mead, 2004).

During processing, surfaces can be contaminated by inappropriate operations. These surfaces and the water used have the potential to contaminate the poultry carcasses further. Although many bacteria are washed away during processing, sufficient remain to cause problems if carcasses are not properly handled (Silverside & Jones, 1992).

1.3 Mozambique

According to the Standards and Trade Development Facility (STDF) 2008, agriculture in Mozambique is the main source of income for more than 65% of the population, contributes significantly to GDP (21,5% in 2005) and along with fisheries, is one of the main export earners. Less than 15% of Mozambique's arable land is under cultivation and the agriculture sector is dominated by smallholders who are predominantly subsistence-oriented. A map of Mozambique is shown below in Fig 1.2.

Fig 1.2 Map of Mozambique

KEY FACTS

- Location - Eastern part of Southern Africa
- Area- 799,390 Km²
- Population- 20,530,714 inhabitants
- Nº of village chickens- 18,080,152
- Climate- Inter tropical
- Capital- Maputo
- Official Language- Portuguese
- Currency- Metical (USD=MZM 26,500)

(National Institute for statistics, 2008)

1.3 Poultry production in Mozambique

Poultry production is not only an important source of income but also a sustainable source of protein for the poor in Mozambique. The agricultural census (INE, 2006) indicated that the majority of the people in rural areas of Mozambique have a tradition of poultry keeping, especially village chickens, of which the number is estimated at 18,080,152.

Village chickens increase food security and poverty alleviation in rural areas and have the following roles:

- Source of income during periods of food shortages.
- One of the most accessible sources of animal protein for the poorest communities.
- Useful for special traditional ceremonies and events in rural areas.

According the Mozambican Association of Poultry (AMA) 22, 647,000 broilers were produced in 2008, representing 81% of the urban and peri-urban market needs. The remaining 19% of the market is assured by imports from several countries with Brazil topping the list. Production is concentrated mainly in Maputo, Manica and Nampula provinces. In Maputo the production of broilers was estimated at 9,484,800 with 60% of it (5,690,400) arising from the small scale producer who is oriented essentially to the informal market (AMA, 2008).

1.4 Problem/Hypothesis

1.4.1 Main problem statement:

Poultry meat safety is a concern in Mozambique; the quality is possibly poor as a result of lack of knowledge and skills among small scale producers and processors. The magnitude of the risk of eating poultry meat processed in the formal abattoirs and in the informal points of slaughter is unknown.

1.4.1.1 Sub-problems:

Concerns that may be applicable to Mozambique include the following:

- Inadequate bio-security/bio-safety measures applied in poultry production
- Lack of knowledge and skills in terms of Good Manufacture Procedures
- Absence of Good Hygiene Practices and Standard Operation Procedures
- Emergence of food borne diseases
- Consumer concerns about low quality and safety of the local product

1.4.2 Hypothesis

The risk to consumers from consuming poultry meat can be assessed, using participatory risk analysis and an integrated food chain approach to describe prerequisites and an appropriate hygiene assessment system for both formal and informal points of slaughter in the poultry industry in Maputo.

1.5 Benefits arising from the project

The results of this study on prerequisites to HACCP could be used by the Veterinary/Municipality Authorities to develop appropriate and affordable hygiene assessment systems (HAS) to improve poultry meat hygiene and safety in formal and informal production and processing along the poultry food chain in Maputo. Specifically, the findings could be used to contribute to:

- Assess the magnitude of microbiological risk in the poultry meat processed in the formal and informal sector in Maputo.
- Assess the point where biological hazards enter in the processing chain.
- Develop a HAS audit checklist (guidelines) to ensure quality and safety in the poultry production and processing chain.
- Reduce the level of microbiological contaminants in poultry meat marketed in Maputo.

1.6 Aims/Objectives

1.6.1 Aim

The ultimate aim is to identify and characterize risks to the safety and quality of poultry meat by investigating the value chains and building flow charts for the informal production and processing of poultry and assess. From this risk mitigation and communication strategies will be developed in order to improve safety of domestic poultry meat.

1.6.2 Objectives:

- Investigate and describe the value chains for informal poultry production in Maputo
- Estimate the proportion of chickens processed in each value chain
- Draw flow charts of the poultry marketing alternatives, indicating possible stages at which there is a risk of a microbiological hazard that will influence the final product
- Estimate the magnitude of this risk using expert opinions, participatory methods, checklists and evidence based on literature reviews
- Develop a sampling frame using a proportionate random sampling method
- Quantify risk by using microbiological risk assessment at point of slaughter
- Develop mitigation strategies especially improved prerequisites
- Communicate mitigation strategies (pre-requisites for HACCP) to relevant stakeholders, in line with Codex Alimentarius risk communication guidelines

CHAPTER 2

LITERATURE REVIEW

2.1 Food borne diseases

According to the South African Directorate of Veterinary Services (DVS, 2007): “a food borne disease (FBD) is an illness in humans in which the food is, or contains, the causative agent”. FBD comprise a broad group of illnesses caused by microbial pathogens, parasites, chemical contaminants and biotoxins (WHO, 2006; Eley, 1994). Food can contain microbiological or chemical agents that cause infections and intoxications, the sources of these agents range from being an inherent constituent of certain food, to inadvertent addition, contamination or multiplication, during food production or processing (Cliver & Riemann, 2002). The causative agent must be present in sufficient numbers (e.g. bacteria) or high enough concentration (toxin) to survive normal handling of the food, in order to be considered as a hazard to food safety.

Infections result from eating food containing a viable potential pathogen that multiplies in the body, while intoxications result if food contains a substance that poisons the body (Cliver & Riemann, 2002). The gastrointestinal (GI) tract, especially the small intestine, is a primary site of action for infectious agents that are ingested with food (Cliver & Riemann, 2002). Multiplication of pathogenic organisms in the consumer usually manifests itself in episodes of gastro-intestinal disease, although other symptoms may also occur if systemic infection results (South African DVS, 2007).

Food borne diseases, especially diarrheal diseases, are an important cause of morbidity and mortality (WHO, 2007; Eley, 1994). Most of the FBD that occur annually are caused by three bacteria: *Campylobacter* spp., nontyphoidal *Salmonella serotypes*, and pathogenic *Escherichia coli*, including *E. coli* O157:H7 (Mead, 2004; Zhao *et al*, 2001; Eley, 1994).

The prevalence of food borne pathogens and epidemiological knowledge of the extent, sources, and causative factors that lead to food borne illness, remain unknown in many parts of the world. Critical to the understanding of food borne illness outbreaks is the identification of both the contaminated food item and the responsible pathogen, allowing trace back to the original source of contamination and subsequent intervention (Dewaal *et al,* 2006). FBD often goes unreported, with the result that the economic and health impacts are greater than the figures suggest (FAO & WHO, 2006). It is known to be greatly under-reported and most reported incidents are “sporadic” with no proven link to a specific food

item (Dewaal *et al,* 2006; Mead, 2004). Reports of food borne illness come mainly from western countries and from a few other countries that collect such statistics (Lund *et al*, 2000). Many countries lack strong surveillance and reporting systems, thus statistical estimates are not available (Dewaal *et al,* 2006). In most developing countries there is insufficient available data to statistically calculate the incidence or prevalence of FBD, but FBD are probably second only to malnutrition as a cause of childhood mortality. (Lund *et al*, 2000). Reliable epidemiological estimates on the burden of these diseases are important in order to assess the impact of food safety measures and advise policy-makers on cost-effective use of resources (Dewaal *et al,* 2006; WHO, 2006).

Outbreaks of FBD can damage trade and tourism, and lead to loss of earnings, unemployment and litigation. Advocacy for producing safer food and increased public health protection may increase the confidence of both national consumers and tourists. This, combined with increased trade, can result in economic growth and national development. Achieving a safe food supply poses major challenges for national food safety authorities, as FBD can cause both human suffering and significant economic losses (FAO & WHO, 2006). In some countries where preventive controls are less rigorous due to deficient employee training, poor plant and equipment sanitation and contamination of raw materials, there is an increased risk of FBD (Sertkaya *et al*, 2006).

2.1.1 Food Borne Disease in relation to poultry meat

Meat has traditionally been viewed as a vehicle for a significant proportion of human FBD. The problem has been well illustrated in recent years by human surveillance studies of specific meat-borne pathogens such as *Escherichia coli* O157:H7, *Salmonella* spp., *Campylobacter* spp. and *Yersinia enterocolitica* (CAC, 2005; Eley, 1994). Meat and poultry products are sensitive to microbiological contamination. After becoming contaminated, they provide an excellent medium for bacterial growth, which can affect both food safety and quality and result in FBD in consumers (Zhao *et al*, 2001). Contaminated raw or cooked poultry meats are considered a high risk for FBD (Northcutt & Russel, 2003). Raw poultry meat is also highly susceptible to microbial spoilage when stored in the unfrozen state (Bremner & Johnston, 1996). Spoilage micro-organisms include bacteria that will cause deterioration of foods through breakdown of the food constituents and/or accumulation of undesirable end products of bacterial metabolism (South African DVS, 2007).

2.1.2 Food Borne Disease in developing countries

Up to one third of the population of developed countries may be affected by food-borne diseases each year. The problem is likely to be even more widespread in developing countries, where food and water-borne diarrheal diseases kill an estimated 2.2 million people each year, most of them children.

The links between hazards in foods and illness in humans have sometimes been difficult to establish or quantify and interventions have not always been feasible (FAO & WHO, 2006). Consumers' choices are influenced by many factors, including social, cultural, psychological, religious, spiritual, and biological motivations. Personal perceptions, rather than nutritional value or safety of food are the most important reason for choosing a particular food (Cliver & Riemann, 2002). These cultural, social and religious preferences are even more likely to be a major factor in food choices in developing counties.

The FAO & WHO (2006) have described the food industry as one of the major sectors, accounting for the highest proportion of the gross domestic product (GDP).

According to Taylor, 2001 (cited by FAO & WHO, 2006) the characteristics of small food businesses are:

- They serve local customers.
- They have a limited share of the available market.
- They are owned by one person or by a small group of people.
- They are mostly owner-managed and independent of ownership by larger groups of companies.

The FAO & WHO (2006) defined the term "small and/or less developed businesses" (SLDBs) as "businesses that, because of their size, lack of technical expertise, economic resources, or the nature of their work, encounter difficulties in implementing HACCP in their food business". It is important to note that the term "less developed business" refers to the status of the food safety management system and not to the number of staff, or volume of production.

The FAO/WHO (2005) defined street-vended foods as: "those foods prepared on the street or other public places". There is a general perception that street-vended foods are unsafe, mainly because of the environment under which they are prepared, usually these locations do not meet food safety requirements, exposing the food to numerous potential contaminants. According the FAO (2009) the risk of food poisoning, especially microbiological contamination, from street food remains a threat in many parts of the world. Food borne pathogens pose a serious health hazard, the magnitude of which is determined by the type of food as well as the methods of preparation and preservation.

Street food vending has, however, become increasingly important in the economies of many African countries in the last years because of urban population growth and the venders often engage in this business mainly to escape poverty (FAO/WHO, 2005). Unfortunately, knowledge and expertise in handling street food are often limited; food products sold on markets or from other outlets are not always of good quality and hazards often arise from a lack of clean water for food preparation, washing of utensils or personal hygiene. In some areas operators have to use well-water that is often contaminated,

especially with bacteria. Even in areas with good clean water, food handlers often use that water inappropriately (FAO, 2009).

The major concern with street foods is their microbiological safety, mainly because vending is done in places that may have poor sanitation (FAO/WHO, 2005). Vendors operate from places such as open markets and street corners where the clientele are guaranteed. Vending sites are seldom included in the town plans and amenities such as processing facilities and refuse collection are not available. Traders produce all kinds of refuse which can endanger consumer health by being a source of contamination for food products.

The preparation and sale of street food provides a regular source of income to millions of men and women in developing countries. Street food also provides outlets for agricultural producers and local food processors, contributing to local and national economic growth (FAO, 2009). Recognizing the socio-economic importance of the informal street food sector, the FAO has undertaken actions to improve the hygiene of food produced and sold on the street, using an integrated approach that embraces all the stakeholders. These actions focus on awareness and training of stakeholders in this complex system, emphasizing the practical implementation of the Codex Alimentarius guidelines, especially the general principles of food hygiene and analysis of critical control points applied to street food, together with revised regional guidelines for measures to control street food in Africa (FAO, 2009).

These guidelines are used to assist relevant authorities in upgrading the operation of the street food industry, to ensure that the population has available wholesome, safe and nutritious foods in accessible places, laying a firm foundation for ensuring food hygiene. They specify the general hygienic requirements and practices to be recommended for inclusion in Codes of Practice for the preparation and sale of street foods (CAC, 1999).

2.1.3 Food borne disease and antimicrobial resistance

Antimicrobial resistance is of global public and animal health concern and is impacted by antimicrobial usage in humans and animals. Managing human health risks arising from non-human usage of antimicrobials and the resulting antimicrobial-resistant bacteria requires national and international interdisciplinary cooperation (FAO/OIE/WHO, 2004). The CAC (2005) code of practice to minimize and contain antimicrobial resistance defines the respective responsibilities of authorities and a group involved in the authorization, production, control, distribution and use of veterinary antimicrobials and provides additional guidance for the responsible and prudent use of antimicrobials in food-producing animals. The FAO/OIE/WHO (2004) publication emphasizes the usefulness of internationally established procedures and principles, such as good agricultural practices (GAP), good manufacturing practices(GMP), good

veterinary practices(GVP), and the prudent use of antimicrobials and risk analysis to minimize the hazard of antimicrobial resistance at national and international levels (Davidson et al, 2005).

According to EFSA (2007), relatively high proportions of *Campylobacter* and *Salmonella* isolates from food of animal origin, were found to be resistant to antimicrobials commonly used in treatment of human diseases. The FBD caused by these resistant bacteria poses a particular risk to humans due to possible treatment failure. In *Campylobacter* spp. isolated from people, resistance to ciprofloxacin was reported to range from 37% (*C. jejuni*) to 48% (*C. coli*). In isolates from animals, resistance to ciprofloxacin and tetracycline was even higher, ranging between 94% and 99%, respectively. Resistance to erythromycin and streptomycin in *C. coli* ranged between 72% and 90%, respectively. Antimicrobial resistances in *Salmonella* spp isolated from animals, including poultry, were reported to be relatively higher to ampicillin, tetracycline and sulphonamides. In addition, 27% of the *S. typhimurium* isolated from humans were resistant to more than four of the antimicrobials tested (EFSA, 2007).

In the interests of food safety and public health, antimicrobial resistance is a critical aspect. National governments are responsible for the registration of antimicrobials. Farmers and veterinarians have a key responsibility to prevent and minimize the antimicrobial resistance that may have an effect on human and animal health and should use only registered stock remedies and veterinary medicines on food animals (FAO/OIE/WHO, 2004).

2.1.4 Food borne diseases and control of microbial hazards during production and processing

Microbial hazards derive from contamination, survival or growth of the causative agents at any stage of the supply chain (Mead, 2004). To significantly reduce FBD, the presence of the main causative agents, should be reduced in food animals. In poultry meat, major hazards include *Campylobacter* spp, *Salmonella* spp and velogenic *Escherichia coli* (Zhao *et al*, 2001). Other food borne pathogens that are often present on carcasses include *Clostridium perfringens,* a common intestinal organism, and *Staphylococcus aureus,* carried on the skin and in the nasopharynx of birds. As these organisms occur in low numbers the meat is unlikely to become hazardous unless it is temperature abused and extensive multiplication occurs (Bremner & Johnston, 1996; Lund *et al*, 2000).

Monitoring of this wide range of potentially hazardous organisms in food of animal origin, including poultry meat, requires sophisticated laboratory techniques and expensive infrastructure. In the food industry internationally, detection and counting of indicator organisms (Coliforms and *E. coli* Type 1) is used to estimate the number of faecally derived pathogens in food as well as *S.aureus* (CAC, 2011; CAC, 1999; WHO & FAO, 2008; WHO, 2008).

Prevention of food borne infections and diseases is a multifactorial or integrated process, requiring establishment of quality and safety controls throughout the whole supply or value chain (Zhao *et al*, 2001). Where control of microbiological contamination cannot be achieved at a primary production stage, control can sometimes be achieved later, during a manufacturing or processing step, for instance cooking at high temperatures (Lund *et al*, 2000).

2.1.5 Food Borne Diseases in Mozambique

According to the CIA World Factbook (updated July 2012), the most important food and water borne diseases in Mozambique include Hepatitis A and E as well as typhoid and leptospirosis[1]. These FBD, although they are not primarily diseases of poultry, could also be prevented by quality and safety controls through the whole value chain for poultry products.

According to the Epidemiological Bulletin from MoH (2009), diarrheal diseases remain one of the most important cause of mortalities in the country. Due to limited resources to screen the agents involved in the diarrheal diseases the Health Authority priories those caused by *Vibrio cholera.* Table 2.1 below reflects information that was obtained from the Mozambique Health Information System and reflects the risks of gastro-intestinal disease to consumers.

Table 2.1: Cases of diarrheal diseases notified to the Health Information System (SIS) in 2009

Disease	**Agent**	**Cases**	**Deaths**	**Deaths rate (%)**
Cholera	*Vibrio Cholera*	19,843	160	0,8
Typhoid fever	*Salmonella typhi*	130	17	13,0
Dysentery	Unknown	185,217	20	1,1
Diarrhea	Unknown	858,663	782	9,1

However, data from the laboratory of microbiology of Maputo Central Hospital (HCM), shows that *E.coli* and *Salmonella* spp were the main bacteria isolated from the samples sent by different hospital departments for diagnosis purpose (Table 2.2).

[1] Https//www.cia.gov/library/publications/the-world-factbook/fields/2193.html, accessed online September, 2012

Table 2.2: Results of samples from human patients collected and processed in the Laboratory of Microbiology of Maputo Central Hospital (HCM), during 2010 and 2011.

Agent	2010		2011	
	Adults	Children	Adults	Children
E.coli	76	33	179	108
Salmonella sp	4	2	4	5

Additionally Rotavirus and parasites were detected in feces samples processed at Laboratory of Maputo Central Hospital (HCM), during the second semester 2011 (Table 2.3).

Table 2.3: Results from feces samples collected and processed in the Laboratory of Parasitology of Maputo Central Hospital (HCM), during the second semester 2011.

Month	Samples processed	*Giar*	*E.Col*	*E.Hist*	*Tric*	*Anc*	*Str*	*Asc*	*Rotavirus*
Jul/11	443	6	14		4	1	5	1	90
Aug/11	571	10	14		14	0	6	6	23
Sept/11	267	4	2		2	2	1	4	6
Oct/11	276	8	1		6	1	5	3	1
Nov/11	386	5	1		9	3	6	2	0
Dec/11	300	10	2		9	1	8	3	3
	2243	43	34		44	8	31	19	123

Diarrheal diseases as well as many other diseases like fever and respiratory tract infections probably remain under-reported in Mozambique.

2.2. Hazards during poultry production and processing

During production and processing, the risk of food borne diseases are often related to general hygiene principles, or prerequisites, that include use of non-potable water, lack of hygiene during handling and processing, as well as secondary contamination. Pathogens associated with consumption of poultry meat may also arise from infections of the live bird, or be introduced during or after slaughter. These are discussed in more detail below.

2.2.1 Non-potable or dirty water

Potable water is an international set of standards for water suitable for consumption by food producing animals and humans. It is also required for processing animal products (WHO, 2010)[2]. According to South African DVS (VPN/16/2007-1) the standards for the microbiological monitoring of water are given in the table 2.1.

Table 2.4: Microbiological monitoring of water

Parameter	**Value**
E.coli 37°±1°C incubation 24hrs	0/100 ml
Total coliforms37°±1°C incubation 24hrs	0/100 ml
Faecal streptococci 37°±1°C incubation 48hrs	0/100 ml
Total plate count 35°±2°C incubation 48hrs	100/ml

According to WHO (2008), the greatest risk of FBD is associated with water that is contaminated with human or animal excreta, although other sources and routes of exposure may also be significant. As there is no simple routine way of culturing most of the intestinal pathogens in water contaminated by sewage (for example cholera, typhoid, hepatitis viruses), there is no absolute way of distinguishing between safe and unsafe water. The accepted method therefore is to culture for bacteria normally found in the intestinal tract as indicators of pollution with feces. There is a direct link between the proportion of coliform bacteria in a sample from water contaminated with raw sewage and the probable number of *Salmonella Typhosa* bacteria. The risk is also related to the prevalence of typhoid in the human population in the area where the water sample originates (WHO, 2010; WHO, 2012[3]) Infectious diseases caused by pathogenic bacteria, viruses and parasites are the most common and widespread health risk associated with polluted water. A breakdown in the supply of potable water may lead to large-scale disease outbreaks when people drink it, or it is used in preparation or processing of foods.

The lack of potable water and basic sanitation, together with the poor housing and malnutrition associated with urban poverty, results in a high potential risk of FBD in developing nations. An adequate supply of safe drinking water is not available in many countries globally (Lund *et al*., 2000, WHO, 2010)[1]. Pathogens that may be transmitted through contaminated drinking-water include Hepatitis A and E,

[2] http://www.who.int/water_sanitation_health/WHS_WWD2010_guidelines_2010_6_en.pdf

[3] Http://whqlibdoc.who.int/monograph/WHO_MONO_49_(chp9-13).pdf, accessed September 2012

Campylobacter spp*, E. coli, Salmonella* spp, *Vibrio* cholerae*, Shigella* spp*, Yersinia* spp, and *Cryptosporidium* (CIA 2012; WHO, 2010[4]).

Pathogens transmitted by the fecal-oral route are particularly important, thus water intended for human consumption should contain no indicator organisms (Fecal coliform and *E. coli* Type 1) that suggest faecal contamination. Improvements in quality and availability of potable water quality and effective disposal of human and animal excreta as well as general hygiene are essential for reducing faecal-oral disease transmission (WHO, 2008; WHO, 2010). The CAC (2005) has proscribed standards for monitoring and maintaining water potability, including storage, temperature control, water distribution and disposal of waste water or effluents.

According to Fanatico (2003) a great deal of water is used in processing poultry, especially for scalding, washing carcasses, chilling, and cleaning of facilities. Wastewater from poultry processing facilities cannot simply be discharged into lakes and rivers because of the relatively high content of organic matter such as protein and fat and the microorganisms present. Treating abattoir effluent (waste water) starts with sieving (screening) out larger chunks (off cuts, feathers etc) and ends with the breakdown of dissolved organic matter by microorganisms.

Steps in effluent management at a poultry abattoir are:

1. Preliminary treatment to remove large chunks by screening or sieving.
2. Primary treatment to remove small particles suspended in the water. Fats and fine solids are removed by means of fat traps or dissolved air flotation.
3. After primary treatment, the wastewater is either discharged onto land ("land treatment") or given secondary treatment. Land treatment requires large tracts of land and may not be an option near urban areas because of odor and pollution of underground water. Secondary treatment or biological treatment uses microorganisms to break down the organic matter suspended in the water and is more used particularly in peri-urban or industrial areas.

