CHARACTERISATION AND PUBLIC HEALTH SIGNIFICANCE OF SELECTED ENTEROPATHOGENIC BACTERIA ISOLATED FROM JAPANESE QUAILS (*Cortunix cortunix japonica*) IN LUSAKA, ZAMBIA

By

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DECLARATION

I, Mukachikwikwi Hamakoko, do hereby declare that the contents of this thesis being submitted herein are my original work and they have not been previously submitted to any University for the award of a degree or any other qualification.

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CERTIFICATE OF APPROVAL

The Board of Examiners has approved the dissertation of **MUKACHIKWIKWI HAMAKOKO** as partial fulfilment of the requirements for the award of the Degree of Master of Science in Epidemiology of the University of Zambia.

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ABSTRACT

Animals are known to harbour different pathogenic bacteria with potential for zoonosis especially with food producing animals like poultry. Currently quail farming is rapidly gaining momentum in Zambia, as a source of protein in the form of meat and eggs. The study aimed at evaluating the prevalence of enteropathogenic bacteria (i.e. *Proteus* spp., *Escherichia coli*, *Salmonella* spp.). This work was a cross sectional study in Lusaka where consented fifteen quail farmers were sampled. These fifteen farms were selected from within Lusaka which had the quails going into market for sale. The aim of the study was to identify *Salmonella* and other entero-pathogenic bacteria on their prevalence in quails. The specific objectives were to characterise isolates and determine the public health significance for *Salmonella*, *E. coli* and *Proteus* bacteria positive farms. The sampling method done was probability proportional to size and systematic sampling at individual farms at set intervals. The study findings indicate that *Salmonella* was absent from the faecal samples collected however other bacteria of public health significance such as *Proteus* and *E. coli* were isolated. From the *E. coli* species isolated, six isolates were identified and shown to have resistant genes CTX-M. Extended Spectrum Beta-Lactamase gram negative organisms are associated with antimicrobial resistance, which are part of an emerging problem worldwide. *E.coli* isolates were resistant to cephalosporins 100% Cefotaxime and 86% Cefoxitin. The findings also suggested that quail farmers that take up veterinary services experience very low isolates of bacterial contamination among their birds.. The study showed no *Salmonella* recovered from the faecal samples collected however other bacteria of public health significance such as ESBL *E.coli* with resistant genes are present that could become an important threat to food safety.

DEDICATION

This work is dedicated to my loving and caring husband Chapita Mbuzi, my children Nkosazana and Mazuba for their patience, encouragement and all the support they rendered to me during my studies.

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ABBREVIATIONS

AIDS	Acquired immunodeficiency Syndrome
ELISA	Enzyme-linked immunosorbent assay
HIV	Human Immunodeficiency Virus
LPS	Lipopolysaccharides
NTS	Non- typhoid Salmonellosis
P.C.R	Polymerase Chain Reaction
TT	Tetrathionate
XLD	Xylose Lysine Deoxycholate agar
W.H.O	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Enterobacteria are a public health concern of great importance around the world affecting both humans and animals with considerable economic impact. Several organisms considered in this group of gram negative bacteria include *Salmonella, E. coli, Proteus, klebsiella* and *Campylobacter.* Other organisms include gram positive such as *Staphylococcus* and *Streptococcus* species. It is widely reported that these organisms *Salmonella* and *E. coli* are widely responsible for enteric infections known in man after consumption of poultry meat products. Foodborne pathogens including bacteria with zoonotic potential are in focus worldwide because of immense health loss and costs that arise from foodborne infection associated with bacteria, such as *Salmonella*, *E. coli* and *Campylobacter* species (Reich, 2013; Chen *et al*., 2010)

Salmonellosis is a commonly and widely distributed food borne illness according to Botti *et al.,* (2013). It is a condition that refers to infections that are caused by *Salmonella* species according to Kauffmann-White scheme (Hendriksen *et al*., 2011). The strains of *Salmonella* are classified into serovars on the basis of lipopolysaccharide (LPS) antigens (O) and flagellar protein (H) and currently over 2600 serovars are recognized (Hendriksen *et al*., 2011; Suez *et al*., 2013). Bacteria of the genus *Salmonella* are gram negative, facultative anaerobic, non-spore forming, usually motile rods (peritrichous flagella) belonging to the Enterobacteriaceae family, which are associated with alimentary tract of animals. *Salmonella* can also be considered a common commensal of the gut micro flora of animals including mammals, birds, reptiles, amphibians, fish and shell fish. Meat animals can be infected and act as reservoirs of *Salmonella*.