There is an intrinsic connection between the availability of safe water and safe food. As water is used in production, processing, and preparation of food, contaminated water can easily affect the food supply. Maintaining safe drinking water and disposal of human and animal feces safely is fundamental to the health of any society (Cliver & Riemann, 2002).

[4] Http://www.who.int/water_sanitation_health/WHS_WWD2010_guidelines_2010_6_en.pdf

2.2.2 Poor hygiene

Meat hygiene programs have traditionally been based on good hygienic practices (GHP), which provides a baseline food control program and are related to standard operating practices that are audited at particular steps in the food processing chain. GHP consist of a qualitative description of all practices regarding the conditions and measures necessary to ensure the safety and suitability of food at all stage of the food chain (FAO & WHO, 2006). Hygiene measures should be applied at those points in the food chain where they will be of greatest value in reducing food-borne risks to consumers (CAC, 2005). Microbiological monitoring (particularly the level of coliform organism, faecal *E.coli* Type 1 and aerobic plate counts or total plate counts) are used to indicate food safety and quality of the end product (Williams *et al.* 2011; WHO, 2012) .

These GHP tend to be lacking in small and less developed businesses (SLDBs) mentioned previously in Section 2.1. They are also often lacking in the informal marketing sector for animal derived foods found in many developing countries (FAO, 2004; FAO/WHO, 2005). It is common for SLDBs, both formal and informal, to face a variety of problems such as:

- inadequate location and facilities;
- incorrect layout or size;
- non-cleanable structures, old non-cleanable equipment; and
- poor staff training.

They may find it hard to obtain raw materials from reliable and affordable sources. Some countries face basic sanitation problems, such as easy access to potable water and safe disposal of waste (FAO & WHO, 2006). Suitable provision must be made for the removal and storage of waste, which must not be allowed to accumulate in food handling, food storage, and other working areas. The waste stores must also be kept appropriately clean (CAC, 2003). The owners often lack the technical competence and business skills necessary to operate an effective prerequisite program and set up a HACCP system as envisaged in the CAC general principles of food hygiene (CAC, 2003).

People working in small premises are more likely to be engaged in more than one task, so stringent hygiene precautions should be in place if a person goes from one task to another, for instance, collecting money from customers and cooking food for sale. This is particular dangerous if the person goes from a dirtier to a cleaner area, without taking the proper precautions such as cleaning their hands and wearing protective clothing (Bremner & Johnston, 1996).

According to Lund *et al*. (2000) good hygiene management practices include basic requirements for hygienic design, construction, operation, and sanitation of food production premises and equipment; and

the education and training of operatives in good personal hygiene and good hygiene practices. These are also considered prerequisites for HACCP during the processing of food in large scale commercial enterprises in developed countries.

Slaughter of poultry in suitable environment by hygienic methods is essential for the production of wholesome meat, extension of the shelf life of the product, reduction of post harvest losses and protection of public health. Personnel hygiene facilities and toilets should be available to ensure that an appropriate degree of personal hygiene can be maintained and to avoid contaminating meat by food handlers (CAC, 1999). Food handlers should maintain a high degree of personal cleanliness and, where appropriate, wear suitable protective clothing, head covering, and footwear (CAC, 2003). Audit checklists are generally used by inspectors or veterinarians, to maintain a suitable level of standards in any commercial poultry processing plant.

Poultry used for the commercial production of chicken meat are known as “broilers”. Generally the production of healthy broilers depends on the maintenance of health and hygiene throughout the entire production system. Birds are normally kept in an environment that potentially contains micro-organisms, insects, parasites, dirt, dust, feces, all manner of filth and feedstuff among their feathers and on their skin (Silverside & Jones, 1992). Guidelines exist and are implemented during intensive broiler production to minimize these potential hazards to safe and hygienic poultry meat, but are generally not implemented by informal producers (Foodsafety website, 2012)[5].

The possibilities for contamination of carcasses in the processing plant are strongly influenced by the microbiological status of the live bird and this, in turn, is related to husbandry practices and the efficacy of hygiene control measures throughout the production chain, including stress during the growing phase, transport and slaughter (Lund *et al*, 2000). It is inevitable that there will be some microbial contamination of the carcasses due to the nature of poultry meat and the means by which it is produced, but this needs to be minimized by effective hygiene control both prior to and in the processing plant (Bremner & Johnston, 1996). Effective hygiene control is vital to avoid the adverse human health and economic consequences of food borne illness, food borne injury, and food spoilage (CAC, 2003).

Good hygienic practices at the level of primary production should involve the health and hygiene of the birds records of treatments, feed and feed ingredients and relevant environmental factors. Poultry identification practices should allow that the origin of meat can be traced back from the abattoir or

[5] Http://www.foodsafety.gov.nz/industry/sectors/poultry accessed September, 2012

establishment to the place of the poultry production to facilitate investigation when necessary (CAC, 2005).

Broilers are normally kept on litter in housing where the environmental temperature and ventilation rate can be controlled. From the point of view of hygiene control, all birds should be removed from the house at the end of the rearing period (the “all-in, all-out” principle). The controlled environment conditions that are necessary for optimal poultry production can also be exploited to prevent flock infection. In this respect, the necessary measures to achieve effective cleaning and disinfection, high standard of bio-security, immunization, feed and water hygiene, feed additive and poultry health monitoring, must be in place (Lund *et al*, 2000; Bilgili, 2010).

2.2.3 Risk of contamination prior to and during slaughter

According to the South African DVS (2007) the slaughtering process should be aimed at keeping the microbiological load on the newly exposed meat surface, as low as possible and all efforts should be made to prevent bacteria from being deposited on the carcass. Microbial contamination of poultry meat is a natural consequence of the transformation of live poultry into retail products (Bilgili, 2010). The organisms are carried asymptomatically in the intestines of a varying proportion of all healthy birds which makes difficult the control of these organisms at source (Mead, 2004), and they frequently became contaminants of processed carcasses. Prior to slaughter, ante-mortem inspection should be effectively carried out taking into account all relevant information submitted by the primary producer who sent the birds to slaughter (DVS, 2007).

The CAC (1999) has highlighted the necessary utilities that must be in places where birds are slaughtered, such as potable water, drainage and solid/water waste disposal. In addition, standards developed by the International Standards Organization (ISO) have been developed and adopted by all member countries, for abattoirs (processing plants) for poultry. The ISO 22000 standard is used for HACCP and includes processing standards for broilers. Although ISO standards which are linked to integrated hygiene management and assessment systems are progressively being introduced into the poultry industry in developed countries, they are not yet achievable in some developing countries due to deficiencies in infrastructure and capital.

Poultry is transported to the processing plant in vehicles that can be readily unloaded. The transport facilities should ensure that cross-contamination with fecal material is minimized and new hazards are not introduced during transport. The birds should not be loaded for transport to the abattoir when the degree of contamination of its external surfaces is likely to compromise the hygienic slaughter and processing (CAC, 2005). After unloaded the trucks are washed and thoroughly cleaned and disinfected (sterilized),

before return to farms to collect more birds. Even the size and shape of containers for transporting birds are detailed in South Africa pertaining to the Meat Safety Act of 2000. In South Africa, slaughter of poultry falls under the regulations pertaining to the HACCP system in terms of Section 15 (1) of the Foodstuffs, Cosmetics and Disinfectants Act 54 of 1972 (NDA website, 2012[6])

Transport crates and vehicles can be a potent source of flock to flock transmission for enteric food borne pathogens, they need to be properly cleaned and disinfected after delivering birds to the processing plant (Bremner & Johnston, 1996). In addition to the environment, employees, the facility and equipment and all raw materials used in the production and processing can serve as source of food borne hazards in poultry products (Bilgili, 2010).

When birds arrive at the processing plant, they are heavily contaminated with a wide variety of microorganisms, which are carried on the skin, among the feathers, and in the alimentary tract (Bremner & Johnston, 1996). It is important that birds are starved prior to slaughter so that the intestines are not distended, thereby reducing the risk of gut breakage during evisceration (Lund *et al,* 2000; Sutherland *et al,* 1986). Live birds represent the single largest source of biological hazards during processing; they serve as "silent carriers" for many human enteric pathogens that thrive in and are usually confined in the digestive tract (Bilgili, 2010).

The source of spoilage organisms is mainly the rearing environment of the birds and they are brought into the plant on the outside of the birds. The principal spoilage bacteria of aerobically stored poultry are pigmented and non-pigmented strains of Pseudomonas spp., that grow faster when the temperature is similar to that linked to their optimum growth (25°C). Inadequate food temperature control is one of the most common causes of food spoilage. In the absence of suitable temperature, humidity and other environmental controls, meat is particularly vulnerable to survival and growth of spoilage micro-organisms (CAC, 2005). Food spoilage is wasteful, costly and can adversely affect trade and consumer confidence. Systems should be in place to ensure that temperature is controlled effectively where it is critical to the safety and suitability of food (CAC, 1969, CAC 2005).

2.2.4 Contamination during processing

During commercial processing of poultry, further contamination can occur at any stage, especially during scalding, plucking, and evisceration (FAO, 2009; Northcutt & Russel, 2003) and can also contaminate processing equipment, aerosols and the hands of operatives. Water in scalding tanks should be managed

[6] Http:// www.nda.agric.za accessed January 2011

so that it is not excessively contaminated (CAC, 2005; Northcutt & Russel, 2003; Sutherland *et al*, 1986). According to Mckee (2007) factors that affect the antimicrobial capacity of the scalder include the level of organic load, scalder configuration, water flow rate and counter-current flow, pH and temperature. At 50°C, bacteria survive in the scald water, and organisms such as *Salmonella* may persist long enough to cause cross-contamination of carcasses (Lund *et al*, 2000; Sutherland *et al*, 1986). A "hard" scald at *ca*. 60°C is used for poultry intended for freezing and a "soft" scald at ca. 50°C for birds to be sold in the "fresh chill" state (Mead, 2004 and Sutherland *et al*, 1986). However scalding at 60°C appears to have a beneficial effect in reducing carcass contamination and can lead to total viable counts from skin of 10^3 c.f.u./cm^2 or less and counts of coliform bacteria below 10^2 c.f.u./cm^2 (Bremner & Johnston, 1996).

Plucking or "defeathering" follows scalding. After de-feathering, the poultry carcass can only be effectively cleaned of dust and other contaminants by the application of potable water (CAC, 2005; Lund *et al,* 2000). The cleaning process removes only some of the microbial contaminants attached to the skin. Washing is not a very effective way of removing attached bacteria, sufficient remain to cause problems if carcasses are not properly handled. Bacteria are found in the bottom of feather follicles, positions which are difficult to clean and conducive to microbial growth (Silverside & Jones, 1992). Washing of the bird carcasses as soon as possible, after each contaminating step and before attachment can occur, reduces the adherence of bacteria to the skin which can minimize overall carcass contamination (CAC, 2005; Lund *et al,* 2000 and Sutherland *et al*, 1986).

Evisceration follows plucking. With high-rate processing, the carcasses on the line are very close together and cross-contamination occurs readily, allowing little opportunity to sanitize implements or equipment between carcasses. Also, the carcass remains whole throughout the process, which means that the viscera must be removed rapidly through a relatively small opening in the abdomen. Evisceration often leads to gut breakage and contamination of the carcass with gut contents, with a high risk of carcass contamination (CAC, 2005; Bremner & Johnston, 1996; Silverside & Jones, 1992).It is difficult to clean the abdominal cavity of an entire carcass effectively. The alimentary tract can split easily, spilling its contents over the carcass on both the outside and inside. Processed water, such as used in washing, chilling and further processing can become easily contaminated. Surfaces in the processing plant, the hands of operators and their processing implements can easily become contaminated (Silverside & Jones, 1992).

2.2.5 Chlorination to reduce risk of spoilage organisms and pathogens

In small processing plants, with manual evisceration, opportunities for increased carcass contamination and for cross-contamination depend upon the measures taken to control plant hygiene (Lund *et al*, 2000). In fully automated plants, the machines must be set carefully and adjusted as required. A fine spray of

water, often well chlorinated, is commonly used in the eviscerating machines, and carcasses may be spray washed at more than one stage in the evisceration process. Chlorine concentrations of 20-50mg l^{-1} may be employed in these washes as they have proved effective in limiting the multiplication and viability of faecally derived organisms. The use of super chlorinated water at 50 mg/l or more or free chlorine is recommended by Bremner & Johnston (1996), to reduce the risk of spoilage organisms and pathogens in poultry carcasses. This is a standard procedure currently in commercial poultry processing plants globally, but is not a feature of small scale and informal poultry slaughter.

Manual evisceration is normally carried out in several stages and cross-contamination by spoilage and by potential pathogenic bacteria readily occurs at this stage: incomplete evisceration can also lead to rapid spoilage and there should be an inspection point after evisceration to remove such carcasses form the slaughter line (Sutherland *et al,* 1986). Microbial monitoring is frequently used to detect whether hygienic measures are effective during processing, these are usually done on the finished product. Microbiological limits should take into consideration the risk associated with the microorganisms, and the conditions under which the food is expected to be handled and consumed (CAC, 1997). Food can become microbiologically hazardous to the consumer when the principles of hygiene and sanitation are not met or when it becomes contaminated by pathogens from humans or from the environment during production or processing. Examination of food samples allows us to determine the presence of these hazards.

2.2.6 Post mortem examination of carcasses

Post-mortem inspection should be science based and be tailored to relevant risks, where indicated by public health concerns, routine screening of carcasses and other relevant parts by methods other than organoleptic inspection (such as microbial counts) may be required for suspected hazards (CAC, 2005).

The Official State Veterinarian must record the checks of the monitoring of all carcasses, as well as, the corrective action taken in cases of non-compliance, ensuring that the abattoir meets all standards required by legislation (South African DVS, VPN/11/2007-1). Condemned poultry products must be clearly separated from the rest of the products and by definition, the word “condemned” used during poultry meat processing, means the meat is unfit for human consumption (Bilgili, 2010).

2.2.7 Chilling to reduce risk of microbiological contamination

Rapid chilling of the carcass after slaughter is essential to reduce microbial growth (Bucher *et al,* 2012; Northcutt *et al*, 2003; Silverside & Jones, 1992). Chilling must be carried out promptly after washing; the deep muscle temperature after washing is ca. 30 °C and if chilling is delayed both spoilage and

pathogenic bacteria can develop (Sutherland *et al*, 1986). In the spin chiller there is a continuous cold water inflow through an aerated water bath at not more than 4 °C and in the air chiller cold air at ± 0 °C is blown over the carcasses at 0.75 meters per second. The carcass deep bone temperature of carcasses leaving the chiller must be less than 7 °C (South African DVS, 2007). In adequately controlled chilling systems, the washing effect on carcasses leads to a net reduction in microbial contamination (Bilgili, 2010) and reduces both aerobic plate counts and counts of coliform bacteria by 50% to 90% (Lund *et al*, 2000). Water used in the spin chiller must have a chorine concentration of not less than 50 p.p.m. (South African DVS, 2007).The washing-off effect of chilling water was estimated to result in lower risks to consumers compared with those generated from air-chilled chickens, but there was uncertainty around the effect of cross-contamination in chill water (Northcutt & *et al*, 2003; FAO/WHO, 2003).

Where reduction in count has not been observed in chilled compared to non-chilled carcasses, either the chilling system was not properly controlled or carcass contamination prior to chilling was unusually high. An obvious disadvantage of chilling water is the opportunity for cross-contamination because large numbers of carcasses are chilled together in the same tank. Because there is no washing effect, air chilling carcasses tend to carry more microorganisms than those that are water chilled in a properly controlled system, but the process is less likely to cause microbial cross-contamination (Lund *et al,* 2000).

Carcasses should be chilled to a maximum of 4 °C, a process that takes 1.5-2.0 hours for a 1.5-2.0 kg carcass (Mead, 2004). Refrigeration is now one of the most widely practiced methods of controlling microbial growth in perishable foods and good storage procedures must be in place to ensure that such foods will achieve the required shelf life and will be safe to the consumer (Lund *et al*, 2000).

The injection of a solutions of polyphosphates or brine (sodium chloride) results in a weight gain (effectively added water) and has been the subject of much critical opinion, although processors claim it produces a more succulent bird of higher quality[7]. More recently, a process involving injection of butter, oil or a mixture of fats has been developed to produce self basting birds, which now command a considerable share of the market. Any form of injection into poultry raises the possibility of introducing deep muscle contamination either by transfer of the microorganisms from the skin or by contamination of the substance being injected (Sutherland *et al*, 1986)**.**

2.2.8 *Salmonella* as a potential microbial pathogen

Salmonella is a genus of Gram-negative facultative rod-shaped bacterium from the Enterobacteriaceae family. They are facultative anaerobic, nonlactose fermenting, nonspore forming, and most are motile

[7] Http://www.daff.gov.za/doaDev/articles/BrineInjectionProject.html, accessed October 2012

(Eissen & Heijden, 2010; Cliver & Riemann, 2002). The *Salmonella* family includes over 2,300 serotypes, the most common types for the majority of human infections are Salmonella enteritidis and *Salmonella typhimurium* (Eissen & Heijden, 2010). They can be found free living in nature, as part of the indigenous flora of human and animal intestinal canals, and are also known to survive in a desiccated state. They grow readily in many foods, as well as in water contaminated with feed or feces (Cliver & Riemann, 2002).

Salmonellosis is a leading cause of food borne illness in many countries, with eggs and poultry meat being important vehicles of transmission (Eissen & Heijden, 2010; Sluis, 2010, and WHO & FAO, 2002). The clinical pattern of salmonellosis can be divided into four disease patterns: gastro-enteritis, enteric fever, bacteremia with or without focal extra intestinal infection and the asymptomatic carrier state (Cliver & Riemann, 2002). The symptoms of Salmonellosis include fever, headache, nausea, vomiting, abdominal pain and diarrhea (WHO, 2007). According Eissen & Heijden (2010), in more serious cases, *Salmonella* can escape from the intestine to the bloodstream and travel to other organs, leading to more severe consequences.

Although *Salmonella* is recognized as the most important food borne pathogen associated with poultry meat, the extent to which consumption of poultry meat is responsible for human salmonellosis is presently unknown, because most cases are sporadic and cannot be traced to source (Lund *et al,* 2000). The species of *Salmonella* is also very important to the level of disease caused in humans and definite links with mortalities have been made in the case of certain serotypes, particularly those that infect eggs. As a result, certain *Salmonella* spp are listed as controlled diseases by the Organisation of Animal Health (OIE) as controlled diseases. For instance, *Salmonella enterica* Serovar Typhi (called *S. Typhi* for simplicity sake, below, as this is the name in earlier literature) which causes typhoid, can also be a contaminant as it is transmitted by human carriers and water sources contaminated with human feces (WHO website, 2012).

Most *Salmonella* infections are zoonotic and are transmitted from healthy carrier animals to humans through contaminated food (Eissen & Heijden, 2010). In the USA, the rate of human disease attributed to salmonellosis has increased, in spite of progress made by the poultry industry in reducing the levels on raw poultry carcasses (O'keefe, 2010). *S. Typhi* is specifically of human origin and contaminates foodstuffs during the primary production via sewage, as a result of poor personal hygiene by a food handler who is a carrier (Lund *et al,* 2000). *S. enterica* Serovar Typhimurium is classically carried by rodents and causes enteric disease in a variety of animals and also in humans. It is also becoming more prevalent in broilers and may play a role in FBD (Larrison *et al*, 2010). Suggestions for control of *Salmonella* throughout the poultry food chain have been published by both the OIE and CAC.

The most pathogenic serotypes for humans and poultry are those that are invasive and capable of reaching internal organs, such as the liver, spleen and reproductive tract. *S.Enterica* Serovar Enteridis is an example of an invasive, highly pathogenic *Salmonella* that can be vertically transmitted and infect eggs (Mead, 2004).

The European Food Safety Association (EFSA, 2007), mentioned salmonellosis as the second most frequent zoonosis in the EU, with 176,395 reported human cases, despite the fall of 9.5% to an incidence rate of 38.2 compared to 2004. The EU legislation focuses on control of *Salmonella* spp within the poultry production chain and the main driver here is financially penalizing poultry meat and egg producers who have high levels of on farm (Eissen & Heijden, 2010). Data from the past 13 years, in USA, suggested that there has not been a correlation between reductions in the incidence rate of carcasses positive for *Salmonella* spp coming out of the chiller and the human illness rate for salmonellosis (O'keefe, 2010). *Salmonella* spp are associated with fecal contamination and the levels of *E. coli* Type 1 in raw poultry meat can act as indicators for the presence of *Salmonella.*

Salmonella spp flourish best in the alimentary tract of young birds, where microbial competition is less during the period that the natural microflora is developing (Mead, 2004). They are transmitted from bird to bird at all stages of growth, handling and transport of the live bird, via the oro-faecal route (Eissen & Heijden, 2010; Silverside & Jones, 1992; Bremner & Johnston, 1996).

Broilers are the main type of chicken consumed in many countries. Large percentages are colonized by salmonellae during growth, the skin and meat carcasses are frequently contaminated by pathogens during slaughter and processing (Mckee, 2007; WHO & FAO, 2002). Salmonella can be prevalent in poultry litter and can be a source of contamination for chicks newly arrived at poultry house (Larrison *et al*, 2010). Salmonellae also multiply if the bird is stressed and high levels in carcasses can be associated with poor transport and slaughter practices that increase stress in birds. Deficiencies in hygiene management of crates used to transport birds to the processing plant were also implicated as a potential source of contamination (Mead, 2004). According to Mead, (2004); Lund *et al*, (2000); Bremner & Johnston, (1996) broiler chicken may acquire *Salmonella* infections from three main sources: breeding stock via the hatchery, the rearing environment, and contaminated feed.

Salmonella control strategies must start before the processing plant (Russell, 2007). Numerous factors during breeding, hatching grow out and transportation can increase the level of *Salmonella* in the finished product and various companies have spent enormous amounts of time and money attempting to reduce this. In the EU, many member states have been able to substantially reduce the prevalence of *Salmonella* in poultry meat by improving the biosecurity measures and the breeder flock status (Sluis, 2010). The OIE

website[8] has guidelines for control of Salmonellosis in broilers and layers throughout the food chain and describes the new classification and nomenclature of Salmonellae as well as listing Serovars which are hazardous for human and animal health.

The introduction of mass processing techniques has increased the incidence of salmonella. It is important to realize that while Salmonella can spread during processing this pathogen does not originate in the processing plant (Sutherland *et al*, 1986). Russel (2007) demonstrated that by changing the management of the broilers prior to slaughter and processing, the prevalence of *Salmonella* can be significantly reduced. Mckee (2007) showed that when 3% to 4% of birds entering a plant tested positive for Salmonella, 20 % to 35% tested positive after processing. Thus, cross contamination is very important.

Integrated hygiene management must be implemented to reduce the level of salmonella in poultry meat. Facilities which have multi-stage scalding systems with a counter-current configuration allow birds to move towards cleaner water when they exit the scalder (Mckee, 2007). Chilling is important as this inhibits the growth of Salmonellae and at temperatures below 7 °C hardly at all (Bucher *et al,* 2012; Northcutt & Russell, 2003; Bremner & Johnston, 1996; Silverside & Jones, 1992). Chlorine is also effective; particularly when the water pH is optimal, water with a high pH will render chlorine ineffective (Mckee, 2007). Feed withdrawal prior to catching birds is a necessary step to prevent fecal contamination of carcasses during processing and same companies controlled successful *Salmonella* in the crop by acidifying the bird's drinking water during the feed withdrawal process (Russel, 2007). Mckee (2007) recommends eight hours as an optimal feed withdrawal time.