It is also known now that enterobacteria are a major cause of foodborne diarrhoeal illness in humans and are the most common bacteria that cause gastroenteritis worldwide (WHO 2013; Crump and Heyderman, 2014; Rothick *et al* ., 2015). Many infections are due to ingestion of food contaminated with *Salmonella* species. A variety of foods have been implicated as vehicles transmitting salmonellosis to humans including poultry, beef, pork, eggs, cheese, resh vegetables and sea food that affect public health and food safety (Espi *et al*., 2005; Crump and Heyderman , 2005 McEntire *et al*., 2014). *Salmonella* can still be divided into

two groups typhoidal and non typhoidal *Salmonella* serovars. Typhoidal serovars include *Salmonella typhi* and *Salmonella paratyphi A*, which are adapted to humans and do not occur in animals (Suez *et al*., 2013). However there are two main types of systemic avian salmonellosis: the chicken adapted *Salmonella*- adapted *Salmonella* serovar *gallinarum* biovars *pullorum* and *gallinarum* which are responsible for *pullorum* disease and fowl typhoid, respectively all over the world (Barrow and Neto, 2011). In addition to these two clinically systemic salmonella, birds will be infected by other *Salmonella* serovars like Salmonella *enterica* serotype *enteriditis*, in this case, these birds may become asymptomatic carriers and potential sources of human salmonellosis

1.2 *Salmonella enterica serotype enteritidis* as a Zoonotic pathogen

Salmonella enterica is a zoonotic pathogen which can readily pass from animal to humans through the consumption of contaminated meat, animal products or other food products after contamination with animal faecal material. Infections caused by *Salmonella enterica serovar enteriditis* have increased worldwide beginning as early the 1970s and subsequently by the 1990s it was reported by Baumer *et al* (2000) as the primary cause of salmonellosis in the world. The ability of salmonella species to cause human infection involves attachment and colonization of intestinal columnar epithelial cells and specialized microfold cells overlying Peyers patches. Symptoms of salmonellosis include vomiting, diarrhoea, abdominal pain and nausea lasting 1 to 7 days and the condition is self- limiting in healthy adults with a mortality of < 1% (Berkely *et al.,* 2005). In severe cases, infection may progress to septicaemia and death unless the person is promptly treated with appropriate antimicrobials presently of fluoroquinolones, macrolides and third generation cephalosporins.

Antibiotics which are very essential drugs for human and animal health are often used by veterinary surgeons and farmers on pets and farm animals for therapeutic and prophylactic treatment and also to promote growth (Graham *et al*., 2002). These routine practices are important factors in the emergence of antibiotic- resistant bacteria that subsequently can be transmitted from animals to humans through the food chain. Most antimicrobial- resistant *Salmonella* infections are acquired from eating contaminated foods of animal origin. Infections with antimicrobial- resistant strains may comprise treatment outcomes thus resulting in increased morbidity and mortality.

In Africa , multi drug resistant Non- typhoidal Salmonellosis are one of the leading causes of morbidity and mortality in children under 5 years of age (Kariuki *et al*., 2006). Individuals

infected with NTS experience mild forms of diarrhoea, abdominal cramps, fever and vomiting. Infections are acquired as food poisoning and are self- limiting but can lead to severe illness in immune-compromised individuals.

Poor hygiene and sanitation as well as close proximity to animals in developing countries all contribute to easy acquisition of the enteric pathogen. Reports of salmonellosis in developing countries (Padungton *et al.,* 2003; Uaboi-Egbenni *et al.,* 2013), point to an urgent need to explore prevalence rates and antibiograms activities in animals because of the zoonotic nature of infections and for proper planning of effective prevention and control measures (Oporto et al., 2009; Botti *et al.,* 2013).

1.3 Other Enteropathogenic Organisms in Poultry

Food products of animal origin are thought to be the main source of zoonoses. *Salmonella* and *Campylobacter* species are the main pathogens contributing to zoonosis regarding poultry and responsible to gastroenteritis in human populations (Mead *et al*., 1999). In other studies it was shown that *Salmonella* and *E. coli* were isolated from raw poultry meat *(*Doyle *and* Schoeni, *1987;* Chen *et al., 2010).* Salmonellosis appears to be most prevalent in intensive animal husbandry such as in pigs and calves as well as poultry reared in confinement according to O.I.E 2010. Enteropathogenic infections of food animals play an important role in both public health and food safety. In Netherlands, it was found the most resistant faecal *E. coli* of food animals is related to antibiotics given on veterinary prescription (Bogaard *et al*., 2001). Its common practice to use antimicrobials in feed supplementation to decrease disease incidents and enhance growth performance in poultry. These antimicrobials tend to favour the growth of antimicrobial resistant bacteria in animals. A source of concern is carcass contamination from these pathogens that may occur hence infect man directly or indirectly. These resistant organisms may then colonize the intestinal tract and contribute resistant genes to the endogenous flora in man, posing a challenge to antimicrobial therapeutic treatments in man. Contamination of carcasses can occur at different stages through the food chain at production, processing, retail sales and handling itself. *Campylobacter, Salmonella,* and pathogenic *E. coli* all colonize the gastrointestinal tracts of a wide range of wild and domestic animals, especially animals raised for human consumption. When these organisms contaminate raw or undercooked poultry and red meats then become particularly important in transmitting these food-borne pathogens (Zhao *et al*.2003)

In an increasing concern on the emergence of multi-resistant food borne pathogens from food sources including poultry, the study aimed to evaluate enterobacteria specifically in quails where there is limited information on the new poultry meat in Zambia.

1.4 Statement of the Problem

In Zambia, the prevalence rates of *Salmonella* and other entero-pathogenic organisms *a*re not well known in quail species and how wide the distribution. Little is known about the role the quails play in the spread of zoonotic pathogens. This is despite reports that most foodborne infections acquired by man is through poultry products such as meat and eggs. Studies have shown that quails have played a role in the circulation of some zoonotic pathogens threatening human health and domestic animals (Abulreeshet *et al*.,2007; Benskin *et al*., 2009). Zoonotic pathogens such as *Staphylococcus* spp and *Proteus* spp have been isolated from migratory quails (Mohamed et al., 2001; Effat and Morsi 2005). Other bacterial organisms such as *Escherichia coli* and *Salmonella* are considered to cause severe losses through mortality in the poultry industry and potentially could be found also in the other species of poultry such as quails.

Currently, quail farming is rapidly gaining momentum in Zambia (Kanyinji and Chionile 2014) as this source of protein requires little feed in production. The increase in production comes with its own pressure hence the possible indiscriminate use of antimicrobials as growth promoters and hence reduction of disease incidence in the flocks with better market weights of birds. On the other side, development of antimicrobial resistance of zoonotic bacteria suggests a far greater public health risk giving rise to treatment failures in human populations.

Studies done in Zambia show prevalence rates of *Salmonella* in chickens and no information is given in other species such as quails. However, anecdoctal evidence suggests that this could also be a problem of *Salmonella* and other entero-pathogenic organisms in these quail poultry species. The economic and health effects of the condition are considerable. WHO (2013) Annual reports in Zambia, show that diarrhoea is one of the leading causes of death in children under 5 years of age. However, it is made difficult by the lack of human salmonellosis information in the population. Most diarrheal conditions are not investigated to determine the causative agents whether biological or otherwise, responsible for the diarrhoea. The regional epidemiological picture within Africa also tells very little about the status quo of salmonellosis and the extent of the problem. The cost of medical expenses and time lost by

adults to look after the sick children is considerable. Adults that are sick may be affected by their ability to work and in some cases even result in the loss of life. In a study done by Mshana *et al., (*2013), the study showed that there is an urgent need for sustainable surveillance on antimicrobial resistance in human and animal pathogens. The study was done in Zambia, DRC, Tanzania and Mozambique which showed an increasing trend in the incidence of antimicrobial resistance for organisms such as *Escherichia coli*, *Klebsiella pneumonia, Staphylococcus aureus* and *Vibrio cholera* as well as non-typhoid *Salmonella*.