Considering the major role eggs and poultry have as vehicle of human cases of salmonellosis, an assessment of different factors affecting the prevalence, growth and transmission of *Salmonella* in eggs and on broiler chicken carcasses and the related risk of human illness should be useful to risk managers in identifying the intervention strategies that would have the greatest impact on reducing human infections (WHO & FAO, 2002). Risk assessment for *Salmonella* in meats can be qualitative, quantitative or semi-quantitative based on the detection of the bacterium (negligible, acceptable, harmful or high), and on the evaluation of Salmonella in meat products (Bonardi *et al*, 2008). As Salmonellae are mainly faecal or water-borne pathogens, *E. coli* Type 1 counts that indicate the presence of fecal organisms are a simple and affordable way of screening food for the risk (likelihood) of contamination with *Salmonella* spp.

Most salmonellae are confined to the intestines of infected birds and excreted in the faeces, however not all serotypes are pathogenic and likely to cause FBD. In the case of flocks that are infected with highly

[8] Http://www.oie.int

pathogenic salmonellae, it is important to avoid the cycle of infection and re-infection (Eissen & Heijden, 2010). Once introduced, infection may be spread via drinking water, feces or feed that rapidly becomes contaminated. Potential vectors include wild birds, rodents, humans, insects and pets.

Any type of poultry is susceptible to a *Salmonella* challenge from the rearing environment. Hence the importance of comprehensive biosecurity measures, which involve the effective use of hygiene precautions and include control of vectors and limit visitor access to the house (Mead, 2004). Significant associations were found between the contamination level and hygiene control in the broiler house, its feed and water supplies.

Sutherland *et al* (1986) emphasized that control of *Salmonella* must be a total operation involving elimination from both the breeding and rearing flocks, which includes safe sources of feed, water and bedding etc. Eissen & Heijden (2010) also included good hygiene, biosecurity and management practices. Control of rodents and wild birds is also essential. Proper sewage treatment and potable /chlorinated water (monitored using coliform counts) are all important (Cliver & Riemann, 2002).

Ultimately prevention of Salmonellosis must lie in safe handling of poultry before and after processing to avoid cross-contamination. According to the FAO (2009), the most important control measures for pathogenic Salmonella during primary production are:

1. Elimination in grandparent and parent flocks.
2. All-in and all-out production at broiler farms, to avoid any carry over during production and later, processing.
3. Logistic slaughter planning scheduled to avoid pathogens being transferred from contaminated processing equipment to another flock.
4. Satisfactory cleaning for transport crates.

Rodenburg *et al* (2004) cited Van De Giessen *et al* (1998) who outlined that the risk of spreading *Salmonella* on the broiler flock can be reduced by applying hygiene measures, like changing footwear when entering a broiler house and washing hands before tending the flocks.

2.2.9 *Campylobacter* as a potential microbial pathogen

Campylobacteriosis is a worldwide disease of animals that can be transmitted secondarily to humans. *Campylobacter,* first identified as animal pathogens about 80 years ago, became recognized widely in the 1970s for causing human illness. *C. Jejuni* was isolated first from human diarrheal stools in 1971 (Hui *et al,* 1994). *Campylobacter* spp. can cause serious complications related to acute bacterial enteric disease

in humans (Rahimi & Tajbakhsh, 2008). In both developed and developing countries, it is a cause of more cases of diarrhea than food borne *Salmonella* bacteria. Campylobacteriosis is responsible for severe illness, complications and hospitalizations for previously well persons in all age groups, becoming an expensive disease. An outbreak affects the economy adversely due to medical costs for treatment and productivity loss (Hui *et al*, 1994, WHO, 2000).

Acute health effects of Campylobacteriosis include severe abdominal pain, fever, nausea and diarrhea (Rahimi, 2008; WHO, 2007; Mead, 2004). The symptoms typically last three to six days, a fatal outcome is rare and is usually confined to very young or elderly patients, or to those already suffering from another serious disease such as AIDS (WHO, 2000). In 2 -10% of cases, the infection may lead to chronic health problems, including reactive arthritis and neurological disorders (WHO, 2007; Mead, 2004).

Campylobacter spp are small, non-spore forming, gram-negative bacteria, with a characteristics curved, S-Shaped, or spiral morphology. The most important pathogenic strains for humans belong to the thermo-tolerant group *(C. jejuni* and *C. coli)*. *Campylobacter jejuni* has recently been recognized as one of the leading causes of gastroenteritis in humans. The infective dose in humans is low, ranging from 500 to 10000 organisms (Cliver & Riemann, 2002).

Poultry is often considered to be the primary source of infection, but others include faecally contaminated water supplies, raw milk and contact with other animals (Cliver & Riemann, 2002). *Campylobacter* organisms are commonly found as commensals of the gastrointestinal tract of wild or domestic animals, including all classes of poultry (Hui *et al,* 1994). *Campylobacter* shed in faeces from the gastrointestinal tract are able to survive for considerable periods in the environment, but are not known to grow under those conditions (Cox *et al*, 2010; FAO/WHO, 2009[2]). Live birds, including chickens, are commonly infected with *C. jejuni*, but *C. coli* predominantly found in pigs, has also been isolated from poultry (FAO, 2009[3]).

Its presence in every stage of the commercial broiler operations has been clearly demonstrated, starting on the breeder farm, passing to the hatchery, then to the broiler flock and finally to the processing plant (Cox *et al,* 2010). The contamination of poultry houses by carrier flocks can lead to contamination of subsequent generations of poultry if cleaning and disinfection between removal of market-ready birds and introduction of new flocks is ineffective. Other potential routes of entry into flock include contamination of feed and water, and wild and game birds. *C. jejuni* has been also isolated from stream and river water and from the effluent of poultry processing plants. The infection of poultry is often without clinical

manifestations (Hui *et al,* 1994). The OIE website[9] gives guidelines for prevention and control of Campylobacteriosis throughout the production and processing chain for broilers and layers

Campylobacter in the environment of poultry operations serve as a nidus for reinfection of flocks, reservoirs within the poultry environment include darkling beetles and houseflies. The use of unchlorinated drinking water facilitates colonization of poultry flocks. Surface waters often harbor *Campylobacter,* which may originate from fecal contamination by humans, terrestrial animals and birds (Cliver & Riemann, 2002).

Campylobacter spp. is a common contaminant of poultry carcasses in poultry processing plants and constitutes a risk for consumers (Ellerbroek *et al,* 2010; Rahimi & Tajbakhsh, 2008; Mead, 2004; Lund *et al*, 2000). Contamination of processing plants due to ineffective sanitation can contribute to the contamination of carcasses from the following slaughtering process (Ellerbroek *et al,* 2010; Hui *et al,* 1994). Cox *et al* (2010) also maintained that campylobacter contamination continues to plague the poultry industry and its source still confusing, making control very difficulty.

Damage to the integrity of the intestinal tract during slaughtering, can lead to direct contamination (Northcutt *et al*, 2003). Contamination can also occur through air, bird to bird, via equipment and water. Exposure to *Campylobacter* spp. has been linked to FAD through undercooking, endogenous contamination of poultry meat and cross-contamination with ready-to-eat foods due to mishandling (Cliver & Riemann, 2002). There is a one-to-one relationship between the number of human illnesses and a reduction in positive carcasses at the processing plant. Thus preventing carcasses from becoming campylobacter-positive would have a greater impact on reducing illness than reducing overall campylobacter population on carcasses (Lindqvist & Lindblad, 2008).

Data from sporadic cases suggest that handling and preparation of chicken and poultry liver, and the consumption of undercooked chicken are particular risk factors (Lund *et al*, 2000). The human contamination also can occur by contaminated water, contact with sick people or other animals. Fecal-oral person-to-person infection has been reported for *C. jejuni* and those in contact with excreta of infected people are at high risk (Hui *et al*, 1994). At least some of the strains present appear to be invasive in the bird and can infect certain organs, such as the liver. Contamination of processed giblets (heart, liver, gizzard and neck) can be particularly high (Mead, 2004). *C. jejuni* is sensitive to drying or freezing temperatures, characteristics that limit its transmission, but the organism survives in milk and water kept at 4°C for several weeks (Hui *et al*, 1994).

[9] Http://www.oie.int

The EFSA (2007) analyzed 2005 data that highlighted campylobacteriosis as the most frequently reported zoonotic disease in humans within the EU. Reported *Campylobacter* cases increased by 7.8% compared to the previous year rising to an incidence rate of 51.6 cases per 100,000 people and to a total of 197,363 recorded cases. The same study from EFSA, (2007) mentioned that amongst foodstuffs, the highest proportion of *Campylobacter* positive samples was reported for fresh poultry meat, where up to 66% samples were found positive.

Contamination of poultry carcasses with *Campylobacter* can be reduced by dipping or spraying of carcasses using chlorinated water, acidified sodium chlorite or acetic or lactic acids (FAO, 2009). Hygienic slaughter and processing procedures will reduce cross-contamination, while adequate cooling and aeration will cause decrease in the microbial load (Hui *et al*, 1994). Processing steps that have been reported to reduce bacterial counts include chilling (Northcutt *et al*, 2003), scalding and washing. Use of chlorinated sprays will maintain clean working surfaces and also reduce carcass contamination (Northcutt *et al* , 2003; Cliver & Riemann, 2002).

Hui *et al* (1994) emphasize that preventive and control measures must include the use of existing resources to reduce the level of infection in animals and subsequently in humans. At farm level the producer should practice sound sanitation around and within the farm, maintain disease-free birds, use only feeds tested and found free of *Campylobacter*, and prevent contamination of birds before or during transfer from farm to market. Using campylobacter-free parent birds and raising Campylobacter-free birds for slaughter will help to protect the consumer from food borne infection. The use of antibiotics to decrease flock levels of this organism is not a good practice as it can lead to higher levels of antimicrobial resistance.

Strict management of poultry production may reduce carriers. Suggested management practices to improve biosecurity include restricting contact between different age groups or houses, requiring disinfection of boots, washing hands, and changing into clean clothing before entering housing areas. The use of chlorinated drinking water in food animal production holds promise. Treatment of chicks with cecal colonizing bacteria may also reduce *C. jejuni* colonization (Cliver & Riemann, 2002).

2.2.10 *Escherichia coli*

According FAO (2011); Kotoski (1997) total coliform bacteria are a collection of relatively harmless microorganisms that live in large numbers in the intestines of man and warm and cold-blooded animals. They aid in the digestion of food. The “coliforms” constitute a group of bacteria that are capable of fermenting lactose with the production of acid and gas at 35°C within 48 hours (DVS, 2007). A specific subgroup of this collection includes the fecal coliform bacteria, the most common member being

Escherichia coli. E. coli belongs to the family Enterobacteriaceae and it is present in the intestinal tract of vertebrate animals; in humans it forms about 1% of the total bacterial biomass (DVS, 2007). *E. coli* is the predominant facultative anaerobe in the human bowel and helps to maintain the normal physiologic function of the intestine. These organisms may be separated from the total coliform group by their ability to grow at elevated temperatures and are associated only with the fecal material of warm-blooded animals.

Their presence in water or food indicates contamination with feces as they are fecal organisms. Faecal contamination of the internal and external surfaces of poultry represents the most important cause of microbial contamination during slaughter (Bilgili, 2010). Slaughter plants will be required to test for generic *E. coli* on carcasses to verify that they are preventing and removing fecal contamination from the final products (Buzby & Crutchfield, 1997). Thus they can be used as an indicator of poor hygienic practices during processing of poultry carcasses. According to Döpfer *et al,* 2012; FAO (2011) *E. coli* (O157:H7) strain is the most important EHEC serotype since, as members of an indicator group, they are also linked to a high incidence of enterohaemorrhagic infections and deaths each year.

The usual method to test for the presence of pathogens is to use the coliform count. It tests for the presence of harmless indicator organisms (*Escherichia coli*) that might indicate the presence of intestinal pathogenic organisms. Due to its high prevalence in the gut, *E. coli* is used as the preferred indicator to detect and measure faecal contamination in the assessment of food and water safety (FAO, 2011; DVS 2007). The coliform count is usually reported as the number of coliform bacteria estimated to be present in 100 cm^3 of water. For water to be considered safe, this parameter must be zero.

The presence of faecal coliform bacteria in aquatic environments indicates that the water has been contaminated with the fecal material of man or other animals, the source of water might have been contaminated by pathogens or disease producing bacteria or viruses which can also exist in fecal material and it can be a potential health risk for individuals exposed to this water (Kotoski, 1997). *E. coli* Type 1 is a faecal organism and its presence shows that other pathogenic organisms found in faeces eg *Campylobacter, E. coli* 0157 or *Salmonella*, may also be present.

The presence of small numbers of *E.coli* in raw meat is not surprising since faecal contamination is always a risk associated with slaughter and dressing. Globally it is suggested that *E. coli* on meat and meat products must be kept as low as possible or eliminated by improving hygiene and using potable or chlorinated water during processing (Stopforth *et al,* 2007; DVS, 2007).

Since this is a fecal organism, its control in the processing plant would depend upon the control of fecal contamination in general during the slaughter process (Stopforth *et al,* 2007; Bremner & Johnston, 1996).

Certain strains of *E. coli* are pathogenic and can cause diarrhea, cramps, nausea, headaches, or other symptoms. These pathogenic *E. coli* types may pose a special health risk for infants, young children, and people with severely compromised immune systems (EPA, 2003). According to Gray (1995) five distinct groups of *E.coli* cause gastrointestinal illnesses ranging from mild diarrhea to cholera like diarrhea with potentially fatal complications such as hemolytic uremic syndrome (HUS), as shown in Table 2.5 below.

Table 2.5: *E.coli* trains that causes enteric infections

Strain	Pathogenic mechanisms	Enteric infections	Common clinical presentations	Common age group
Enterotoxigenic *E. coli* (ETEC)	Produces heat-stable (ST) and heat-labile (LT) enterotoxins	Diarrhea; traveler's diarrhea	Profuse watery diarrhea, cramps, nausea and dehydration	Adults and children
Enteropathogenic *E. coli* (EPEC)	Adherence factor; attachment to and effacement of intestinal epithelium	Acute diarrhea	Watery diarrhea, fever, vomiting and mucus in stool	Adults and children < 2 yrs old
Enteroinvasive *E. coli* (EIEC)	Invasion and destruction of intestinal mucosal epithelium	Dysentery similar to *Shigela* dysentery	Dysentery; scant stool; blood, mucus and leukocytes in stool; fever and cramps	Adults
Enterohemorrhagic *E. coli* (EHEC)	Produces Shiga-like toxins	Diarrhea; hemorrhagic colitis	Diarrhea; abdominal cramps; blood in stool; fever, HUS	Children and elderly
Enteroaggregative *E. coli* (EAggEC)	Unknown	Chronic and acute diarrheas	Watery diarrhea, vomiting	All ages

The FAO (2011); Cliver & Riemann (2002) classified the *E. coli* strains that cause diarrhea into six categories: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), entero-aggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and enterohemorrhagic *E. coli* (EHEC). The mechanisms by which diarrhea is produced vary for each type of pathogenic *E. coli* and include bacterial attachment to host cells, invasion of intestinal cells, and production of toxins. The EPEC

strain causes watery diarrhea illness, with low-grade fever and vomiting in young infants; while the ETEC strains causes a non-inflammatory diarrhea, accompanied by nausea, mild to moderate abdominal cramping, but little if any fever. The signs are similar to the severe form of cholera (*Vibrio cholerae* infection) with a severe dehydrating diarrhea and attendant high mortality. The EIEC infection may a also resemble human dysentery caused by *Shigella* (Hui *et al*, 1994).

Akkaya (2006); Döpfer *et al* (2012) recognized that the enterohaemorrhagic *E. coli* (EHEC) is an important agent of bloody, and none bloody diarrhea in humans. Cliver & Riemann (2002) mentioned that toxins are produced by several serotypes of *E. coli*, but bloody diarrheal diseases are mostly caused by *E. coli* O157:H7. The term EHEC refers to *E. coli* serotypes that share the same clinical, pathogenic and epidemiologic features with *E. coli* O157:H7. Shiga toxin- producing *E. coli* (STEC), particularly *E. coli* O157:H7 can cause serious human diseases such as bloody diarrhea and hemolytic uremic syndrome (Döpfer *et al* 2012; Mead, 2004; Zhao *et al*, 2001; Bremner & Johnston, 1996) and can lead to chronic kidney failure and death (Mead, 2004; Buzby & Crutchfield, 1997). It is the most common bacterial pathogen isolated from bloody stools (Gray, 1995). *E. coli* O157:H7 infection can cause a wide spectrum of gastrointestinal illnesses, from asymptomatic infection to mild non-bloody diarrhea and even severe hemorrhagic colitis, often associated with profound abdominal pain (Döpfer *et al* 2012; Cliver & Riemann, 2002; Gray, 1995).

E. coli O157:H7 can normally be found in the gastrointestinal system of a range of domestic animals. Poultry meat can also be a source of velogenic *E. coli* infections for humans. It was reported that *E. coli* O157:H7 could readily colonize the caeca of chickens and it was excreted in feces from infected birds for several months. The presence of *E. coli* O157:H7 on chicken carcasses, may result either from cross-contamination during slaughter, processing and/or during transportation of birds. Contamination may also arise from non-potable water during slaughtering and processing of poultry (Akkaya, 2006).

Due to the low infectious dose and life-threatening complications of *E. coli* O157:H7, the organism has emerged as an important zoonotic agent, causing serious public health problems. *E. coli* O157:H7 is regarded to maintain its natural long life cycle in the environment by its resistance to cooling, freezing and to acidic conditions. Appropriate control measures should be developed and implemented to eliminate this human pathogen from foods of animal origin, including poultry, in order to protect human health (Akkaya, 2006).

According CAC (1997) the microbiological limits should take into consideration the risk associated with the microorganisms, and the conditions under which the food is expected to be handled and consumed.

2.3 Risk analysis

According to the FAO (2009), over the past decade, risk analysis has emerged as a structured model for improving food safety control systems, with the objectives of producing safer food, reducing the numbers of food borne illnesses and facilitating domestic and international trade in food.

A contemporary risk-based approach to meat hygiene requires that hygiene measures should be applied at those points in the food chain where they will be of greatest value in reducing FBD risks to consumers (CAC, 2005). The Codex Alimentarius Commission (1999) defines Microbiological Risk Analysis as a process consisting of three components: Risk Assessment, Risk Management, and Risk Communication, which has the overall objective to ensure the protection of public health.

Figure 2.2: Components of risk analysis according the CAC (1999): Risk Assessment, Risk Management, and Risk Communication

Risk analysis is used to develop an estimate of the risks to human health and safety, to identify and implement appropriate measures to control the risks, and communicate with stakeholders about the risks and measures applied. It can be used to support and improve the development of standards, as well as to address food safety issues that result from emerging hazards or breakdowns in food control systems (FAO & WHO, 2006).

Cliver & Riemann (2002) defined risk analysis as a standardized process to assess, manage, and communicate about risk. Risk analysis is beneficial in evaluating the impact of the factors that influence the chance of development the food borne illness, including the food production techniques, the nature of the pathogen, qualities relating to the individual consuming the food, and the condition under which the food is consumed.

2.3.1 Risk assessment

Risk assessment provides us with a framework for organizing all data and information to better understand the interaction between microorganisms, food and human illnesses. And it also gives a tool with which we can compare and evaluate different scenarios as well as identify what types of data are necessary for estimating and optimizing mitigation interventions (FAO, 2009).

Risk assessment is a structured process to evaluate the probability of the occurrence and severity of adverse health effects resulting from human exposure to hazards (Cliver & Riemann, 2002). Different risk assessment methods are used in different countries and within countries, and different methods may be used to assess different kinds of food safety problems. Methods vary according to the class of hazard, the food safety scenario and the time and resources available.

The USDA (2003) definition of risk assessment is: *"a scientifically based process of evaluating hazards and the likelihood of exposure to those hazards, then estimating the resulting public health impact*". Lund et al (2000) described risk assessment as "*the science of understanding hazards, the likelihood that they will occur, and the consequences of their occurrence*". For Bilgili (2010) risk assessment is: "*the structured measurement of risks associated with any type of hazard in food and identification of factors that influence it*".

The OIE (2007) defines risk assessment (mainly for purposes of trade harmonization, import and export) as a process which consists of four interrelated steps:

- release assessment;
- exposure assessment;
- consequence assessment; and
- risk estimation.

Biological risk assessments typically use a quantitative model to describe the baseline food safety situation and estimate the level of consumer protection currently afforded (FAO & WHO, 2006). Microbiological risk assessment (MRA) is an emerging tool for the evaluation of the safety of food and water supplies. FAO and WHO have important tasks in developing and standardizing MRA at an international level, and informing risk managers at national and international level. According to Codex (CAC, 1999), microbiological risk assessment is a scientifically based process conducted according to a structured approach which includes the following four steps:

2.3.1.1 Hazard identification

Identify the microorganisms or the microbial toxins of concern in food, (this is predominately a qualitative process). Hazards can be identified from relevant data sources such as: clinical studies, epidemiological studies and surveillance, laboratory animal studies, investigations of the characteristics of microorganisms, the interaction between microorganisms and their environment through the food chain from primary production up to and including consumption, and studies on analogous microorganisms and situations.

2.3.1.2 Exposure Assessments

An exposure Assessment should describe the pathway from production to consumption and estimates the level of microbiological pathogens or microbiological toxins, and the likelihood of their occurrence in foods at the time of consumption. Scenarios can be constructed to predict the range of possible exposures and might reflect effects of processing, such as hygienic design, cleaning and disinfection, the time/temperature, food handling and consumption patterns. The presence, growth, survival, or death of pathogenic microorganisms in foods, are influenced by processing and packaging, the storage environment including the temperature and the relative humidity.

2.3.1.3 Hazard Characterization

Provides a qualitative or quantitative description of the severity and duration of adverse effects that may result from the ingestion of a microorganism or its toxin in food. Important factors need to be considered in Hazard Characterization: the virulence and infectivity of microorganisms can change depending on their interaction with the host and the environment; microorganisms can be spread through secondary and tertiary transmission; the microorganisms can persist in certain individuals leading to continued excretion of the microorganism and continued risk of spread of infection; individual host susceptibility characteristics; population characteristics, and persistence of the organism in the population.

2.3.1.4 Risk characterization

Represents the integration of the first three steps, providing a qualitative or quantitative estimate of the likelihood and severity of the adverse effects which could occur in a given population, including a description of the uncertainties associated with these estimates that can be assessed by comparison with independent epidemiological data that relate hazards to disease prevalence. Risk Characterization depends on available data and expert judgments.

The above four steps fit into the ambit of participatory risk assessment (Grace *et al,* 2008) as stakeholders, role players and end users can be consulted using participatory and qualitative methods, in order to describe and estimate the risks, hazards and likely level of exposure. This is particularly applicable to informal food chains where there is no existing data available.