The bacteria *Salmonella* continues to lead as a cause of non-typhoidal Salmonellosis in the developing countries. It was seen as a cause of bacteraemia and septicaemia in children in tropical Africa particularly in a study done in Rwanda which showed up to 47% of isolates from *S. enterica enteriditis* and *S. enterica tyhimurium* (Graham *et al*., 2002, Maclannen *et al.,* 2008). The condition was a source of concern in children less than 5 years of age and indeed immuno-comprised individuals such as those suffering from HIV-AIDS (Magwedere *et al.,* 2015).

1.5 Justification

Zoonotic pathogens such as *Staphylococcus* spp and *Proteus* spp have been isolated from migratory quails (Mohamed et al., 2001; Effat and Morsi 2005). In other studies, it was shown that *Salmonella* and *E. coli* were isolated from raw poultry meat (Hang'ombe et al., 1999; Kilonzo-Nthenge et al., 2008). While these organisms colonize the gut of these animals, they could potentially contaminate raw or undercooked poultry meats and become particularly important in the transmission of food borne pathogens (Reich and Klein, 2013). Furthermore, an emerging problem now is the antimicrobial resistant organisms such as *E. coli* found in quails (Roy et al., 2006; Paulsen et al., 2012). Therefore there is need to understand that to effectively control salmonellosis or other emergent infections, epidemiological surveys largely need to be employed to understand the infection status in flocks of quails with reference to entero bacteria. The status of entero pathogens in quail farms and possible sources at small scale production needs to be assessed and documented for better control strategies. In other studies it has been reported the existence of quails especially those that are now avian species for commercial purposes may have potential pathogens of zoonotic nature that present as a serious public health risk (Youssef and Mansour, 2014). While this is documented, currently in Zambia, information on entero bacteria in quail species is limited.

Most surveys that have been conducted in Zambia have generally looked at prevalence rates of *Salmonella* in chicken broiler poultry units as well as layer breeding units otherwise information on entero bacteria in other poultry species is unknown. The purpose of this study is in identifying *Salmonella* and other selected enteropathogens strains present in quails from selected farms in Lusaka. This information would then help identify for possible zoonotic strains of the pathogens with their molecular characterisation in quails and the risk factors associated with *entero pathogens* infected quails entering the market for sale.

The study will generate the knowledge on selected *enteropathogens,* in quails and if there is any antimicrobial resistance of the organism. The information will show an important update on the status of entero-pathogens in quails and if it presents as an emerging problem in these avian species and bring into play the deliberate actions to control antimicrobial resistance in animal populations that may spill over into the human population. It will be important to identify resistant strains of *Salmonella, E. coli* and *Proteus* organisms that can spread from this birds to humans and if this can complicate the treatment of the disease in humans. Consequently NTS is a target of integrated surveillance system of foodborne pathogens and taking a One Health Approach and implemented along the farm-to-folk continuum (Magwedere, 2015).

1.6. Main Objective

1. To characterize and determine the public health significance of selected entero pathogenic bacteria isolated from Quails in Lusaka, Zambia.

1.7 Specific Objectives

1. To isolate and identify *Salmonella, E. coli* and *Proteus* from quails in Lusaka.
2. To identify molecular characteristics of *Salmonella, E. coli* and *Proteus* isolates from quails in Lusaka.
3. To assess for antimicrobial susceptibility of *Salmonella, E. coli* and *Proteus* isolates from quails in Lusaka.
4. To establish the public health significance of the selected enterobacteria identified

1.8 Conceptual Framework

To answer the objectives of the study, it is also important to consider the risks factors associated with *Salmonella* and other enteropathogenic isolates infected quails at farm level. It is known in developed countries, increase in demand of meat products has led countries to

produce animal or their products with higher efficiency and in turn, massive production has caused the increase in food borne pathogens (Koluman 2012). Risk factors have been documented in chicken poultry species as suggested by Mollenhorst *et al.,* (2005) that risks for *Salmonella* in laying hens include housing, flock size and different ages of birds. Managerial skills, general hygiene and environmental status were assessed in a study conducted in French commercial broiler flocks that showed an association of risk of *Salmonella* in birds (Rose *et al.,* 1999). By using the mentioned set of factors, a proposed frame work was generated as shown in Appendix II.