Although risk assessment is current best practice and the keystone of international trade, its use in developing countries has been limited. In particular, it has not been applied to the domestic markets where most poor people sell and buy food, yet where levels of hygiene and safety are lowest, and vulnerability to food-borne disease highest (Grace *et al*, 2008). One of the problems is the general absence of sound data on the relationship between a particular concentration of pathogenic microorganism and its probability of causing illness if it is consumed in a food (Lund *et al*, 2000).

The FAO/WHO risk assessment of *Salmonella* in broiler chickens (Surak & Wilson, 2007; FAO, 2002a) estimated that a percentage change in contamination of chickens at the end of processing would result in the same percentage change in risks to consumers. Individual aspects of process control were not modeled, but any intervention that significantly and sustainably reduced levels of *Salmonella* contamination prior to the end of processing would be expected to be an effective risk management measure.

2.3.2 Risk management

A process of weighing policy alternatives in the light of the results of risk assessment and, if required, selecting and implementing appropriate control options including regulatory measures (CAC, 1999). In order to facilitate a broader understanding by interested parties, Microbiological Risk Management (MRM) process should be transparent and fully documented (CAC, 2007). Risk management it is also known as “risk mitigation”. The goal of the risk management process is to establish the significance of the estimated risk, to compare the costs of reducing this risk with the benefits gained, to compare the estimated risks with the societal benefits derived from incurring the risk and to carry out the political and institutional process of reducing the risk (WHO, 2000). The outcome of the risk management process is the development of standards, guidelines and other recommendations for food safety. These can be incorporated into integrated food safety management systems, where standard operating procedures and hygiene audits are routinely used in food processing plants (CAC, 1999).

In the risk evaluation step risk managers determine whether the current level of risk is acceptable. If the risk is acceptable no further action is required, but if the risk is unacceptable managers need to consider appropriate interventions (WHO, 2000).

Microbiological Risk Management (MRM) should take into account the diversity of production methods and processes, inspection, monitoring and verifications systems, sampling and testing methods, distribution and marketing systems, consumer food use patterns, consumers' perception and the prevalence of specific adverse health effect. The MRM should be an iterative process and decisions made should be subject to timely review, taking into account all relevant newly generated data, with a goal toward further risk reduction and public health improvement (CAC, 2007). Risk management refers to the development and implementation of strategies to control identified risks (Bilgili, 2010). Risk management compares alternatives in light of the results of risk assessment (Cliver & Riemann, 2002).The risk management phase identifies, selects, and implements measures that can be applied to reduce the risk identified during the assessment (USDA, 2003).

FSIS USDA guidance on minimizing risks due to *Salmonella* and *E. coli* O157:H7 (FSIS USDA, 2002) is strongly focused on interventions during process control that minimize carcass contamination. While advocating a production-to-consumption approach, risk management interventions are based to a large extent on hygiene procedures and intervention methods that prevent carcass contamination during processing. A zero tolerance for visible fecal contamination is a regulatory requirement

2.3.3 Risk communication

According the CAC (1999) risk communication is an interactive process of exchange of information and opinion among risk assessors, risk managers and other interested parties. Risk communication provides the private and public sector with the information necessary for preventing, reducing or minimizing food risks to acceptable levels through systems of food quality and safety management by either mandatory or voluntary means. At community level risk communication is an important part of veterinary public health and medical health education systems. Participatory methods can be used to develop a message to mitigate risk of FBD that is in line with cultural norms and relevant perceptions (McCrindle, 2005).

Novak et al (2006) mentioned that risk communication includes the interactive process among interested parties or between employees within an organization for identifying risk, projecting its relevance and potential impact and enacting practices to eliminate or minimize the threat. Risk communication refers to communicating the results of the risk analysis to the general public, and ongoing communication among risk assessors, managers, scientists, regulators, and various stakeholders during the entire process to ensure that all affected parties fully understand the process of and information generated by the risk analysis (USDA, 2003).

Since no food product is risk-free, including poultry meat, innovative risk communication methods must be used to educate producers, processors, distributors, retails, as well as consumers on their role in the safety of the food supply (Bilgili, 2010).

2.3.4 Participatory methods in risk analysis

According to the Livestock Development Group (2003) participatory methodologies' (PRA) have been defined as a group of approaches that favour change in both individuals and institutions. Participatory risk assessment has been proposed by Grace *et al* (2008) as a method for estimating the magnitude of the risk to consumers in developing countries. This is in line with Risk Analysis methodology published by the CAC under general principles for food borne antimicrobial resistance (AMR) risk analysis (CAC/GL, 2011). This can be adapted for maintaining general quality and safety in food of animal origin, however, instead of expert teams, participatory methods are used to interrogate the knowledge and opinions of stakeholders, particularly in informal markets. Key informant methodologies (Catley & Admassu, 2003) and focus groups are techniques that can be used to assist with participatory risk assessment.

2.3.4.1 Key-informant methodology

The farmers generally are recognized as knowledgeable about animal health matters, but certain people are known to possess special livestock knowledge and skills. These local experts are important key informants for participatory epidemiologists (Catley & Admassu, 2003).

The key-informant methodology is the single most powerful ethnographic data-gathering tool. The key-informant is an *expert*, who provides important information to the interviewer and must be interviewed more than once, so that a social relationship develops between the interviewer and key-informant. Ideally, the relationships of researchers to their key-informants continue throughout the duration of the project and in later phases of information gathering, it is very useful for the interviewer to try out hypotheses with the best key-informants.

2.3.4.2 Focus Groups (FG)

In the context of the wider study, focus groups are utilized to gain basic information regarding livestock production, animal health, livelihood activities, and perceptions of poverty in order to gather background information regarding the community. The participants in the focus groups generally participated on a voluntary basis and the discussion are conducted by a member of the core research team with the help of a local translator.

2.3.4.3 Direct observation and photographs

Direct observation of the poultry producers and processors, their relationships within poultry production value chain, the infrastructures, equipments and material used for poultry processing. Record those observations through photographs to obtain a better picture of the poultry value chain in Maputo and producers perceptions on food safety.

2.4 Prerequisites to HACCP

Wallace & Williams (2000) cited the 1998 definition of HACCP Prerequisites by the Canadian Food Inspection Agency, as "*the universal steps or procedures that control the operational conditions within a food establishment allowing for environmental conditions that are favorable for the production of safe food*". They also quoted the definition of WHO 1999 as "*practices and conditions needed prior to and during the implementation of HACCP, which are essential in food safety*".

According the FAO & WHO (2006), prerequisite programs outline the measures taken to ensure that premises, equipment, transport and employees do not contribute to or become food safety hazards. Many potential hazards can be eliminated or reduced to a "non-hazardous" state through well-defined and effective prerequisite programs (Newslow, 2002). Without these basic principles (e.g. sanitation, pest control, personnel practices), a risk-based system such as HACCP will fail. The basic Good Hygiene Practices (GHP) program is of prime importance for food safety. Prerequisite programs to HACCP, including training, should be well established, fully operational and verified, in order to facilitate the successful application and implementation of the HACCP system.

In addition, the programs developed for prerequisites may be a useful starting point for companies who have a long way to go to achieve HACCP. Experience within international companies has shown that in certain factories in less developed countries, prerequisites are a good place to prioritize activity (Wallace & Williams, 2000). This is the reason that this research into improving small scale poultry processing in Mozambique is based mainly on prerequisites. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) (1997) has defined prerequisite programs as: "*Procedures, including GMP that addresses operational conditions, providing the foundation for the HACCP system*". Surak & Wilson (2007) and Newslow (2002) emphasized that well-designed prerequisite programs provide a solid foundation for every effective HACCP plan. Each segment of the food industry must provide the necessary conditions to protect food while it is under their control [10].

[10] Http:www.cfsan.fda.gov/~comm/nacmcfp.html accessed September 2012

The production of safe food products requires that the HACCP system be built on a solid foundation of prerequisite programs, traditionally accomplished through the application of GMP. According to Newslow (2002) the GMP program requirements should be defined clearly and include an effective method (i.e., internal audit program) to monitor compliance and overall effectiveness.

GMP may be defined in one overall program or divided into subprograms (Keener, 2009; Newslow, 2002):

- Building design and maintenance;
- Loose jewelry and hair constraints;
- Food hygiene, which may include hand washing and sanitation facilities;
- Water quality and water treatment;
- Traffic control (to protect against cross contamination);
- Adequate lighting and lighting protection;
- Foreign material control;
- Product storage and distribution including a first-in-first-out shelf life system; and
- Pest control.

Prerequisite programs provide the basic environmental and operating conditions that are necessary for the production of safe and wholesome food. Prerequisites are clearly inked to integrated hygiene and food safety management systems (Surak & Wilson, 2007; FAO, 2004[1]). Prerequisite program requires that employees be trained in food safety practices and that wholesome meat and poultry products are produced under sanitary conditions (Keener, 2009; Surak & Wilson, 2007).

Common prerequisite programs may include (NACMCF, 1997):

- **Facilities:** located, constructed, and maintained according to sanitary design principles. There should be linear product flow and traffic control to minimize cross-contamination of the finished products.
- **Supplier Control:** Assure that the suppliers have in place effective GMP and food safety programs.
- **Specifications:** Written specifications for all ingredients, products, and packaging materials.
- **Production Equipment*:*** Constructed and installed according to sanitary design principles. Preventive maintenance and calibration schedules should be established and documented.
- **Cleaning and Sanitation:** Written and followed all procedures for cleaning and sanitation of the equipment and the facility. A master sanitation schedule in place.

- **Personal Hygiene:** All employees and other persons who enter the manufacturing plant should follow the requirements for personal hygiene.
- **Training:** All employees receive documented training in personal hygiene, GMP, cleaning and sanitation procedures and personal safety.
- **Chemical Control:** Documented procedures in place to ensure the segregation and proper use of nonfood chemicals in the plant, including cleaning chemicals, fumigants, and pesticides or baits used in or around the plant.
- **Receiving, Storage and Shipping:** All raw materials and products stored under sanitary conditions and the proper environmental conditions such as temperature and humidity to assure their safety and wholesomeness.
- **Traceability and Recall:** All raw materials and products lot-coded and a recall system in place so that rapid and complete traces and recalls can be done when product retrieval is necessary.
- **Pest Control:** Effective pest control in place.

The South African DVS (2007) highlighted personal hygiene and health of food handlers as one of the most important aspects to deliver a safe product of high quality to the consumer. Personal hygiene practices should prevent undue general contamination, and prevent cross-contamination with human pathogens that may cause FBD (CAC, 2005). Workers should be medically examined before employment in order to determine if they do not suffer from transmissible diseases, which can be transmitted through the food they handle to the consumer. Workers must be issued daily with clean clothes in a good condition in order to protect the food from contamination and also to protect them against potential dangers. Provision of water, soap, toilet paper, hand wash basins etc. are all basic hygiene requirements.

According to Zhao et al (2001) to diminish *Campylobacter*, *E. coli*, and *Salmonella* contamination rates in retail meats, it is critical that risk reduction strategies are used throughout the food chain. These strategies include on-farm practices that reduce pathogen carriage, increased hygiene at both slaughter and meat processing, and increased consumer education efforts (Larrison *et al*, 2010). Reducing pathogens before processing can be beneficial in that it may allow processing plant to lower the cost of production by decreasing the risk and cross-contamination.

Where GHP is inadequate, the initial objective of HACCP in SLDB's should be basic hygiene improvement. Hazard analysis can help focus on priority areas where improved hygiene is necessary. Application of GHP during the poultry processing helps to ensure that the contamination of broiler carcasses remains as low as possible (FAO, 2009). GHP represents the "foundation" that most food safety systems are built upon. Food borne disease outbreaks occur mainly due to failure in applying and enforcing the well-established sanitation and hygiene principles (Bilgili, 2010).

According to South African DVS (2007) the Hygiene Assessment System (HAS) is a nationally standardized evaluation system that quantifies the standard of hygiene management at abattoirs, i e, assess the hygiene status of the abattoir. The HAS is 'a pre-determined standard to which the manager or senior inspector can measure the hygiene standard at the abattoir, against essential national standards. Progressive improvement or deterioration can easily be monitored and corrective actions can be documented according to the marks scored.

The Hygiene Management System (HMS) is control measures or programs required to monitor identified control points, including the methods of monitoring or checking these control points. Management must provide documented Hygiene Control Programs (HCP) approved by the competent authority, to prevent, eliminate or reduce the hazards. Periodic auditing by state inspectors is essential in order to maintain set standards. The FAO/WHO (2003) and Newslow (2002) define audit as a systematic examination to determine whether what is actually happening complies with documented procedures.

Codex provides principles for the establishment and application of microbiological criteria for food. As defined by the Codex, a microbiological criterion for food defines the acceptability of a product or a food lot (batch), based on the absence or presence, or number of microorganisms, including parasites, and/or quantity of their toxin/metabolites, per unit(s) of mass, volume, area (CAC, 1997).

These microbiological criteria may be used for the examination of foods, including raw materials and ingredients, of unknown or uncertain origin, or when other means of verifying the efficacy of HACCP-based systems and GHP are not available. The microbiological criterion should be established and applied only where it is practical and where there is a definite need, with evidence that the food under consideration may represent a public health risk and that a criterion is meaningful for consumer protection.

The HACCP system should not be implemented until a food business is operating in accordance with GHPs and in compliance with appropriate food safety requirements (Keerner, 2009; FAO, 2004[1]). According to Newslow (2002) the success of the HACCP system depends on prerequisite programs that are both active and effective. Initial responsibility for HACCP lies within the food industry management and in addition governments are responsible for creating a scientific, technical and financial environment favorable to HACCP implementation, with specific consideration for SLDBs important contribution to the national food supply (FAO & WHO, 2006):

- Food must be produced in a hygienic manner.
- The source of incoming raw materials must be considered.
- A risk-based approach must be implemented to achieve food safety.

Figure 2.3: International organizations call for a production to processing approach that includes the applications of good hygienic practices, risk assessment, food safety objectives (FSO) and HACCP (Bilgili, 2010)

Food safety objective (FSO) defines the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP). Wherever possible and practical, competent authorities should formulate food safety objectives (FSOs) according to a risk-based approach so as to objectively express the level of hazards control that is required to meet public health goals (Bilgili, 2010; Larrison *et al,* 2010; CAC, 2007; CAC, 2005; WHO, 2000) (See Fig 2.3).

Improvements in the protection of public health rely on improvements in the safety of food. In this regard, governments, the food industry and consumers have a shared responsibility to adopt the best practices for the control of food safety hazards (FAO & WHO, 2006; Northcutt & Russel, 2003).

2.5 Using HACCP for poultry processing

The HACCP system is a more sophisticated food control system than GHP, which "identifies, evaluates, and controls hazards which are significant for food safety" (Keener, 2009; Northcutt & Russel, 2003; FAO & WHO, 1999). The HACCP system, as it applies to food safety management, uses the approach of controlling critical points in food handling to prevent food safety problems. The system, which is science-

based and systematic, identifies specific hazards and measures for their control to ensure the safety of food.

The FDA/USDA/NACMCF, (1997) defined HACCP as “a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product”. And Northcutt & Russel (2003) defined HACCP as “a system of extensive evaluation and control over an entire food production process for the sole purpose of reducing potential food-related health risks to consumers”.

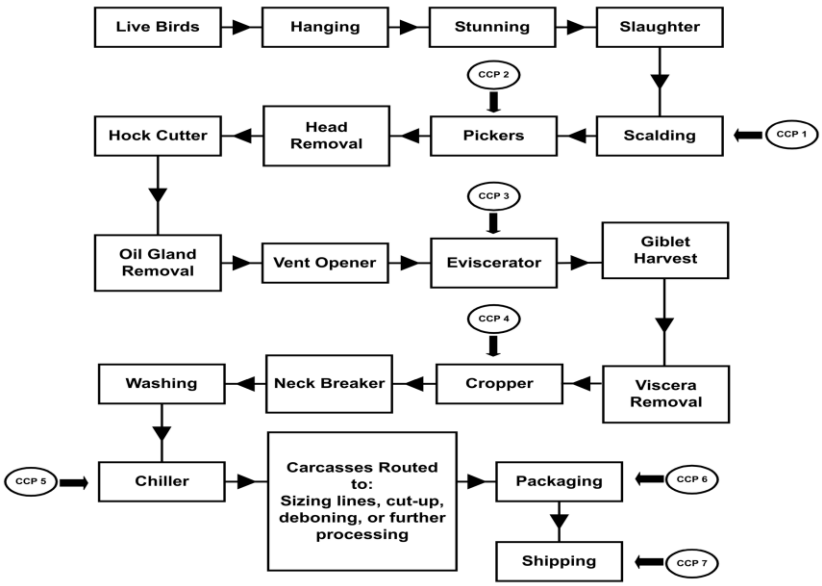
The principles of the HACCP system have been adopted by CAC and guidelines to its application are provided in an Annex to the General Principles of Food Hygiene. According the FAO & WHO (2006) the Codex committees recommend food safety standards in the role of risk managers, i.e. the Commission is the decision-making body of the Joint FAO/WHO Food Standards Program.

Participation in Codex and use of Codex standards assists policy-makers in building a sound national food control system to provide food of adequate quality and to protect the consumer (FAO & WHO, 2005). Within Codex, the Codex Committee on Food Hygiene (CCFH) is responsible for the elaboration of standards, guidelines and recommendations for the management of microbiological hazards in foods (FAO & WHO, 2008).

Buzby & Crutchfield (1997) cited USDA’s Economic Research Service (ERS) who worked with FSIS to estimate the potential savings in medical costs and lost productivity associated with the new meat and poultry inspection systems when they are fully implemented. The overall benefits of pathogen reduction will depend on how successful HACCP is in reducing pathogens and preventing new cases of food borne disease. Using the low estimate of medical and productivity costs, FSIS determined that if HACCP reduces these illnesses by 15 to 17 % then the benefits of HACCP outweigh the costs.

To improve product safety, the meat and poultry industries are adopting HACCP as an important process control system. The HACCP program maintains safety and wholesomeness of meat and poultry products because potential hazards that may occur during processing are anticipated, evaluated, controlled and prevented. The HACCP system provides clear benefits to food businesses, enhances the safety of food and reduces cases of food-borne disease; and provides a basis for defense against litigation and can bring reduced insurance costs (FAO & WHO, 2006). A hazard is defined as any biological, physical or chemical property that could cause a product to be unsafe for consumption (Keener, 2009).

Silverside & Jones (1992) appointed four areas in an abattoir as the most important to monitor cross-contamination of poultry carcasses by micro-organisms: the reception areas for the live birds, the scalding tank area, the de-feathering machine and the chilling tank. However, according the FAO (2009) the operations known to increase the contamination are scalding, plucking and evisceration. The feather plucker is the most important critical control point in the process in relation to contamination, but also evisceration can pose a big risk as a consequence of gut rupture.



CCP= Critical Control Points

Figure 2.4: Example of Poultry Processing HACCP Flow Diagram (Northcutt & Russel, 2003).

CHAPTER 3

MATERIALS AND METHODS

3.1 Background

Microbiological control requirements for foodstuffs should be firmly based on an understanding of the microbiological risks that need to be controlled (Lund *et al*, 2000). Poultry slaughter establishments are required to test carcasses for generic *E. coli* as a means of verifying if the carcasses are produced in a hygienic manner and using an adequately controlled process.

3.2 Indicator organisms

An indicator organism for foods is one that, if found in given products at specified levels, provides a warning that a safety or spoilage hazard either may exist or be imminent (Hui *et al*, 1994). Pathogens can be detected directly and reliably, by testing for them in preference to testing for indicator organisms. If a test for an indicator organism is applied, there should be a clear statement whether the test is used to indicate unsatisfactory hygienic practices or a health hazard (CAC, 1997). Coliformis, fecal coliformis and *E.coli* Type I have emerged as most useful safety microbial indicators.

3.2.1 *E. coli* type I / Coliform count

The group of fecal coliforms is defined by the production of acid and gas in *E. coli* broth, and for practical purposes a fecal coliform is *E. coli* type I (Hui *et al*, 1994). According WHO (2008); EPA (2003), the indicator organism of choice for faecal pollution is *E.coli.* Generic *E.coli* was selected because of the scientific consensus that it is an excellent indicator of fecal contamination, because the analysis is relatively easy and inexpensive to perform and the levels of *E.coli* contamination can be quantified.

3.2.2 Aerobic Plate counts

According the DFS (2002) the Aerobic Plate Count (APC) is used as an indicator of the level of bacteria in a food product and it does not measure the entire bacterial population, but rather the number of bacteria that grow in the presence of oxygen (aerobically) and at medium range (mesophilic) temperatures. The APC can be used to evaluate the sanitary condition of a food product or equipment. A high APC may be an indication of poor sanitation or of problems with process control or ingredients, but a low APC, is not a guarantee that the sample is free of pathogens.

APC's of perishable refrigerated foods such as poultry meat may reflect the microbiological condition of the raw food, the effectiveness of the processing method(s), the sanitary condition of equipment and utensils and the temperature/time profile of storage (DVS, 2007).

3.3 Model system for research

The method used was participatory risk analysis (Grace *et al*, 2008), including semi structured interviews using a check list with key informants, various focus group discussions with poultry value chain actors and an observational study of poultry production and processing, including photographs.

The magnitude of the risk of microbiological hazards of the final product was estimated using laboratory analysis, more specifically 3M™ Petrifilm™ Plates[1] (See Addendum 1, Interpretation guide for 3M Petrifilm : . *coli* / Coliform and Aerobic Plate Count) for *E. coli* Type I as indicators of fecal contamination, coliforms as indicators of environmental contamination and APC as indicators of contamination by spoilage organisms. The agents are indicators of contamination to assess the compliance with Good Hygiene Practices (GHP) and Good Manufacturing Procedures (GMP) that were considered relevant pre-requisites for HACCP. For this purpose, a total of 330 samples of poultry carcasses, water and swab from surfaces and hands of operatives were taken from formal abattoirs (n=110), live bird markets (n=110) and farms (n=110).

3.4 Experimental design and procedures

Key informants, part of the main players in the development of the smallholder poultry industry in Mozambique, involved in this study are:

- Head of Maputo Provincial Livestock Services.
- Head of National Poultry Association (AMA).
- Head of Maputo Poultry Association (ADAM).
- Staff member of Technoserve (American NGO).
- Staff member of Maputo Municipality (market section).
- Managers of the poultry abattoirs.
- Inspectors from the poultry abattoirs.
- Poultry processors.
- Poultry vendors.

Audit list for the formal abattoir was designed to gather information regarding hygiene, sanitation and management practices in place (See Addendum C). Study visits to the poultry farms and live bird markets for data collection

[1] Petrifilm is a Trade Mark of 3M, USA. TM 70-2008-4574-4 (1211) DPI. Http://www.3M.com/microbiology

using a checklist based on FAO guidelines for informal markets, and visual observation were carried out also for the informal processors in each unit visited.

To get participation of the producers and processors in risk analysis of the broiler food chain within their own marketing strategies, a workshop of focus group discussion were carried out, centred on following question:

- "What is the value chain for poultry and poultry products?"

The second workshop of focus group discussions centred on the preliminary results of the study. The participants raised and discussed the following issues:

- "How could the risk to consumer health be reduced"
- "What critical steps or procedures would prevent contamination of carcass or growth of bacteria?"
- "Which was the best way to improve food safety during processing among the three sectors?"

3.5 Microbiology

The following rapid test kits were used for microbiological testing:

- 3M™ Petrifilm™ *E. coli* / Coliform Count Plate (EC) 6414
- 3M™ Petrifilm™ Aerobic Count Plates 6406

The 3M™ Petrifilm™ Rapid Coliform /*E. coli* Count Plate is designed to provide a fast, accurate result in 4-24 hours. High coliform contamination can be detected beginning at four hours, with presumptive coliforms being recorded at 14 hours. Faster information means detecting process or contamination problems and being able to act on them to minimize the cost. The Petrifilm *E. coli* Count Plate is one method that gives results comparable to direct violet red bile agar (VRBA) plating and after incubation at 35°C for 24h, *E. coli* colonies appear in blue color and those that appear in red with evidence of gas production are enumerated as coliforms (Hui *et al*, 1994).

The main advantage of the Petrifilm™ Plate method is its simplicity of use and the fact that many samples can be taken in a short time. They are especially convenient for use where facilities for agar preparation do not exist (DVS, 2007).

3.6 Study area

The study area is represented by different poultry marketing systems around Maputo city and Maputo Province (See map in Figure 3.1). In Maputo 5,690,400 broilers were produced by the small-scale producers in 2008. According to the Maputo Poultry Association (ADAM) 403 members are registered as small-scale poultry producers.

Even in Maputo, which for the most part is urban, backyard poultry farming for home consumption is common, however since this study concentrated on contamination of chicken carcasses marketed through informal and formal poultry value chains, we excluded this type of farming.

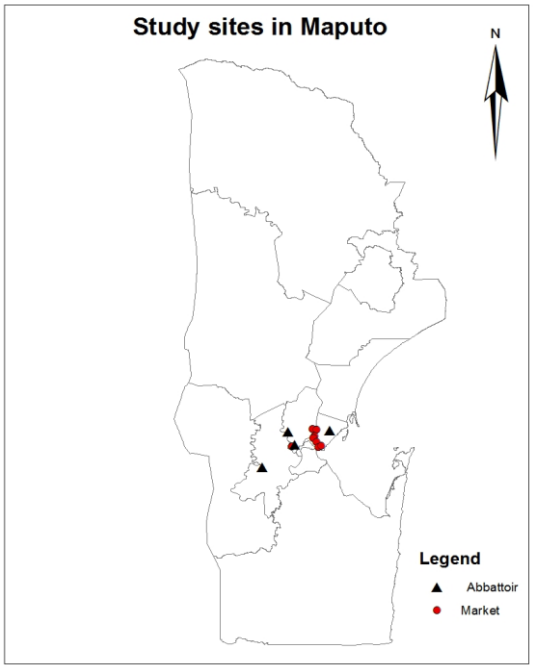


Figure 3.1: Map of Maputo showing poultry markets and abattoirs

3.7 Sampling frame

A sampling frame is the list of all units used from sample collection in the study area (Gummow, 2007). In this study the sampling units were classified into three groups and during the pilot phase of this project, all were counted

- poultry abattoirs (formal point of slaughter), n=4;
- live bird markets (market where birds are slaughtered informally on purchase) n=9; and
- poultry farms (informal point of slaughter), n=40.

All informal markets and three abattoirs were are supplied by poultry coming from small scale farmers many of them registered with the Maputo Poultry Association One abattoir was supplied by a large scale producer. All the farmers in the study were registered with the Provincial Livestock Services in Maputo. In Mozambique all livestock farmers must be registered in terms of the National Animal Health Act of 2008. The sampling frame for processors of poultry was discussed first and proportionate random sampling used to obtain samples (Thusfield, 2005).

Using the above information, it was decided that it was feasible to sample all the abattoirs and live markets. Only those farmers who did not supply abattoirs or markets but did slaughter on farm, were included in the sampling frame for proportional random sampling of carcases (Table 3.1).

Assuming that 51735 chickens corresponding to 190 farms are sold alive per week, the total number of chicken slaughtered per week at abattoirs, farms and markets is 57998 corresponding to 213 farms (Table 3.1). The estimated mean number of chickens produced per farmer per cycle is 2000.

Table 3.1: Fowls slaughtered per week per outlet

Processor category	Number of outlets	% of farms each value chain	Number of farms supplying each outlet	Total fouls slaughtered per week
Slaughter on farm	40	10	40	10943
Slaughter at abattoirs	4	40	161	43772
Slaughter at markets	9	3	12	3283
Sold alive	190	47	190	51735
TOTALS		100	403	109733

From the above table it can be seen that the majority of birds are sold alive to consumers who slaughter at home. However these birds did not form part of this study.

3.8 Microbiological sampling and analysis

3.8.1 Variables tested:

- Water hygiene level and farming system of poultry farm
- Microbiological level of slaughtered carcasses at abattoirs, live birds markets and on-farm vendors (including fresh, chilled and frozen)
- Microbiological level (sanitation) of the equipment and implements used during poultry processing and personal hygiene of the operators

3.8.2 Sample size

The sample size in the limited population was calculated using Episcope 2.0. The expected prevalence was 20% at 95% confidence interval and a precision of 5%. This resulted in a required sample size of 246

per target group or 82 samples per outlet. Considering expected loss to follow-up, the final sample size was increased to 110 per outlet.

In total 330 samples were randomly collected from;

- formal abattoirs (110);
- live bird markets (110); and
- farms (110)

3.9 Methods used for collection of samples

The microbiological samples were taken from carcasses of freshly slaughtered and frozen fowls according to the method described by OIE (2004). Samples were packaged, labelled and immediately transported on ice (cool box) to the laboratory:

- A sample of each carcass was collected as a whole carcass wrapped with the processor wrapping material (as it went to retail).

3.10 Laboratory testing

3.10.1 Sample preparation

Whole slaughtered chicken carcasses were brought to the laboratory in cooler bags, on ice at 4°C. A sample of about 25.00 mg was excised from the carcase using sterile precautions and placed in a marked, sterile Whirl-pac™ bag and 225.00 ml buffered saline was added to give a 1:10 dilution, according to method ISO 6887. Details can be found on the 3M website[2].The sample was then homogenised in a Seward Stomacher Lab-Blender 400 (Merck Pty., Ltd, South Africa)

3.10.2 Culture and evaluation

The following microbial monitoring tests were routinely done for poultry meat and water quality: *E. coli* Type I, Coliforms and Aerobic Plate Count. Fecal coliforms and *E. coli* are bacteria whose presence indicates that the water or the carcass may be contaminated with human or animal wastes. The methods for culture and evaluation of microbiological counts using 3 M Petrifilm are shown in Addendum 1. Plates 3.1, 3.2, and 3.3 illustrate the simplicity of the 3M System.

[2] Http://www.3M.com/microbiology

Plate 3.1 Inoculating Petrifilm

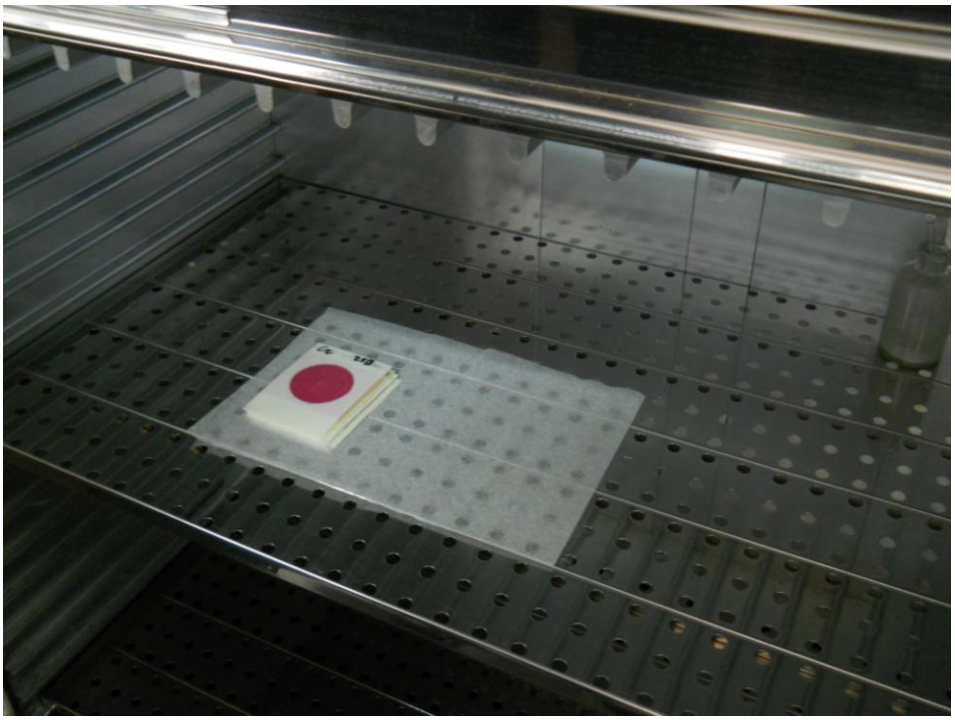


Plate 3.2 Incubating Petrifilms

3.10.3 Ethics

University of Pretoria ethical consent forms were signed by all key informants (n=26). The form is provided in Addendum B.

CHAPTER 4

RESULTS

4.1 Introduction

In Chapter 4, value chains for each category are described and critical points at which contamination could have occurred are highlighted, focusing on points of the food chain that pose highest risk. In addition the hygiene indicator microorganisms in the abattoirs, markets and farms were evaluated during the processing to identify potential CCPs and allow options for monitoring.

Figure 4.1 shows the food value chain as a flow diagram, for each type of processor in Maputo.

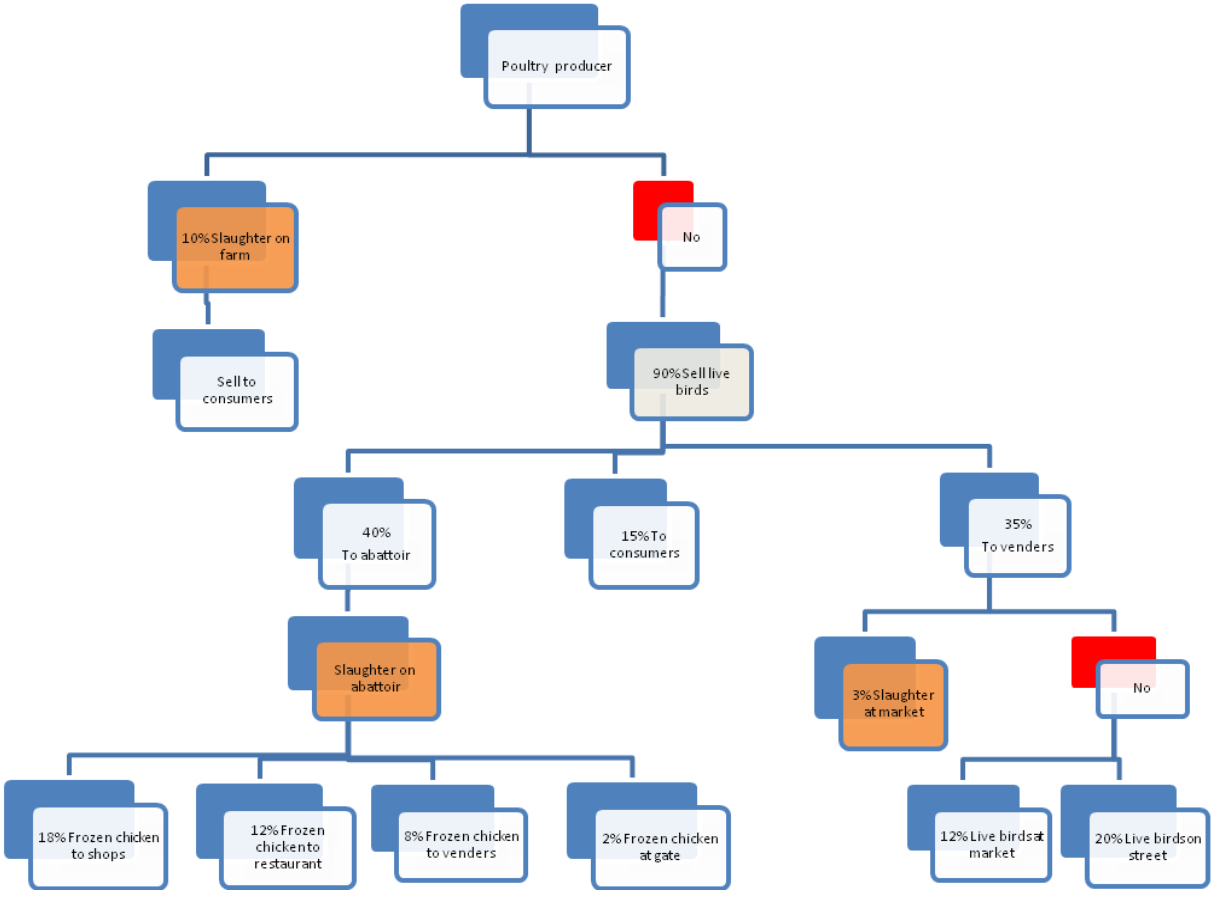


Figure 4.1: Flow diagram value chain for poultry meat in Maputo area based on interviews with a total of 26 key informants

The research showed the categories of processors where poultry are slaughtered for consumption, the proportion and frequency of slaughter in each category (See Table 3.1, Chapter 3):

- Slaughter at markets.
- Slaughter on farm.
- Slaughter at abattoirs.

4.2 Hygiene assessment at each category of outlet

Pictures were taken to document the hygiene situation of each outlet (See Plates 4.1 to 4.20) and are shown and described below.

4.2.1 Live bird markets (9)

See Plates 4.1 to 4.12.
The following problems which could lead to microbiological levels increasing in poultry meat were observed and recorded in the live markets:

- stressful transportation of the birds which can lead to disease or death (Plate 4.1);
- dirty crates where the birds remain for several days until sold (Plate 4.2);
- infrequent changing of the scalding and further processing water (Plate 4.4);
- general lack of hygiene in the live bird market environment (dust, poultry feces, feathers and rubbish)(Plates 4.3; 4.5; 4.6; 4.8; 4.9);
- cross contamination between live birds and slaughtered carcasses (Plates 4.8; 4.10; 4.11; 4.12)
- lack of fly control (Plate 4.7);
- dirty dishes and containers, not covered, used at point of sale (Plates 4.9; 4.10; 4.11)
- lack of ablutions (toilets and hand washing facilities)or hand disinfectants;
- lack of cooling facilities for carcass conservation (Plate 4.12);
- lack of access to clean water leading to use and re-use of dirty water (Plates 4.4; 4.5; 4.8; 4.10)
- handling of carcasses, money, live birds and food without washing hands;
- lack of protective clothing.

Of the total number of vendors (n=87, 82%) showed one or more of the above problems

4.2.2 Farms (n=22)

See plates 4.13 to 4.16.

The following problems which could lead to microbiological levels increasing in poultry meat were observed and recorded during slaughter of birds on farm:

- potable water for slaughtering, de-feathering, washing carcasses was not often present (Plate 4.13);
- lack of infrastructure and facilities, like tables, defeathering machines and storage facilities, for slaughter (Plates 4.14; 4.15)
- general lack of hygiene in slaughter area and during slaughter (Plates 4.15; 4.16)
- scalding water was not changed during processing;
- lack of/ inadequate ablutions (toilets and hand washing facilities) or hand disinfectant;
- handling of carcasses, money, live birds and food without washing hands; and
- lack of protective clothing (Plate 4.14).

4.2.3 Abattoirs (n=4)

See plates 4.17 to 4.20.

At abattoirs, the following steps in the slaughtering process were observed that could have a high risk for microbiological contamination of poultry meat. In line with HACCP, CCP's were assigned to these and are shown below:

- pickers (deficient sterilization of pickers and contaminants attached into skin) – CCP1 (Plate 4.19);
- evisceration (manual evisceration leads to gut breakage and deficient hand washing) – CCP2;
- washing (inadequate water pressure) – CCP3;
- chilling (deficient sterilization of the chiller and inadequate temperature control of water and air) – CCP4 (Plate 4.20);
- packaging (deficient sterilization of implements and equipments, deficient hand washing and injection of brine mix solution) – CCP5;
- deficient drainage of waste water during processing due to inappropriate drain ducts (Plate 4.18);
- deficient cleaning of protective clothing (Plate 4.17);
- inadequate store of packaging material.

4.3 Results of laboratory analysis

The results of microbiological tests on the carcasses sampled are shown below in Tables 4.1, 4.2 and 4.3. According to South African DoH the allowed limits proposed for *E.coli* in raw meat is <10/g.

Table 4.1: Results of *E. coli* Type I growth of the samples taken from different processors in Maputo area

Source	No. of samples	E. coli no growth		E. coli negative≤10		E.coli positive>10		E.coli TNTC*	
		No.	%	No.	%	No.	%	No.	%
Abattoir	107	30	28	29	27	35	33	6	6
Market	106	12	11	27	25	66	62	1	1
Farm	109	23	21	42	39	43	39	1	1

*** TNTC:** too numerous to count

Figure 4.2 shows the results of a qualitative risk assessment for each category of slaughter, based on the levels of *E. coli* Type 1, which is an indicator of human or animal faecal contamination during processing.

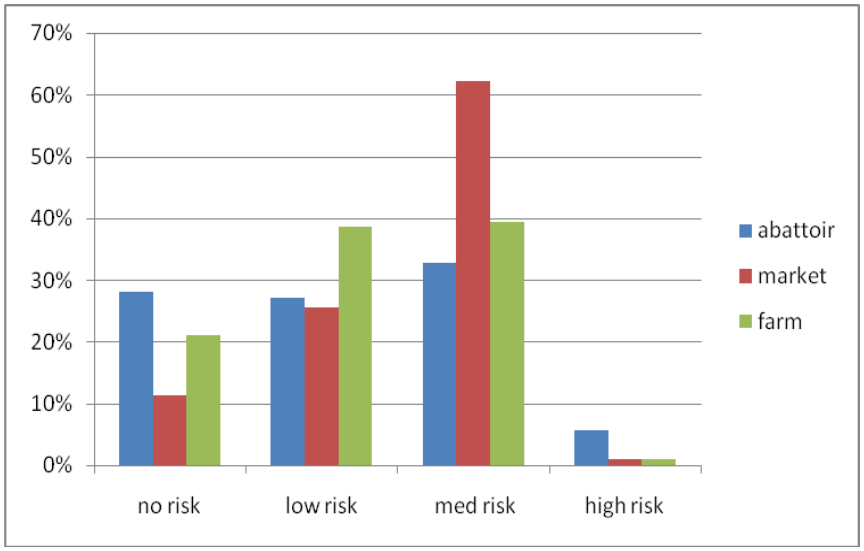


Figure 4.2: Bar chart comparing qualitative risk assessment for presence of *E. coli* Type 1 in carcasses in the abattoirs, live markets and farms investigated

Low numbers of coliforms are usually permitted in sensitive foods at numbers ranging from 1 to not exceeding 100/g or ml (SA Department of Health, 1997).

Table 4.2 below shows the relative frequency of coliform organisms in carcasses sampled (which are indicators of non-potable or dirty water spreading the organisms, or lack of cold chain resulting in growth of coliform spoilage organisms).

Table 4.2: Coliforms in samples taken carcasses from abattoirs, markets and farms in the Maputo area.

Source	No. of samples	Coliforms no growth		Coliforms negative≤100		Coliforms positive>100		Coliforms TNTC	
		No.	%	No.	%	No.	%	No.	%
Abattoir	107	11	10%	53	50%	19	18%	24	23%
Market	106	4	4%	95	90%	4	4%	2	2%
Farm	109	15	14%	62	57%	19	17%	4	4%

Figure 4.3 shows the results of a qualitative risk assessment for each category of slaughter, based on the levels of coliforms.

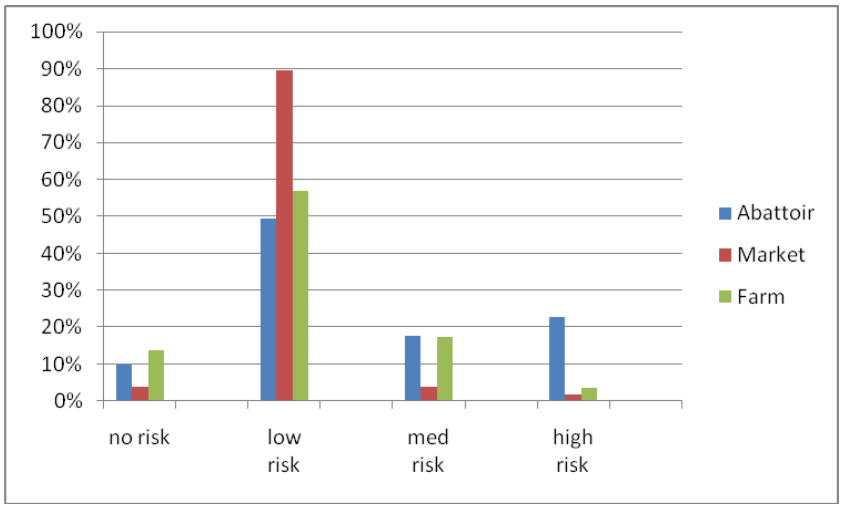


Figure 4.3: Bar chart comparing qualitative risk assessment for presence of Coliforms in carcasses in the abattoirs, live markets and farms investigated

According to South African DVS in VPN/15/2007-1 the maximum allowable levels for APC in chilled export meat is 10,000/g. The maximum allowable level for APC defined by the Ministry of Health of Mozambique for poultry meat is 1,000,000/g.

The low risk at the markets (90%) is probably because they use very little water as Coliforms are usually carried and spread by non-potable or dirty water. At one abattoir, an unacceptable level of Coliform count (TNTC) and APC (+ve) was found in samples of the brine solutions (n=4) and this was therefore made a

CCP. Table 4.3 below shows the APC (which are indicators of environmental contamination or lack of cold chain resulting in growth of spoilage organisms) in carcass samples

Table 4.4: APC in carcasses from abattoirs, markets and farms

Source	No. of samples	APC no growth		APC negative≤10.000		APC positive>10.000		APC TNTC	
		No.	%	No.	%	No.	%	No.	%
Abattoir	100	8	8%	28	28%	0		64	64%
Market	100	2	2%	11	11%	0		87	87%
Farm	100	2	2%	54	54%	0		44	44%

For APC, 10 out of 110 cultures were discarded due to being grossly overgrown by contaminants and thus could not be read.

4.4 Comparison of relative frequency of contamination between the three outlet types

It can be seen from Tables 4.1, 4.2 and 4.3 that the relative frequency of APC, coliforms and *E coli* counts differed between outlets:

- In samples from the abattoir, 64% (64 of 100 samples) were APC positive, while 40.19% (43/107) were positive for coliforms and 38.32% (41/107) were positive for *E. coli* Type 1.
- In those from live markets, 87% (87/100) were positive on APC, 5.66% (6 out of 107) positive for coliforms and 63.2% (67/106) were positive for *E. coli* Type 1.
- Samples from birds slaughtered on farm showed that 44% (44/100) were positive on APC, 21.1% (23/109) positive for coliforms and 40.37% (44/109) were positive for *E. coli*.