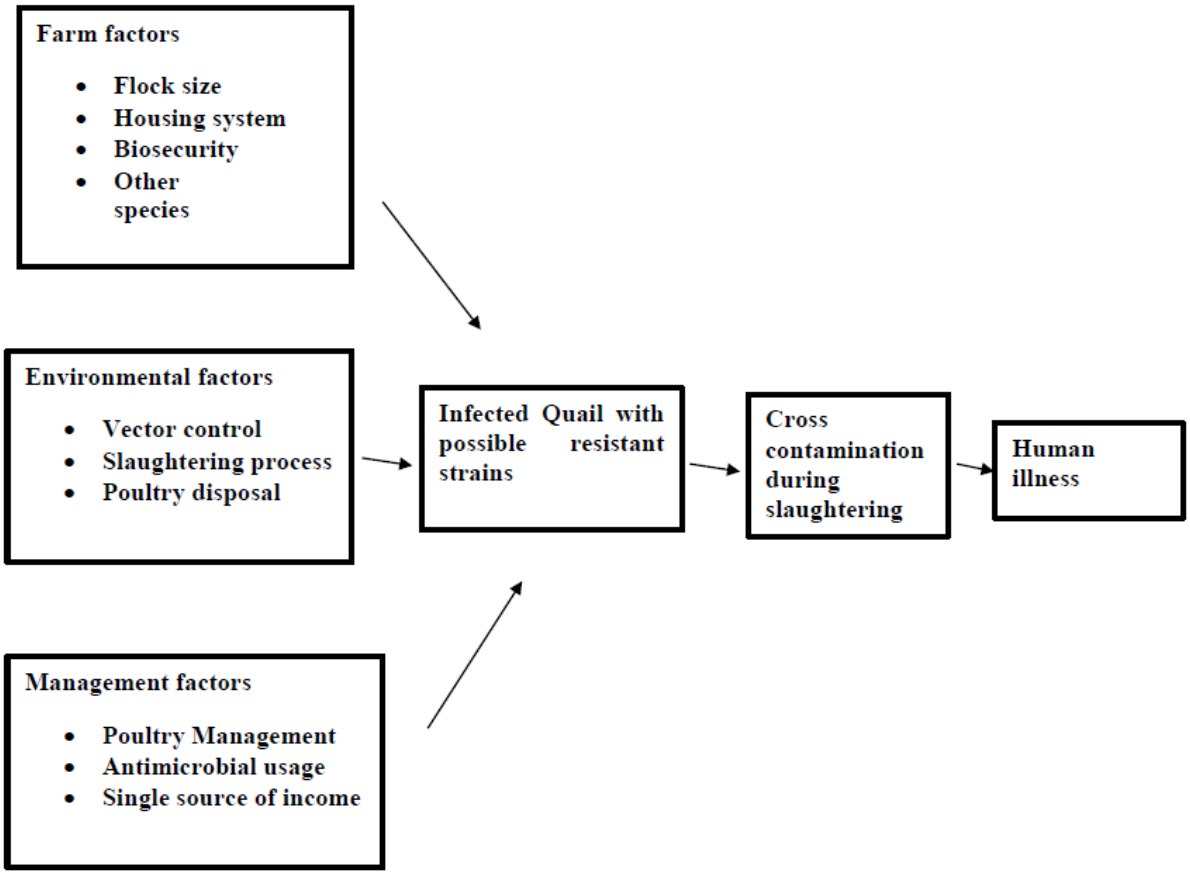

Figure1. Conceptual framework

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview

Enterobacteria are pathogens that will inhabit both humans and animals which are limited to their digestive tracts. These animals include livestock, poultry, birds, reptiles, rodents and pets. These species of pathogens can cause a number of foodborne and waterborne diseases. These include food poisoning (gastroenteritis), typhoid (enteric fever) as well as bacteraemia and septicaemia (Bell and Kyriakides, 2002)

Campylobacters are relatively 'new' zoonotic pathogens and the two species which are most important in food-borne infections of humans with Campylobacter are *C. jejuni* and *C. coli.* The pathogens are ubiquitous in nature and in domestic animals and, as a consequence, are found frequently in the environment and on many raw foods, of both plant and animal origin and bacterial numbers can be very high on certain key foods like raw poultry meat. Although all commercial poultry species can carry *campylobacter*s, the risk is greater from chicken because of the high levels of consumption (Humphrey *et al*., 2007).