Figure 4.4 shows the comparison of relative frequency of bacteria counts between the three outlet types, based on the percentage of positives of APC, coliforms and *E. coli* Type 1.

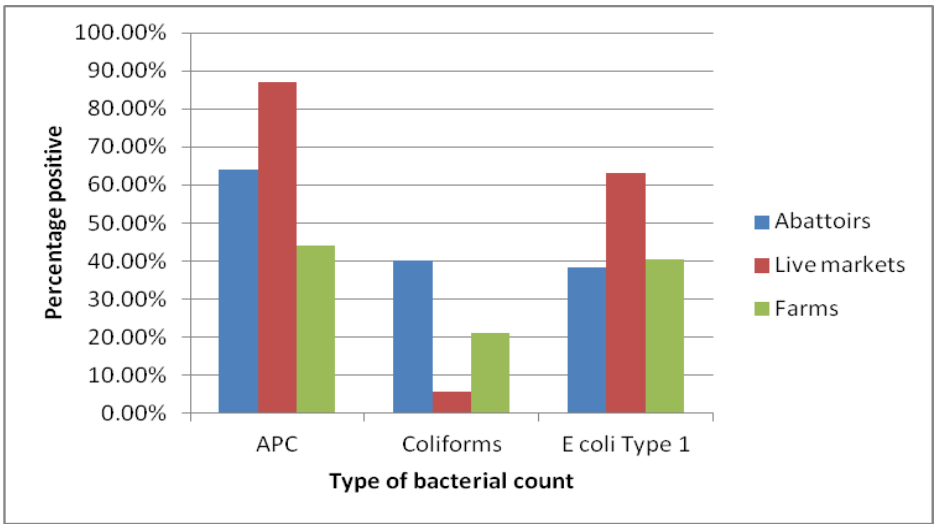


Figure 4.4: Comparison of relative frequency of bacteria counts between the three outlet types

4.5 Magnitude of risk to consumer for different outlets

During risk assessment, the magnitude of the risk to consumers needs to be measured. Of the three bacterial counts that are most likely to prejudice food safety rather than quality, the level of *E. coli* Type 1 is most important as it indicates faecal contamination. Other organisms of concern that are present in human and animal faeces include *Salmonella* spp and *Campylobacter* in poultry faeces, while *Vibrio cholerae*, *Salmonella typhi* and viruses such as Hepatitis A , B and C, as well as, Helminthes such as *Ascaris*, can be found in food contaminated with human faeces.

The estimated number of chicken carcasses per week contaminated with *E. coli* Type 1, entering the market in Maputo, thus possibly compromising the health of consumers is shown per outlet in Table 4.4.

Table 4.4: Estimated numbers of faecally contaminated carcasses being sold to consumers in Maputo weekly

Outlet type	Carcasses/week	Percentage positive for *E. coli* Type 1	Frequency of contaminated carcasses
Farms	10943	40.37	4417
Abattoirs	43772	38.32	16773
Market	3283	63.20	2074

The magnitude of the risk for a single consumer can be estimated from market segmentation, based on Table 3.1 (Chapter 3) which shows the estimated number of fowls slaughtered per week per outlet type (markets, farms and abattoirs) in Maputo.

Thus, although the highest proportion of contaminated carcasses is from the live markets, the largest number of contaminated poultry carcasses comes from the abattoirs. This is why high throughput abattoirs must have good HAS in place.

4.6 Focus group discussions

4.6.1 Initial focus group discussion

The workshop using focus group discussion on mitigation strategies to reduce food safety risks in the poultry industry in Mozambique, involving representatives of poultry industry, veterinary authority and academics, discussed the question “What is the value chain for poultry and poultry products?” and and summarizing the opinions suggested:

- Development of poultry production value chain based on information provided by Key informants and field visits

4.6.2 Second focus group discussion

After the microbiological results were obtained there was feedback to the focus group at a workshop. The following were suggested by the participants to respond the questions:

“How could the risk to consumer health be reduced?”

- It appeared from the results that all three outlets were a risk to human health and a radical change should be made in the approach of authorities, vendors and consumers.

“What critical steps or procedures would prevent contamination of carcass or growth of bacteria?”

- On-going training in Good Hygiene Practice (GHP) including the sanitary measures along the chain of production and poultry processing.
- Public awareness on food safety to educate the consumers in avoiding poultry that is slaughtered at informal markets.

"Which was best way to improve food safety during processing among the three sectors?"

- Reinforce the role of the inspection services and collaboration of inspection authorities with the poultry industry association to improve the quality of final products. Inspection should aim to detect and remove hazards of human health significance from poultry meat.
- Make available a set of Standards containing a code of practices (Good Agricultural Practice - GAP and GHP) in terms of poultry production and processing and the systems such as HMS and HAS to assure the quality of the final product.

4.7 Photographs at outlets

Plates 4.1 to 4.12 show photographs of possible CCPs in markets, Plates 4.13 to 4.16 show photographs of possible CCPs on farm and Plates 4.17 to 4.20 show photographs of possible CCPs in abattoirs in Maputo.

Plate 4.1: Dead on arrival

Plate 4.2: Unhygienic cages

Plate 4.3: Bleeding in dirty environment

Plate 4.4: Dirty pan for scalding

Plate 4.5: Water and environment polluted

Plate 4.6: Defeathering in a dirty place

Plate 4.7: Flies on carcasses

Plate 4.8: Evisceration in dirty water and polluted environment

Plate 4.9: Carcasses in a dirty dish and ready to sell

Plate 4.10: Carcasses on the chicken cages and ready to sell

Plate 4.11: Carcasses at ambient temperature waiting for clientele and exposed to dust

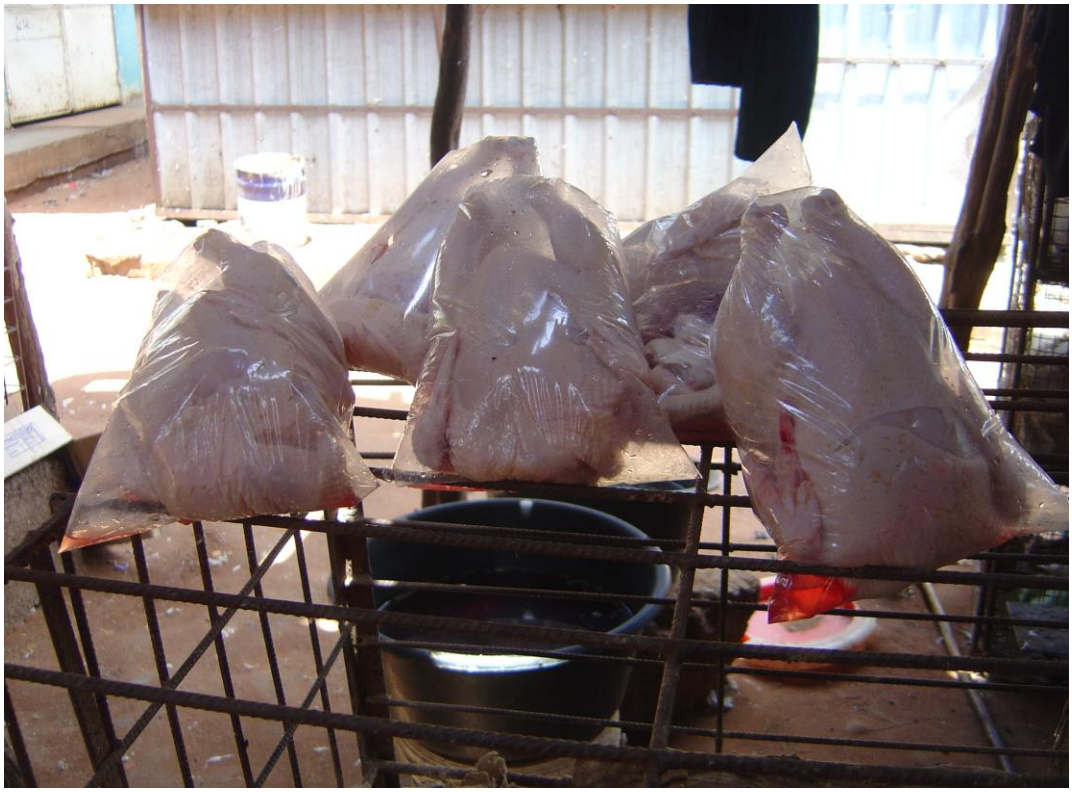


Plate 4.12: Carcasses at ambient temperature waiting for clientele

Plate 4.13: Well water used for poultry processing at farm

Plate 4.14: Defeathering on the ground

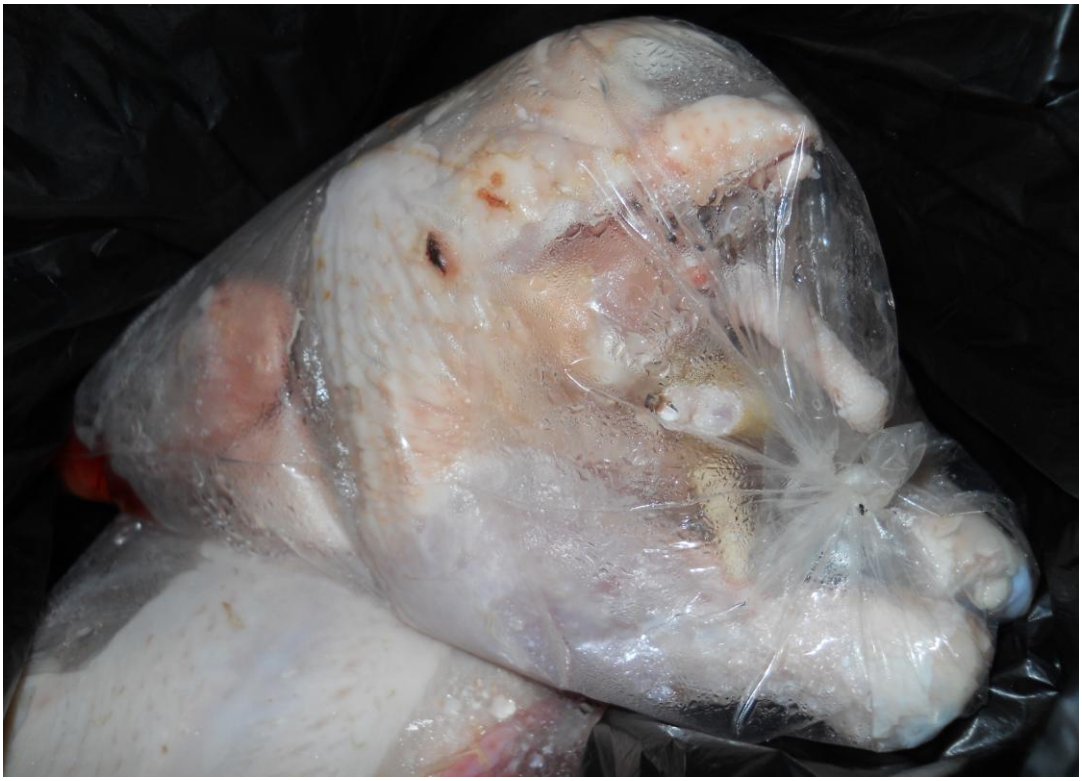


Plate 4.15: Packaged carcasses with feces on-farm

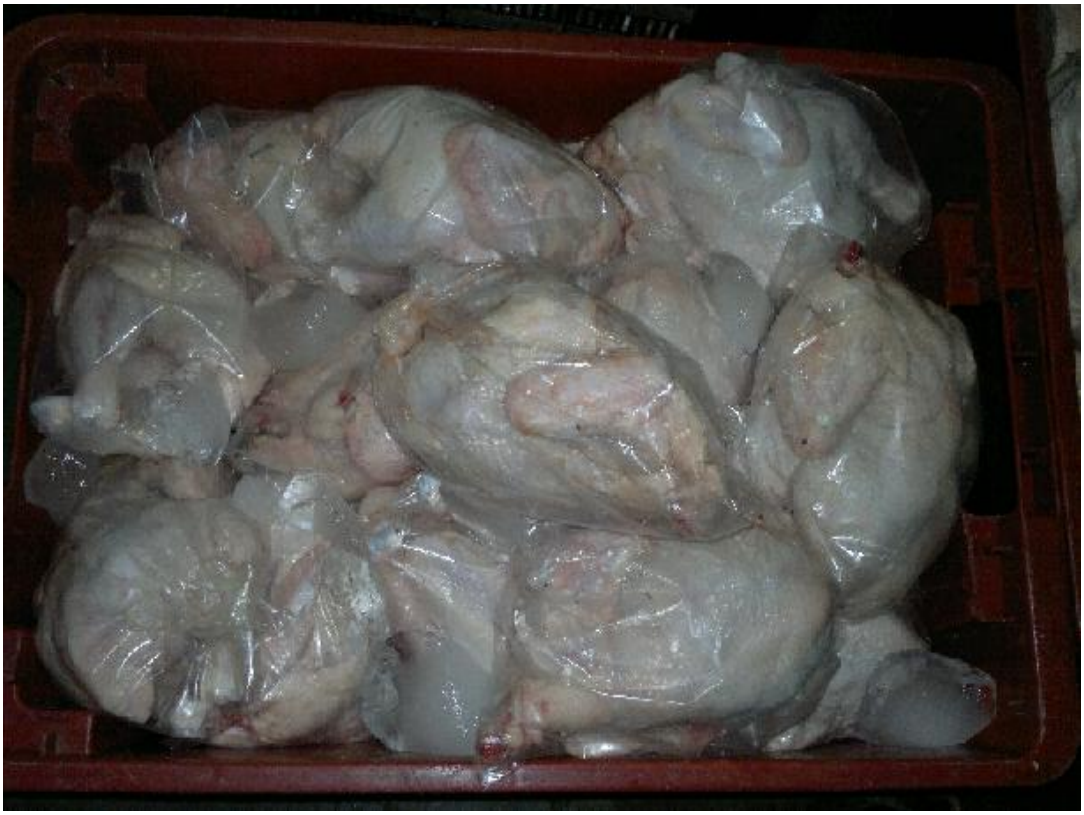


Plate 4.16: Carcasses stored in a dirty cage

Plate 4.17: Dirty personal clothing at abattoir

Plate 4.18: Deficient drainage of waste water and feces

Plate 4.19: Carcasses with feces and feathers in the chilling room

Plate 4.20: Deficient cleaning of chilling tank

CHAPTER 5

DISCUSSION

5.1 Introduction

In Chapter 4 the results were presented and further interpretation and explanation will be presented below in Chapter 5.

5.2 Informal slaughter at markets and farms

At the markets and farms, hygiene systems were not well formulated or observed. The most serious problems requiring attention were:

- Lack of infrastructure compromising basic hygiene principles.
- Environmental pollution: dust, dirt, faeces, feathers and flies in slaughter area.
- Cross contamination between live birds and slaughtered carcasses
- Lack of waste and effluent management
- Poor personal hygiene.
- Poor water quality.
- Lack of traceability.

5.2.1 Lack of infrastructure for processing and environmental safety

The high level of *E.coli* Type I found in fowls from live bird markets was probably due to lack of processing infrastructure. The birds remained for several days in dirty cages until slaughtered. In the markets, inadequate waste management, inappropriate scalding procedures, absence of cooling facilities for carcass conservation and inadequate transport of live birds were observed. At the farms the main problems observed were inadequate waste management, inappropriate scalding procedures and inappropriate or lack of cooling facilities for carcass conservation.

Inadequate refuse disposal facilities led to the accumulation of refuse at food vending sites, leading to an increased pest population that would result in an increased risk of food contamination (FAO/WHO, 2005). All waste should be handled and removed in such a way as to prevent the contamination of food, water and environment (FAO, 2009). According to Bilgili (2010) fecal contamination of the internal and external surfaces of poultry carcass represents the most important cause of microbial contamination during

slaughter. This is in line with our findings. According to the FAO (2009), fresh meat is very perishable and any delay in transport and distribution requires refrigeration in the short term. Meat prepared and kept too long before sale, at ambient temperature, gives bacteria time to grow to unacceptable levels. The ideal temperature for microbial growth is between 10 and 60°C. In all markets visited (n=9,100%) and 15 of the 22 farms (68%), there were no cooling facilities for carcass conservation. Poultry carcasses were kept for up to six hours, at ambient temperatures waiting for clientele to purchase them. In Maputo, according to the BBC Weather Website[1], the ambient summer maximum temperature ranges from 27-41°C in summer and even in winter it is often above 20°C. This climate is favorable for carcass deterioration due to spoilage bacteria.

In line with norms for slaughter of poultry, HMS and HAS need to be developed. In informal markets, these will obviously be less sophisticated than those in a commercial abattoir. However, in line with what is possible; the following categories could be proposed as a minimum requirement to improve infrastructure:

- Delivery area and holding pen.
- Slaughter and bleeding area.
- Processing area and inspection area (remove feathers, heads, feet and offal).
- Disposal area (intestinal waste, blood, feathers, dirty water and condemned material).
- Packaging and dispatch.
- Storage (cold chain).

It may be very difficult to achieve all these steps within an informal market, without compromising the health of both buyers and vendors, including other stall owners and vendors. It may be better to have a small scale abattoir which is located not far from the market and sells directly to the public, or a small “butchery” within the market, where freshly slaughtered birds are brought in daily or even more frequently and kept cool , either in a refrigerator or in insulated freezer boxes with sealed ice-packs.

A small scale three room abattoir can also easily be built on a farm. Another option is that birds are slaughtered and hung overnight to “chill” in a chiller at 7°C, then packed in smaller freezer boxes on the back of a pickup, which delivers them to the vendor stalls early in the morning and then goes to pick up birds for slaughter from farmers. These options will not only provide safer meat, but also take into account the dangers of polluted waste and wastewater, as well as flies, rats and birds, within a crowded informal market.

[1] Http://www. bbc.co.uk/weather/

Mobile slaughter facilities designed for on-farm use (Plate 5.1 and 5.2) would be more appropriate for countries lacking infrastructure or centralized slaughter and processing facilities. These could minimize health risks to consumers and increase product quality through providing processing infrastructure to small scale producers. Successful use could improve bird welfare and the quality of carcasses (Mead, 2004).

Plates 5.1 and **5.2:** Mobile slaughter facilities designed for on-farm use

It is difficult to change perceptions about culture being linked to informal slaughter of fowls. If a housewife kills one or two backyard fowls a week, there is no problem with waste disposal as the amount is minimal. However, when more fowls are slaughtered, at least an elementary hygiene management and assessment system should be present. Table 5.1 below suggests feasible steps in hygiene management and assessment linked to the above categories in informal processing.

In the circumstances of informal trading, it is possible to use “consumer power” to improve hygiene. If a small stall is selling chicken carcasses or pieces that have a better shelf life at the same price, it will attract consumers, especially if it looks neat and clean.

With regard to ante-mortem inspection of fowls at a small abattoir, if the abattoir owner is purchasing these birds he or she can refuse to pay for injured or sick birds. If the abattoir is running as an independent slaughter facility for vendors, who pay a fee to have birds slaughtered and packaged, the vendor will tend to only bring healthy birds in many cases as an unhealthy bird costs the same amount of money to slaughter as a healthy bird and they may not be able to sell a diseased carcass. It is thus proposed that education, training and feedback to vendors and consumers would, in the beginning, be much more successful than legislation. Another important principle is that human beings will always follow the easiest pathway. If it is made easy for them to dispose of waste in a hygienic fashion, this will be

successful. So some pathway must be designed for successful waste disposal by vendors or small scale abattoirs that are appropriate to the situation.

Table 5.1: Suggested HAS steps for informal processing of poultry in Maputo

Category	HAS steps	Implementation through
Delivery area and holding pen	Ante-mortem inspection of all birds Vehicle washed Poultry crates washed	Education and training Consumer power Marketing
Slaughter and bleeding area	Hygienic slaughter and good exsanguinations	Education on improved quality of carcass: Consumer power
Processing area and inspection area (remove feathers, heads, feet, offal) : hygiene focus	Scalding procedures-clean water De-feathering Rinsing process Evisceration Pos-mortem inspection	Education and training on hygienic surroundings, clean water removal of wastewater and feathers using simple, appropriate methods
Disposal area (intestinal waste, blood, feathers, dirty water and condemned material)	Waste storage container Vermin control Entrance kept closed	Finding a waste disposal pathway that does not compromise hygiene, the environment or underground water
Packaging and Storage (cold chain)	Wash basin, soap and paper towel available Packaging material available Cold store management Clean packing tables, walls and floors	Education and training about hygiene Provide appropriate ablutions: hygienic washbasins and toilets close to markets and abattoirs.

In addition a HAS system checklist, based on that used by the South African National Department of Agriculture has been developed and translated into Portuguese, for use in the poultry abattoirs in Mozambique (See Addendum C).

5.2.2 Poor personal hygiene

One of the clear risk factors in the informal market is ignorance of the causes of FBD and the risks to public health that are exacerbated by poor hygiene, inadequate access to clean water and unsanitary surroundings. Facilities and equipment for personal hygiene should be provided, designed and located so that meat safety is not compromised (CAC, 2005[1]). The basic personal hygiene of the vendor is essential.

He should wash his hands after handling dirty material or after going to the toilet (FAO, 2009). During the observational visits to the markets and farms deficient or lack of ablutions (toilets and hand washing facilities) were noted. Scalding and washing water for carcasses were not renewed, important factors contributing to carcass contamination by fecal hazards. The cleanliness of the vendor's clothing is an important measure of hygiene and a good indicator of attention to hygiene and cleanliness (FAO, 2009). In the markets there were no facilities for showering or changing clothes, the vendors wore the same clothes they used to go to market and back home.

Street foods in some African countries have been tested for various microorganisms of public health concern, including fecal coliforms, *E. coli* and *Salmonella* spp. In Harare *E. coli* were recovered in a significant proportion of the food, water, hands and surface swabs tested (FAO/WHO, 2005). These foods were generally prepared and sold under unhygienic conditions, with limited access to safe water, sanitary services, or garbage disposal facilities (FAO/WHO, 2003). *E. coli* have been shown to survive in contaminated soil for up to 20 months, remaining an environmental contaminant for a prolonged period of time (FAO, 2011).

Appropriate personal hygiene facilities are needed to improve personal hygiene and minimize cross-contamination of meat (CAC, 2005[1]):

- changing facilities;
- toilets with hand washing basins and soap;
- potable water and paper towels; and
- protective clothing that can be effectively cleaned.

The availability and functionality of the above hygiene facilities in informal markets is a challenging goal, due to the nature of the vending site and the absence of information about food contamination for the vendors. The perception of the consumer on food safety is also limited; they continue buying even if the vendor doesn't wash the hands or if the carcass is in a dirty environment, without adequate protection from flies and dust. Slaughter of poultry in suitable surroundings by hygienic methods is essential for the production of wholesome meat, extension of the shelf life of the product and protection of public health (Silverside & Jones, 1992). Furthermore, public awareness on food safety must be carried out nationwide, to inform the consumer about the threats that the contaminated food represent for human health.

5.2.3 Poor water quality

Water is often of unknown or neglected sanitary quality and not always available in sufficient quantity. Good hygiene requires abundant use of water for the frequent washing of hands, dishes, premises and

raw materials. Adequate facilities should be provided for distribution of potable water and for the disposal of waste water (CAC, 2005[1]). Both in markets and farms the water for scalding and washing up is used several times over and ends up with an excessive physical and microbial load that makes the washing useless. During informal slaughter at farms some processors use borehole water of unknown quality for producing and processing purposes. The wells lie close to the surface of the ground, are poorly protected and exposed to biological contamination. Only water meeting WHO standards (potable water) should be used when handling and processing food. Potable water is defined as *"water that does not contain a pathogenic or chemical agent in concentrations that can be harmful to health"* (WHO, 2008).

Potable water should be used in food processing whenever necessary to avoid contamination. Water is a major source of potential contamination of street food in Africa, either because of poor quality at origin or because subsequently dirtied and misused. Chlorine based disinfectants are most commonly used to destroy bacteria in water worldwide, using a process known as chlorination. In Mozambique, all municipal water is chlorinated.

5.2.4 Unknown source of raw materials and traceability of fowls

The source of raw materials will determine the sanitary quality of end product. Poor quality of raw materials can cause various forms of food contamination, including food poisoning; microorganisms in food can be the result of contamination of the raw materials used for food preparation (FAO, 2009).

In the markets the poultry venders seldom know about the conditions of poultry production and transport to the vending sites. The majority has never visited suppliers; they only interact with the intermediate trader, who is usually only the transporter. International norms for transport are not followed, thus exposing the fowls to unnecessary stress and injury (Plate 4.1). On- farm processors hold crated birds under shade and there is no need for transport in vehicles.

Fowls should be handled in such a way that they are subjected to minimum stress prior to transportation and are fit to travel with minimum risk of injuries (FAO, 2004[1]). The transport of slaughter animals should be carried out in a manner that does not have an adverse impact on the safety and quality of meat (CAC, 2005[1]) and crowding of birds in crates is an animal welfare issue (Fanatico, 2003).

Traceability is not possible at the markets because the vendors do not know the poultry producer and they also mix fowls from different flocks or suppliers in the crates.

According to the OIE (2011)[1] animal identification and animal traceability systems should be integrated in order to be able to trace slaughtered animals back to their place of origin, and products derived from them

forward through the meat production chain. FAO (2004[1]) recognizes animal traceability as a marketing tool to enable consumers to be certain that purchased food items originate from production practices that are safe and morally acceptable. The identification of animals must be linked to the identification of carcasses so that tracing is possible from the meat to the animal or group of animals from which it was derived (FAO, 2004[1]).

5.3 Abattoirs- formal slaughter

Practices that were observed to be conducive to poor hygiene or a risk to food safety at abattoirs are discussed in more detail below. In general the response to these deficiencies would have to be linked to legislated norms, if the abattoir operator has been made aware of GMP, HMS and HAS in the poultry production cycle. The approach is different to that used in informal production and processing of poultry, but can still be constructive and proactive. The official veterinarian is internationally recognized as the person responsible for monitoring compliance at abattoirs (OIE, 2012). However, as the industry grows, it will be necessary to make sure that the role and powers of the state veterinary services in Mozambique are recognized by the poultry industry.

5.3.1 Deficient cleaning and disinfection of equipment

The equipment and utensils used for poultry processing should be adequately cleaned and disinfected to avoid the contamination of poultry carcasses. Inadequate disinfection procedures can allow pathogens being transferred from contaminated processing equipment to the carcasses. This situation was observed in 3 out of 4 abattoirs visited where pickers, chiller spin and crates used for carcass transportation before packaging were not satisfactory cleaned and samples collected from the surfaces of these equipments registered a high level of bacterial contamination. According to FAO/WHO (2009[2]) the feather plucker is the most important critical control point in the process in relation to contamination.

5.3.2 Inadequate carcass washing

According FAO/WHO (2009[2]) the application of GHP during processing helps to ensure that the contamination of broiler carcasses remains as low as possible. Essential practices include the removal of faecal matter, feathers, etc., from carcasses. On mechanical lines the carcasses goes through an inside/ outside washer with cold water containing a approved bactericidal levels that washes the carcasses under mild pressure. On manual lines the carcass is usually washed with a shower type sprayer. If chlorine is used, the concentration must be 5 p.p.m (South African DVS, 2007).

The step that normally should ensure the reduction of bacteria loads to acceptable level is not done properly, due to lack of water pressure for washing carcasses and sufficient bacteria remain to cause problems. In carcasses considered ready to markets at the abattoir, some were found to be very contaminated with feces due deficient washing. Carcass contamination is preventable by using good evisceration techniques. If a contaminated carcass is found it should be removed from the line and well washed, if necessary using a chlorine solution.

5.3.3 Inadequate chilling temperature

During the observational visits at two abattoirs in the study area, it was observed that the temperature of water and the ambient temperature in the chiller room was the same as the ambient temperature outside. In the first abattoir chilling was done through chiller spin and in the second through airflow. However, neither of these chilling systems had worked for at least two weeks, although the abattoirs continued processing fowls. According to DVS (2007), the chiller temperature must not be more than 4 °C. and the water used in the spin chiller must have a chorine concentration of not less than 50 p.p.m. The chilling can be considered as an important CCP. Rodrigues (2008) obtained results indicating that this procedure was able to significantly reduce the microbiological contamination on carcasses.

5.3.4 Poor personal hygiene

Appropriate ablution facilities should be available to ensure that an appropriate degree of personal hygiene is in place to minimize possible carcass contamination, in particular with faecally derived *E coli*. In one of the four abattoirs investigated, showers were found without soap and running water for at least 2 weeks and the protective clothing used by abattoir workers were also dirty (Plate 4.17). In another abattoir there was no soap for washing hands, in the entrance of processing rooms. It is essential that all abattoir workers should be provided with clean protective clothing and basins for washing their hands during and between operations (FAO, 2004[1]).

According to FAO (2004[1]) persons who come into direct or indirect contact with edible parts of animals or meat should:

- maintain an appropriate standard of personal cleanliness;
- wear protective clothing appropriate to the circumstances;
- immediately wash and sanitize hands when there has been contact with abnormal animal parts that are likely to harbour food-borne pathogens.

5.3.5 Poor management procedures

Injection of brine mix solution into poultry carcasses to induce “plumping” increases the possibility of contamination by transferring microorganisms in the solution being injected. At one abattoir where this process was adopted, the injection was made after chilling, thus increasing the possibility of raising the carcass temperature before packaging. Manual evisceration used by the same abattoir led to gut breakage, resulting in high levels of fecal contamination (4.19). Another poor management practice seen at this abattoir was the lack of appropriate waste water drainage (Plate 4.18).

5.4 Correlation between observational findings and proportion of contaminated carcasses per outlets

As samples from all outlets exceeded the acceptable levels, it is obvious that hygiene and possibly food safety are compromised during processing of poultry meat. Usually high APC indicates spoilage bacteria are proliferating and this is related to time and temperature. According to DVS (2007), an important factor influencing the generation time of bacteria is temperature. The use of refrigeration to lengthen the generation time of unwanted bacteria (e.g. spoilage bacteria) and therefore slow down their multiplication is the most important method of extending the shelf-life of the meat.

According to CAC (2005[1]) in the absence of suitable temperature, humidity and other environmental controls, meat is particularly vulnerable to survival and growth of pathogens and spoilage micro-organisms. It is thus not surprising that the relative frequency is highest in the live market where birds are stored at high ambient temperatures and there is no cold chain. High coliforms are often related to water that is not clean and this may be lower in the live markets as little water is used to wash birds .The *E.coli* Type 1 is related to fecal contamination and this may result from the lack of ablutions in live markets, as well as, the fact that fowls are very soiled with feces when they are piled one above the other in cages.

CHAPTER 6

CONCLUSIONS AND RECOMENADATIONS

6.1 Introduction

The hypothesis of this study stated that risk to consumers from consuming poultry meat can be assessed, using participatory risk analysis and an integrated food chain approach to describe prerequisites and an appropriate hygiene assessment system designed for both formal and informal points of slaughter in the poultry industry in Maputo.

The ultimate aim was to identify and characterize risks to the safety and quality of poultry meat by investigating the value chains and building flow charts for the informal production and processing of poultry. In the sections below, conclusions will be presented on whether this was achieved and recommendations made on the way forward to achieve a better level of food safety through improving pre-requisites at all levels of the poultry production cycle in Maputo.

6.2 Value chains and proportion in each

This study investigated and described the value chains for small scale poultry production in Maputo, indicating possible stages at which there was a risk of a hazard that could influence the final product and estimating the magnitude of this risk by using microbiological risk assessment in poultry meat. It included both the informal and formal producers and processors.

The flow diagram for the value chain has shown the categories of processors where poultry are slaughtered for consumption in Maputo area. In the study area it was estimated that only 40% of total poultry production was processed in the formal abattoirs (AMA, 2009). The remaining 47% were sold live and 13% processed by informal processors at point of sale if the customer asked for the fowl to be slaughtered. This is the first time the market segments of the poultry industry have been described for Mozambique and linking this study to risk analysis means that the situation can be managed to reduce risk.

Considering that is the first study in risk analysis in poultry processing in Mozambique, the results are of importance to improve the quality and safety of poultry meat and can possibly be extrapolated to other

parts of Mozambique, although abattoirs are only found in urban areas, so it is likely that elsewhere there will be a much higher proportion of live bird markets and small scale farmers that slaughter fowls informally.

6.3 Nature and magnitude of risk

The quality of the poultry carcass collected from the three sectors was not satisfactory. Poultry meat from formal abattoirs was not found to be much safer than meat purchased at markets and farms using informal slaughtering processes. To improve prerequisites, HMS and HAS, using an appropriate audit system tailored to the type of processing (ie formal or informal) are proposed for all three value chains with a focus on critical control points.

The presence of *E.coli* might indicate the presence of intestinal pathogenic organisms (*Salmonella* and *Campylobacter*) that in other countries have been shown to be the major causes of FBD. Some strains of *E.coli* can cause severe and life-threatening diarrhea, but in addition, the presence of the fecal derived organisms could be an indicator for even more serious diseases such as typhoid and viral hepatitis. Such diseases affect the poor and vulnerable, including young children, the elderly and those who are immune-compromised, very severely.

According to Bilgili, 2010, fecal contamination of the internal and external surfaces of poultry represents the most important cause of microbial contamination during slaughter and processing. This is in line with our findings.

6.4 Mitigation and communication strategies

6.4.1 Mitigation at community level

A small scale three room abattoir could be built on a farm, where the poultry farmers can easily slaughter the birds and in a place not far from the market so the consumers have easy access. These facilities could be managed by a group of farmers or associations. Where it is possible, mobile slaughter facilities designed for on-farm use would be more appropriate for places lacking infrastructure for slaughter and processing.

In the markets a small “butchery” , with clean washable walls and tables, screened from flies, could be placed within each market, where freshly slaughtered birds are brought in on a daily basis and kept cool in a refrigerator or in insulated freezer boxes with sealed ice-packs. This would be much cleaner and safer than selling live birds. In markets where there is no facility for such a “butchery”, cages and facilities

for live birds must be more hygienic and attention paid to welfare (such as keeping birds for short time periods in the market) to prevent birds being stressed. Stress is known to increase the risk of *Salmonella* and *Campylobacter* in poultry. Informal slaughter should also be discouraged unless the infrastructure is appropriate and can be kept clean. This includes disposal of solid waste and contaminated water. Markets and farms should be under state veterinary supervision, so monitoring of hygiene is ongoing.

Monitoring activities related to measuring the state of public health are the responsibility of national governments. The activities respecting monitoring of microbial hazards may be needed at multiple points along the entire food chain to identify food safety issues and to assess public health and food safety status and trends (CAC, 2007).

6.4.2 Mitigation at abattoirs

At abattoirs legislated norms for poultry abattoirs and the role of veterinary inspector should be reinforced to ensure best practices are observed during slaughtering processes, in line with OIE, CAC and ISO norms, but with understanding that this will not be achieved overnight, the role of the veterinary services would be motivate abattoir owners to produce a more hygienic product with better keeping qualities. Once prerequisites are in place, a move could be made towards introducing HACCP in the long term.

Use of the hygiene audit shown in Addendum C, would probably make a major difference to abattoir hygiene, not only in Maputo but in the whole of Mozambique.

6.4.3 Mitigation using appropriate microbiological monitoring

The 3M Petrifilm system is ideal for monitoring microbiological food safety and quality in a developing country such as Mozambique, where there is little infrastructure. It does not require sophisticated laboratory facilities and can be managed with low cost as it requires only a room that has impervious, washable walls and floors. The laboratory tables can be small and easily made form solid glass or stainless steel as the Petrifilms are small and so this costs less to set up. The laboratory scale, stomacher bag, bunsen burner and incubator required are very affordable. Glassware costs can also be reduced by using disposable pipettes.

Although the Petrifilms look expensive at first glance, the system is affordable because there is such a huge reduction in costs associated with transport of ingredients and disposal of petri dishes and other biological waste. The materials are also easily biodegradable and thus have a “green footprint”.

6.4.4 Communication

A strategy of communicating with consumers could also assist in motivating poultry processors to change. To this end, a joint programme is being developed with the Municipality of Maputo, using the photographs from the informal markets, to educate consumers about the risk they are running when they consume chicken that is contaminated with feces or dirty water. At this stage posters are being placed in the main markets warning consumers to avoid buying carcasses that are informally slaughtered as it can be a risk to their health. The next stage will be to design a TV and radio programme.

In regard to educating food processors to improve hygiene, meetings have been organized and set up by the Municipality Healthy Directorate to mobilize all the stakeholders to educate the consumers in the Maputo area. Participatory methods will be used to improve community health, particularly in regard to the informal slaughter of poultry. This is in line with the aims of participatory risk analysis.

According to CAC (2007) consumers can enhance their personal health by being responsible for, adhering to, being informed of and following food safety-related instructions. Multiple means of providing the information to consumers should be undertaken, such as public education programs, appropriate labeling, and public interest messages.

6.5 Recommendations

The main recommendation of the study is to train the food processors and handlers in basic hygiene practices and food safety. This is important because the number of small poultry farmers/vendors, has grown significantly in recent years and is expected to grow even more and this study shows that this target group is not trained on food safety practices.

Companies should educate employees to observe and correct personal hygiene principles, establish effective rodent and insect controls, limit nonessential traffic in all food-processing areas, remove the waste from the premises on a continuous basis and employ technically competent managers and supervisors who are conscious of and committed to the principles of food hygiene.

The training programs will vary, depending on the responsibility of the person being trained, but all should be given training on basic principles of food hygiene to know how food poisoning organisms can be spread in a food factory. Adequate training of competent personnel is of fundamental importance in the production of meat that is safe and suitable for human consumption. Training programs should provide personnel with the knowledge, skills and ability to carry out specified meat hygiene tasks, e.g., post-mortem inspection (CAC, 2005).

Managers and supervisors of food processes should have the necessary knowledge of food hygiene principles and practices to be able to judge potential risks and take the necessary action to remedy deficiencies. Training programs should be routinely reviewed and updated where necessary. Systems should be in place to ensure that food handlers remain aware of all procedures necessary to maintain the safety and suitability of food (CAC, 2003).

It is recommended that further research into the presence of *Salmonella* and *Campylobacter* as well as strain typing of *E.coli* to assess if the present strain is pathogenic or not.

A public health surveillance system should be able to estimate the proportion of illnesses and death that is truly foodborne disease and the major food vehicles, processes, and food handling practices responsible for each hazard (CAC, 2007).

CHAPTER 7

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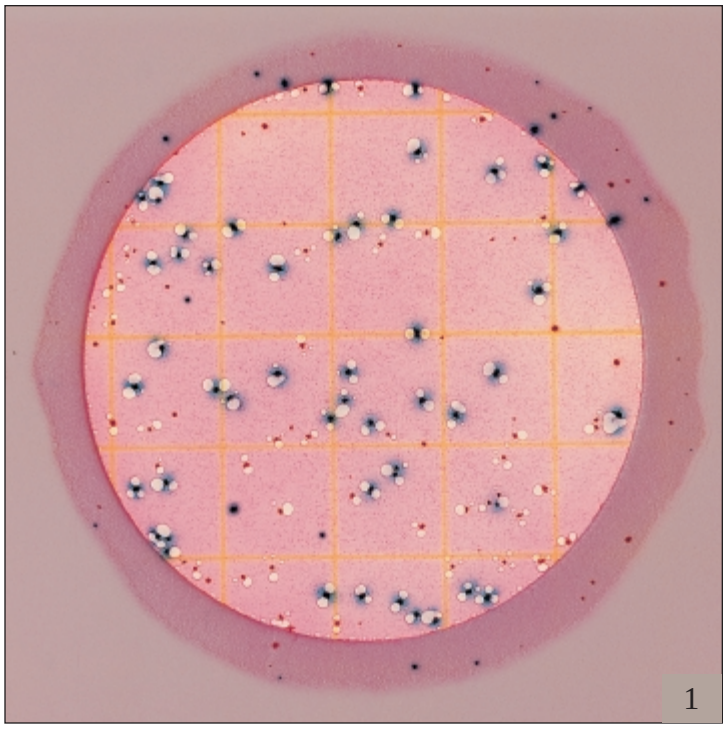
Interpretation Guide

Petrifilm™
E. coli/Coliform Count Plate

This guide familiarizes you with results on 3M™ Petrifilm™ E. coli/Coliform Count plates. For more information, contact the official 3M Microbiology Products representative nearest you.

Petrifilm E. coli/Coliform Count (EC) plates contain Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity, and an indicator that facilitates colony enumeration. Most *E. coli* (about 97%) produce beta-glucuronidase which produces a blue precipitate associated with the colony. The top film traps gas produced by the lactose fermenting coliforms and *E. coli*. About 95% of *E. coli* produce gas, indicated by blue to red-blue colonies associated with entrapped gas on the Petrifilm EC plate (within approximately one colony diameter).

AOAC INTERNATIONAL and U.S. FDA Bacteriological Analytical Manual (BAM) define coliforms as gram-negative rods which produce acid and gas from lactose during metabolic fermentation. Coliform colonies growing on the Petrifilm EC plate produce acid which causes the pH indicator to make the gel color darker red. Gas trapped around red coliform colonies indicates confirmed coliforms.

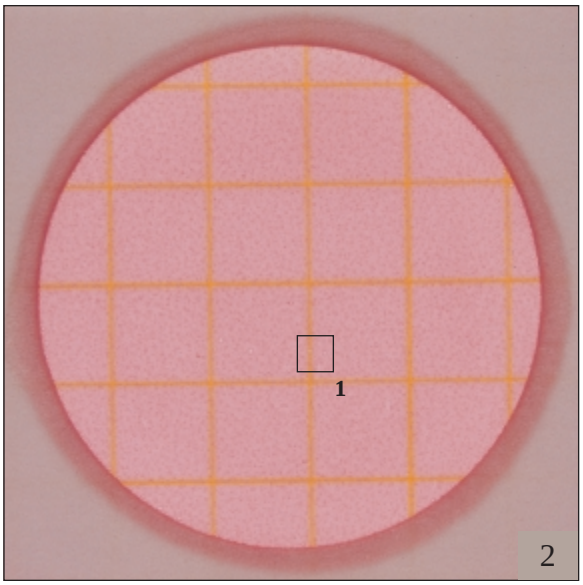

1

The identification of *E. coli* may vary by country (see Reminders for Use section for incubation times and temperatures):

AOAC INTERNATIONAL validated method
***E. coli* = 49** (blue colonies with gas)
Total coliform = 87 (red and blue colonies with gas)

Do not use this plate alone for the detection of *E. coli* O157. Like most other *E. coli*/coliform media, this plate will not specifically indicate whether any O157 strain is present.

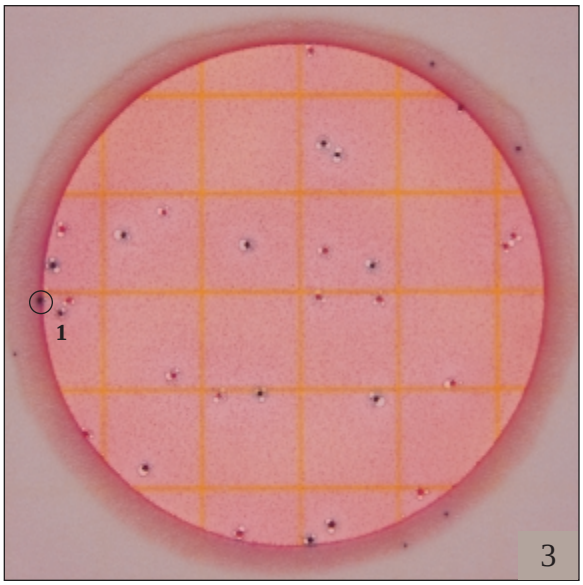
3M™ Petrifilm™ E. coli/Coliform Count Plate



No growth = 0

Notice the changes in gel color in figures 2 through 8. As the *E. coli* or coliform count increases, the color of the gel turns to dark red or purple-blue.

Background bubbles are a characteristic of the gel and are not a result of *E. coli* or coliform growth. See square 1.

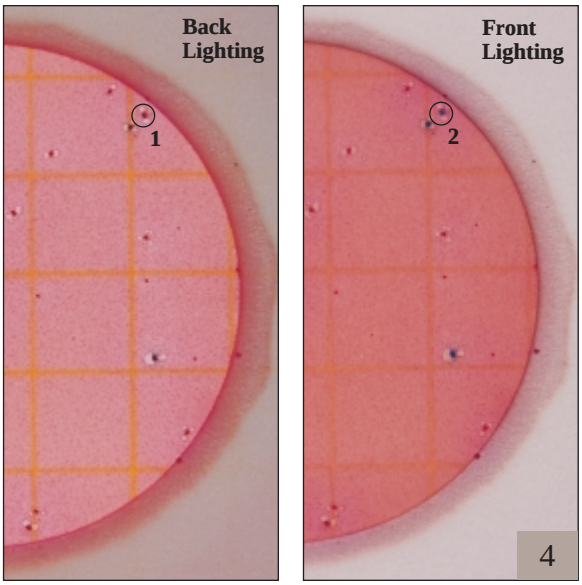


***E. coli* count = 13**

Total coliform count = 28

The counting range for the total population on Petrifilm EC plates is 15–150.

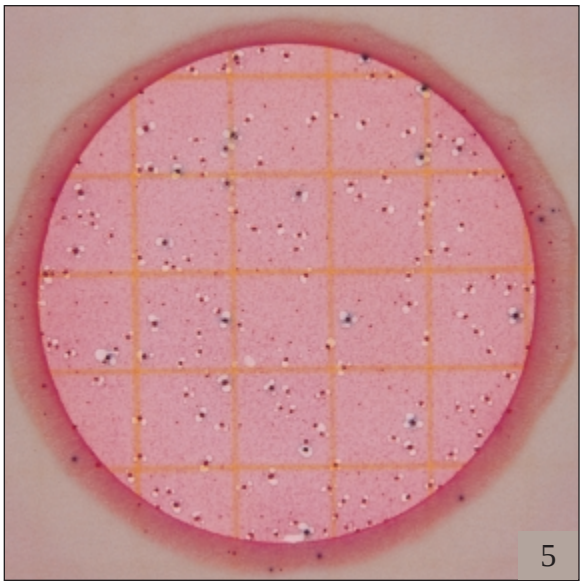
Do not count colonies that appear on the foam barrier because they are removed from the selective influence of the medium. See circle 1.