Escherichia coli is a bacterial commensal of the intestinal microflora of a variety of animals, including humans. However, not all *E. coli* strains are harmless, as some are able to cause diseases in humans as well as in mammals and birds (Dho-Moulin & Fairbrother, 1999; Kaper *et al*., 2004). Pathogenic *E. coli* strains fall into two categories: those that cause intestinal pathologies and those that cause extraintestinal pathologies. Intestinal pathologies mostly consist of more or less severe diarrhoea or enteritis caused by different *E. coli* pathotypes such as enterotoxinogenic, enteropathogenic or enterohaemorragic *E. coli* (ETEC, EPEC and EHEC, respectively), potentially evolving into a haemolytic uremic syndrome (HUS) in the case of EHEC infections in the case of EHEC infections. Comparison of *E. coli* isolates can be a considerable challenge because of the wide genetic diversity resulting from genome remodelling and horizontal acquisition from other pathogenic bacteria according to Ron, 2010.

The enteropathogenic serotypes of *E. coli* (018, 044, 055, 086, 0111, 0114, 0119, 0126, 0127, 0128ab, 0142, 0158) produce toxins adhere to intestinal mucosa, disturbing the function of microvilli, and cause diarrhea. The entero invasive serotypes (028ac, 029, 0124, 0136, 0143, 0144, 0152, 0164, 0167) invade and proliferate within epithelial cells, eventually causing cell

death. The enteroxigenic serotypes (06, 08, 020, 025, 027, 063, 078, 080, 085, 0115, 0128ac, 0139, 0148, 0153, 0159, 0167) and the entero haemorrhagic serotypes (01, 026, 091, 0111, 0113, 0121, 0128, 0145, 0157) of *E. coli* are associated with diarrheal illness.

Escherichia coli has implicated as an etiological agent of food poisoning involving different foods such as raw milk, vegetables, cheese, potatoes, uncooked or poorly cooked meats and poultry. Several strains of *E. coli* have emerged has potent food pathogens. One particular strain such as (0157:H7) has been identified as one of the most dangerous in humans causing a bloody diarrhea and even responsible for kidney failure in children (Ron, 2010)

Salmonella is rod shaped gram negative facultative anaerobic bacteria belonging to the family Enterobacteriaceae that causes Salmonellosis. It's an intracellular bacterium that is primarily intestinal in nature in both animals and man. The bacterium is characterized by the O, H and Vi antigens. The taxonomic classification of *Salmonella* is based on two species which *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is further divided into six subspecies which are I, II, IIa, IIIb, IV and IV (Noyal *et al*., 2009). Most of the Salmonellosis in warm blooded animals in most cases is associated with serovars of *Salmonella enterica* belonging to sub type I including the typhoid and paratyphoid bacilli. The most common type of infection is the carrier state, in which healthy animals carry the pathogen without showing any clinical signs. Clinical signs may take two forms systemic septicaemia and enteritis. Numerous serovars of non-typhoid *Salmonella* (NTS) cause a self-limiting gastroenteritis in healthy humans but may cause serious illness in immune-compromised persons. The most common serovars isolated from humans in the developed world are serovars *typhimurium, enteritidis, virchow and hadar*. They are all considered to be of zoonotic origin and all exhibit resistance to commonly used antibiotics (Phillips *et al*., 2004). In the developing world this is a great challenge, as little is known about bacterial populations in animal hosts.

The basic nature of these pathogens is to invade the intestinal mucosa and associated lymphoid tissue. From the infected intestinal tissues the pathogens are drained to the surrounding lymph nodes where macrophages from the lymphoid tissues form the first line of barrier or defence against systemic infection. If the host is able to limit the infection to the gut then it remains localized. In humans, non- typhoidal *Salmonella* serovars typically cause a localized infection which manifests itself as acute gastroenteritis. On the other hand if the macrophages located in the lymph nodes are unable to localize the infection, *Salmonella* can cause a systemic infection. The systemic disease caused by human adapted serovar

Typhimurium causes typhoid fever. Some *Salmonella* serovars that cause typhoid fever like disease in animals include *S. gallinarum* in poultry (fowl typhoid), *S. choleraesuis* in pigs (porcine paratyphoid), *S. tyhphimurium* and *S. enteriditis* in mice (Wray and Wray 2002).

Salmonella serovars causing localized or systemic illness is complicated by the fact the disease outcome is dependent on the immune status of the host. Most *Salmonella* serovars are able to cause systemic disease in immune-compromised individuals that those at extreme ages, the young and the old as well as those with underlying conditions such as HIV infection (Magwedere *et al.*,2015)

2.2 Non-Typhoid Salmonellosis as an Emerging Problem

The poultry industry is a fast growing sector for both small and large scale commercial farmers over the recent years in Zambia. The industry provides a cheap source of protein in the form of meat as well as eggs. The industry in Zambia largely comprises the chickens readily available on the market. However, due to the demand for other poultry species such as quails, these species of poultry are now sold in supermarkets, local trading markets and farms at point of sale for consumption and as source of revenue for poultry keepers.

In a study conducted by Hangombe *et al.,* (1999), the prevalence rate of *Salmonella* in poultry was estimated at 23%. The study also went to show that the presence of *Salmonella enteritidis* was much higher for Zambia as compared to other parts of the world for chicken poultry products at 4.7%. However, a recent study done by Ulaya (2013) found prevalence rates of Salmonella in poultry species in particular chickens at 16.9%

An emerging problem now is that of antimicrobial resistance globally and in particular Africa (Thakar *et al.,* 2005, Kariuki *et al*., 2006). Routine practices of giving antimicrobial agents to domestic livestock as a means of preventing and treating diseases, as well as promoting growth, is an important factor in the emergence of antibiotic-resistant bacteria that are subsequently transferred to humans through the food chain. Most infections with antimicrobial-resistant *Salmonella* are acquired by eating contaminated foods of animal origin (White *et al.,* 2001).

The burden of invasive *Salmonella* disease in Africa stands at 227 per 100,000 in population according to Gibani 2015. Invasive NTS disease was estimated to cause 3.4 million illnesses and approximately 690 000 deaths in 2010 alone according to Crump 2015. NTS stands as one of the leading enteric pathogens causing bacteraemia in young children in many parts of

the world including Africa (Berkley *et al*., 2006; Ikumapayi *et al*., 2007,). In a meta-analysis of studies done in Africa, *Salmonella* serovars accounted for at least 33.1% of all invasive NTS infections (Keddy *et al.,* 2015). In a study done in Congo, they had shown *Salmonella* enteritidis isolates of up to 79.7% showing multidrug resistance (Kalonji *et al.,* 2015). Whilst in South Africa, NTS is on the increase as an emerging pathogen associated with meningitis among HIV infected persons according to Keddy *et al.,* 2015. Likewise a study done in Zambia by Mwansa *et al.,* 2002, it was found that of 124 adults presented with persistent diarrhea caused by non typhoidal *Salmonella* was accounted for at 5% of the individuals whilst in the same study HIV related persistent diarrhoea in relation to NTS were at 11% and 16% in Rwanda and Kenya respectively. This is in agreement with W.H.O. 2013 reports suggesting that infectious diarrhoea is a frequently occurring worldwide disease and incidence of the condition is high in developing countries. From the findings mentioned above we can see how NTS is a problem in foodborne illness and the challenges it presents. It is for this reason that this study will look at the *Salmonella* strains and other bacterial strains their molecular characteristics present in quail species and how sensitive they are to antibiotics including their risk factors in quail species.

2.3 Quails

Quails have been farmed for a long time in many parts of the world and over the past few decades commercial quail farming has grown in many parts if the world including Africa . This has been seen in response to a fast growing market for the meat and the eggs they produce**.** It has also been documented that quails are now been used as experimental animals in biological research and in particular vaccine production for some diseases which they are seen to be resistant to like Newcastle disease (Alderton, 1992; Shanaway, 1994). The common quail is about 17.5 cm in length and can weigh about 70 to 155 grams in weight (Figure 2).