***E. coli* count = 3**

Any blue in a colony (blue to red-blue) indicates the presence of *E. coli*. Front lighting will enhance the detection of blue precipitate formed by a colony.

Circle 1 shows a red-blue colony counted using back lighting. Circle 2 shows the same colony with front lighting. The blue precipitate is more evident in circle 2.

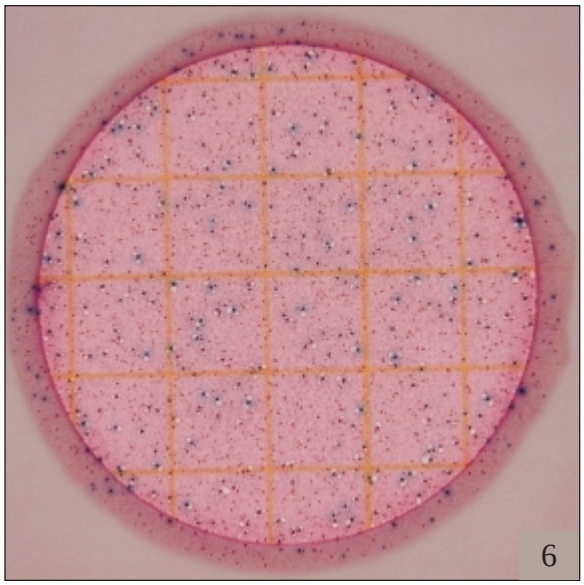


***E. coli* count = 17**

Estimated total coliform count = 150

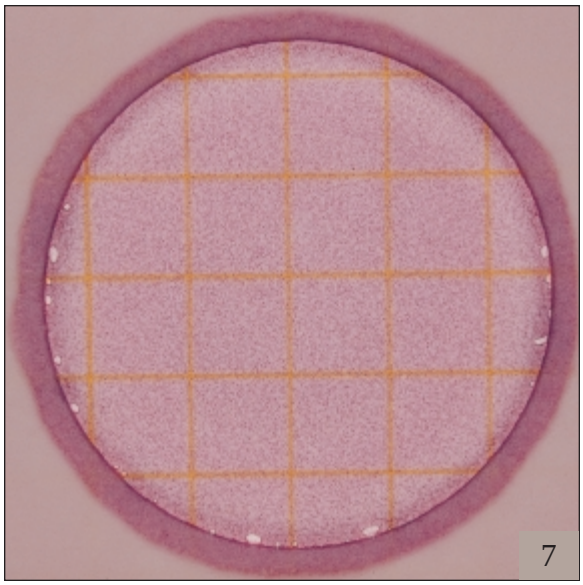
The circular growth area is approximately 20 cm^2. Estimates can be made on plates containing greater than 150 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine the estimated count per plate.

TNTC (Too Numerous to Count) *To obtain a more accurate count, dilute the sample further.*



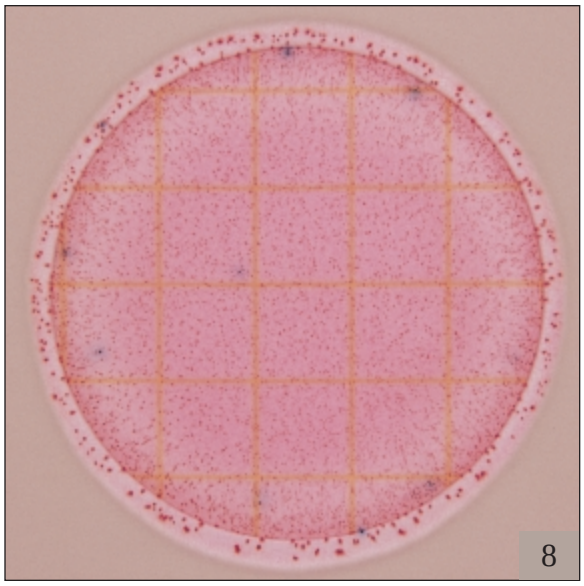
Actual count ~ 10^6

Petrifilm EC plates with colonies that are TNTC have one or more of the following characteristics: many small colonies, many gas bubbles, and a deepening of the gel color from red to purple-blue.



Actual count ~ 10^8

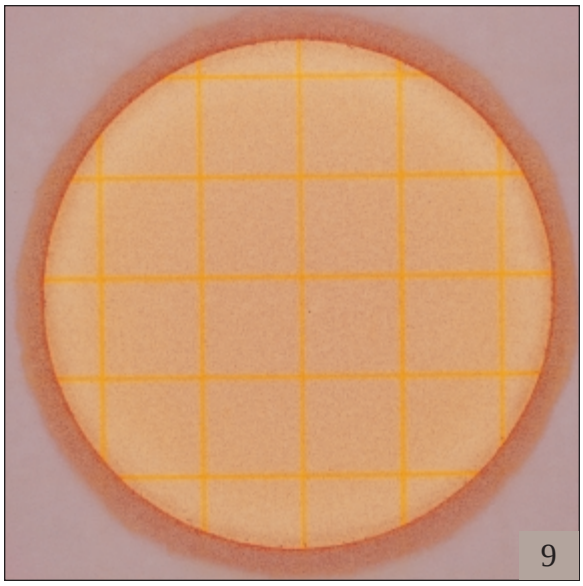
High concentrations of *E. coli* may cause the growth area to turn purple-blue.



Presumptive *E. coli* count ~ 8

Estimated total coliform count ~ 10^8

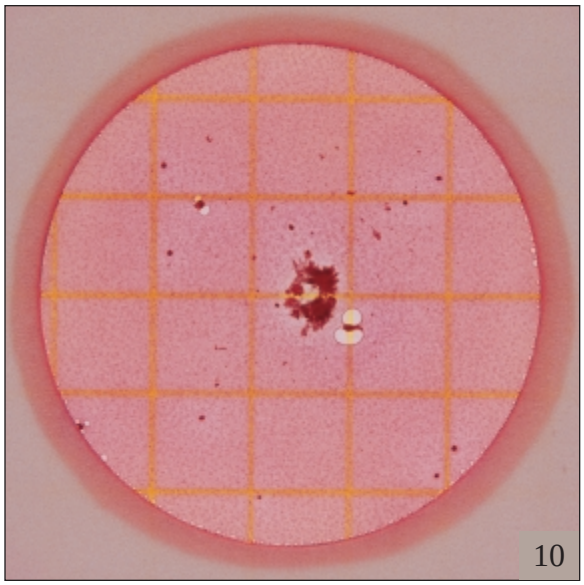
When high levels of coliforms are present (>10^8), some strains of *E. coli* may produce less gas and blue colonies may be less definitive. Count all blue colonies without gas and/or blue zones as presumptive *E. coli.* Pick blue colonies without gas and confirm if necessary.



Actual count ~ 10^8

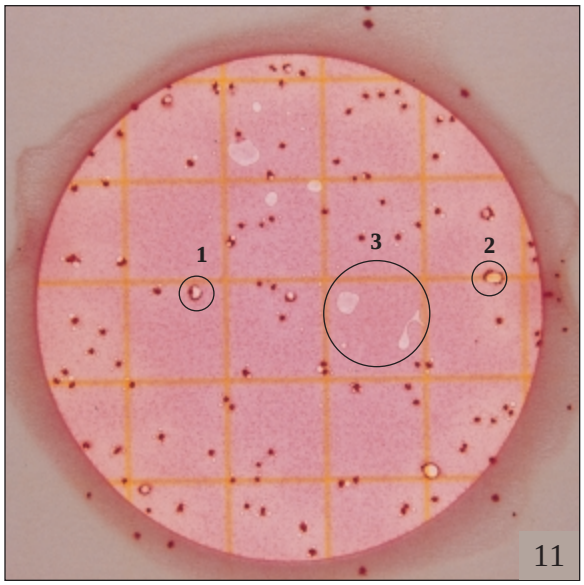
When high numbers of non-coliform organisms such as *Pseudomonas* are present on Petrifilm EC plates, the gel may turn yellow.

Bubbles


10

Total coliform count = 3

Food particles are irregularly shaped and are not associated with gas bubbles.

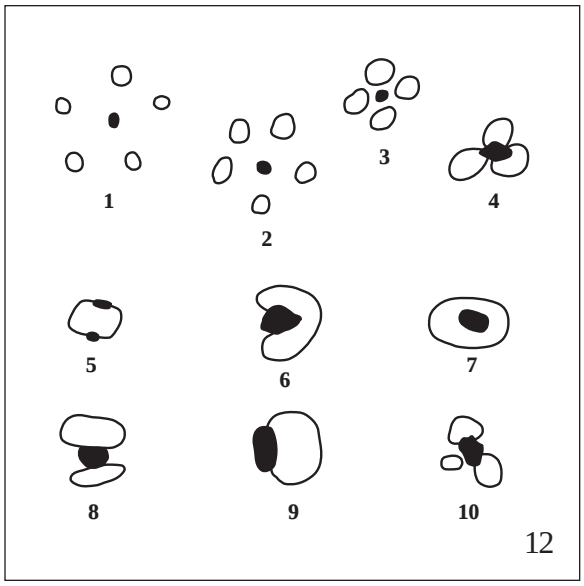


11

Total coliform count = 78

Bubble patterns may vary. Gas may disrupt the colony so that the colony “outlines” the bubble. See circles 1 and 2.

Artifact bubbles may result from improper inoculation or from trapped air within the sample. They are irregularly shaped and are not associated with a colony. See circle 3.



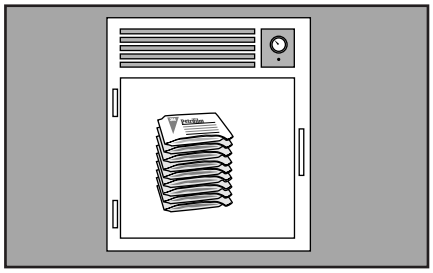
12

Examples 1–10 show various bubble patterns associated with gas producing colonies. All should be enumerated.

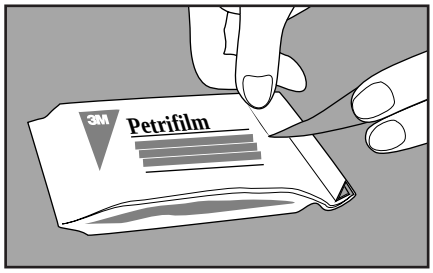
3M Petrifilm™ E. coli / Coliform Count Plates Reminders for Use

For detailed WARNING, CAUTIONS, DISCLAIMER OF WARRANTIES / LIMITED REMEDY, LIMITATION OF 3M LIABILITY, STORAGE AND DISPOSAL information, and INSTRUCTIONS FOR USE see Product's package insert.

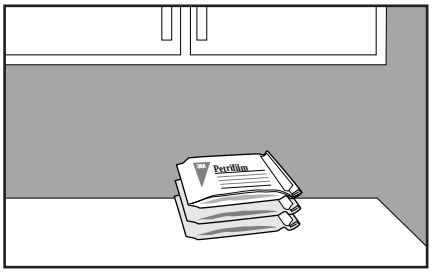
Storage



1 **Store** unopened packages at ≤8°C (≤46°F). Use before expiration date on package. In areas of high humidity where condensate may be an issue, it is best to allow packages to reach room temperature before opening.

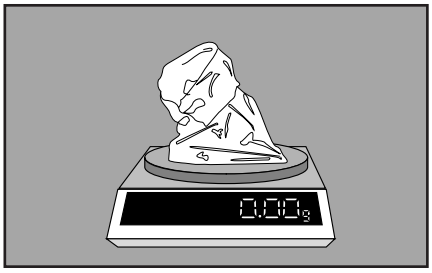


2 To seal opened package, fold end over and tape shut.

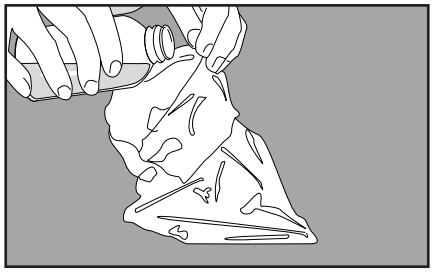


3 Keep resealed package at ≤25°C (≤77°F) and <50%RH. **Do not refrigerate opened packages.** Use Petrifilm plates within one month after opening.

Sample Preparation

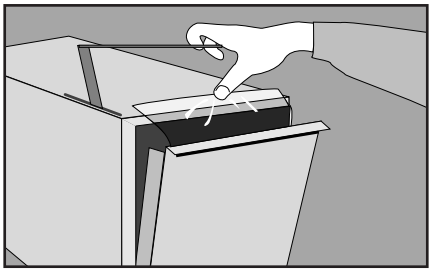


4 Prepare a 1:10 or greater dilution of food product. Weigh or pipette food product into an appropriate container such as a stomacher bag, dilution bottle, Whirl-Pak® bag, or other sterile container.



5 Add appropriate quantity of one of the following sterile diluents: Butterfield's phosphate buffer (IDF phosphate buffer, 0.0425 g/L of KH_2PO_4 adjusted to pH 7.2), 0.1% peptone water, peptone salt diluent (ISO method 6887), buffered peptone water (ISO method 6887-1), saline solution (0.85-0.90%), bisulfite-free letheen broth, or distilled water.

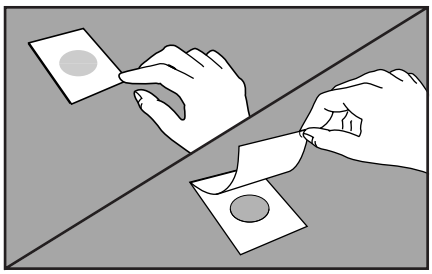
Do not use buffers containing citrate, bisulfite, or thiosulfate; they can inhibit growth.



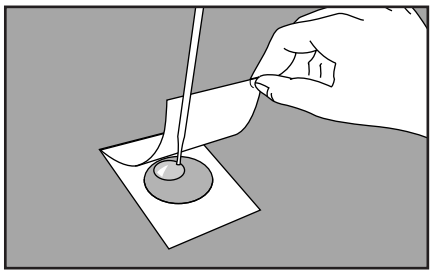
6 Blend or homogenize sample per current procedure.

Adjust pH of the diluted sample between 6.5 and 7.5.
- for acid products, use 1*N* NaOH,
- for alkaline products, use 1*N* HCl.

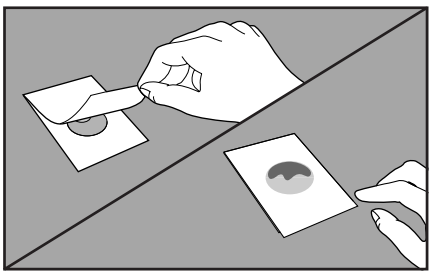
Inoculation



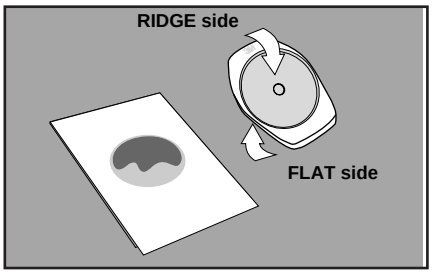
7 Place Petrifilm plate on **level** surface. Lift top film.



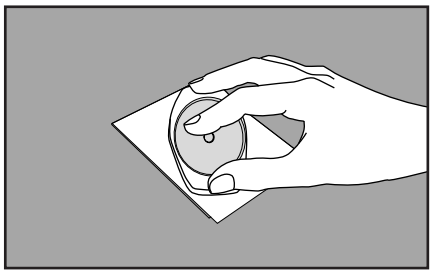
8 With pipette **perpendicular** to Petrifilm plate, place 1 mL of sample onto center of bottom film.



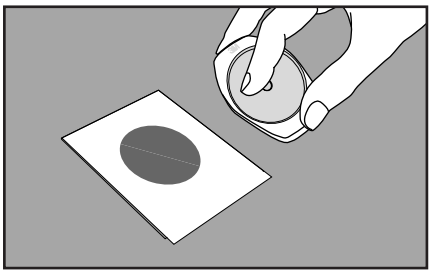
9 Carefully **ROLL** top film down to avoid entrapping air bubbles. Do **NOT** let top film drop.



With **FLAT** side down, place spreader on top film over inoculum.

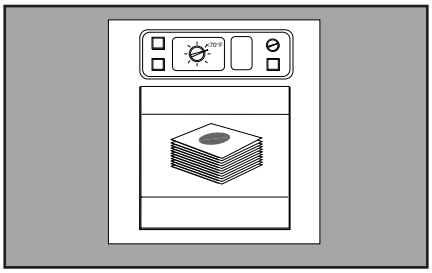


11 **GENTLY** apply pressure on spreader to distribute inoculum over circular area before gel is formed. Do not twist or slide the spreader.



12 Lift spreader. Wait a minimum of one minute for gel to solidify.

Incubation



13 Incubate plates with clear side up in stacks of no more than 20. It may be necessary to humidify incubator to minimize moisture loss.

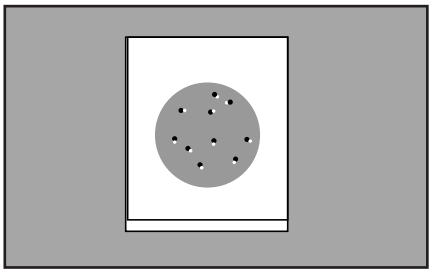
Incubation time and temperature varies by method. Most common approved methods:

AOAC Official Method 991.14
for coliforms: incubate 24h ± 2h at 35°C ± 1°C
for *E.coli:* incubate 48h ± 2h at 35°C ± 1°C

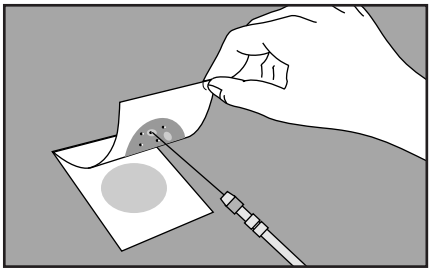
AOAC Official Method 998.08
for *E.coli* (for meat, poultry and seafood)*:*
incubate 24h ± 2h at 35°C ± 1°C

NMKL method (147.1993)
for coliforms: incubate 24h ± 2h at 37°C
for *E.coli:* incubate 48h ± 2h at 37°C

Interpretation



14 Petrifilm plates can be counted on a standard colony counter or other illuminated magnifier. Refer to the *Interpretation Guide* section when reading results.



15 Colonies may be isolated for further identification. Lift top film and pick the colony from the gel.

Additional Comments

- Questions? U.S., Call **1-800-328-6553**, Canada, call **1-800-265-1840 x6574** for technical service.
- To order Petrifilm plates in the U.S., call **1-800-328-1671**
- Latin America / Africa and Asia Pacific regions, call **1-651-733-7562.**

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ADDENDUM B

CONSENT FORM

PREREQUISITES FOR HACCP IN SMALL SCALE POULTRY PRODUCTION

RESEARCH BY DR AMV CAMBAZA DOS MUCHANGOS, REGISTERED AT THE UNIVERSITY OF PRETORIA

I [name of respondent]_____hereby voluntarily grant my permission for participation in the project as explained me by Ana Bela M.V. Cambaza dos Muchangos.

The nature, objective, possible safety and health implication have been explained to me and I understand them.

I understand my right to choose whether to participate in this project and that information furnished will be handled confidentially.

I am aware that the results of the investigation may be used for the purposes of publication.

Upon signature of this form, you will be provided with a copy.

Signed [Respondent]: _____ Date_____

Signed [Researcher]: _____Date _____

ADDENDUM C

MINISTÉRIO DA AGRICULTURA
Direcção Nacional dos Serviços de Veterinária
Departamento de Higiene e Saúde Pública Veterinária

SISTEMA DE AVALIAÇÃO DE HIGIENE

MATADOUROS DE AVES

Matadouro _____
Tipo _____
Localização _____
Data de inspecção _____

NO	ACTIVIDADE	BOM	RAZOÁVEL	POBRE	CONSIDERAÇÕES
A	**ABATTOIR PREMISES**				
1	Limpeza das instalações				
2	Eficácia no controlo de vermes				
3	Limpeza da área de armazenamento do lixo				
4	Controlo da entradas (fechadas)				
5	Separação da área limpa e suja				
B	**ÁREA DE RECEPÇÃO E DESCARGA**				
1	Limpeza e desinfecção geral				
2	Manipulação das aves (sombra & Ventilação)				
3	Inspecção Ante-mortem das aves				
5	Lavagem da viatura de transporte de aves				
6	Lavagem da grades de aves				
C	**ÁREA SUJA**				
1	Lavagem das botas (detergente & Escova)				
2	Esterilizadores @ 82° C				
3	Lavatórios (detergente & Agua quente)				
4	Manipulação das aves				
5	Procedimento de atordoamento/sangramento				
6	Procedimento de escalda				
7	Procedimento de depenagem				
8	Limpeza contínua				
9	Lavagem da carcassa (antes da evisceração)				
D	**ÁREA LIMPA**				
1	Lavagem de botas (Detergente & Escova)				
2	Esterilizadores @ 82° C				
3	Lavatórios (Detergente & Àgua quente)				

4	Procedimentos evisceração				
5	Limpeza contínua				
6	Lavagem final das carcassas				
E	**INSPECÇÃO**				
1	Inspecção primária da carcassas				
2	Correlação entre as carcassas e miúdezas				
3	Manipulação do material condenado				
4	Aproveitamento das carcassas				
5	Inspecção final				
F	**PROCESSAMENTO DE MIÚDEZAS**				
1	Lavatórios (Detergente & Agua quente)				
2	Manipulação de miúdezas				
4	Limpeza eficaz de moelas				
5	Limpeza contínua				
6	Material de empacotamento e plastificação				
G	**ARREFECIMENTO, PORCIONAMENTO, EMPACOTAMENTO E ARMAZENAMENTO**				
1	Arrefecimento das carcassas para <10°				
2	Procedimentos higiénicos de porcionamento				
3	Lavatórios (Detergente & Agua quente)				
4	Material de empacotamento e plastificação				
5	Limpeza geral				
6	Gestão do armazém frio				
7	Manipulação da saídas				
9	Limpeza contínua				
10	Limpeza da viatura de transporte de carnes				
H	**REFEITÓRIO, BALNEÁRIO, VESTIÁRIO & CASAS DE BANHO**				
1	Limpeza geral				
2	Condições dos cacifos/armários				
3	Papel higiénico nas casas de banho				
4	Detergente em lavatórios de mão				
5	Toalhas de papel/secadores de mão				
I	**MANUTENÇÃO**				
1	Manutenção de equipamentos				
2	Manutenção da intensidade de luz				
J	**PESSOAL**				
1	Condições de roupa sanitária				
2	Roupa protectora completa				
3	Higiene pessoal dos trabalhadores				
4	Condições físicas dos trabalhadores				
K	**GESTÃO DE LIXO**				
1	Protocolo aprovado para cada categoria de lixo				

Comentários

_____

Assinatura _____ _____

Inspector Gestor do Matadouro