Figure 2. Quail species

Common quail are terrestrial, temperate and tropical birds. Grasslands are the general habitat of common quail. Dense, tall vegetation is preferred, while forest edges and hedgerows are avoided. Cultivated fields of winter wheat, clover, and small grain crops are also used as nesting cover (Johnsgard, 1988). The diet of quails consists of grains, seeds, nuts and insects

These birds have been considered as game animals for a long time until more recently commercialized for their meat and eggs for consumption. The common quail is closely related to the Japanese quail from the east which has well documented in other studies. According to Chege (2014) protein deficiency remains a major challenge all over the world and quail farming has been shown to be cheap and this has been used to fill up the nutrition gap.

These birds are known to produce rapidly and are easy to keep in confinement although they have not been commercially grown on a large scale as compared to broiler or layer chickens here in Zambia.

Commercialized quails such as Japanese quail (*Cortunix japonica)* are raised for meat and eggs. They reach market weight at 5- 6 weeks of age and they begin to lay their eggs at 6-7 weeks of age much earlier than broiler chickens at 22-24 weeks (Farooq et al., 2014) .

In developing countries, quail farming offers a viable and sustainable way of addressing the problem of animal protein shortage hence offering an alternative source of protein other than chicken production (Chege, 2014).

2.4 Bacteria and Antimicrobial Resistance in quails

In a study done by Roy *et al.,* (2006), bacteria *E. coli* was isolated from diseased quails and the bacteria was shown to be resistant to antimicrobials such as tetracyclines, ampicillin/cloxacillin, cotrimoxazole, chloramphenicol and nitrofuratoin. In other studies done in Iran isolates of *Campylobacter* were seen in quails at 43% of raw carcasses examined with antimicrobial resistance to tetracyclines and nalidixic acid. These studies then show that even as quails have been commercialized they are susceptible to bacterial infections and the organisms been shown to be resistant to antimicrobials, presenting as a serious concern.

The global food-products trade is expected to increase in the future. Thus, attempts to improve food safety must emphasize detection of antimicrobial drug–resistant bacteria

Salmonella species resistant to multiple antimicrobials agents have emerged worldwide according to Jiang *et al.,* 2005. This was shown with 3 *Salmonella* isolates that were isolated from quails as recovered under the Danish Institute for Veterinary Research in October, 2003. The serotypes isolated were of Virchow showing antimicrobial resistance to ampicillin, ceftiofur, nalidixic acid, and tetracycline and with reduced susceptibility to ciprofloxacin (MICs >0.125 µg/mL). Other studies have been done in quails located in the wild in Europe as opposed to quails commercialized for sale of their meat and their eggs. In a study done by Paulsen *et al*., 2012, a number of *Salmonella enterica* serovar isolates were recovered with highest prevalence rates been *S. typhimurium* and *S. enteriditis*. The study also showed that breeding game animals under intensive farming like the quails can create new epidemiological situations of *Salmonella* in the birds and transmission of these pathogens into other farm animal species.

2.5 Virulence and Rapid Detection of *Salmonella*

Characterisation of mechanisms underlying invasive manifestations by NTS is essential to understanding the real depth in the biology and pathogenicity of *Salmonella* (Suez *et al.,*

2013). Laboratory techniques such as PCR assays have now been recognised as means of detecting *Salmonella* and other pathogens. The molecular methods such as PCR were standardised as a tool for detection of food borne pathogens including *Salmonella* species as reported in study done by Malorny *et al.,* 2002. The PCR can also be used to amplify segments of particular genes such as *invA* that is responsible for the invasive characteristic of *Salmonella* to cause disease (Mercanoglu *et al.,* 2005). Other methods of identifying *Salmonella* include dot blot hybridization and Enzyme Linked Immuno-sorbent Assay (ELISA).

There is an epidemiologically important connection between poultry products and human infections because many of the serotypes that are most prevalent in humans (such as *Salmonella typhimurium* and *Salmonella enteritidis*) are similarly common in poultry Richard (2007). Serotyping is important in determining what strains of the organism are present and put in place appropriate interventions to control the disease in poultry species. A changing epidemiological pattern and their dynamics can also be addressed by knowing the specific strains present in specific poultry species. These bacteria can contaminate animal carcasses at slaughter leading to potential human illness through raw consumption of eggs or undercooked meat.

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Study Design

The study used a quantitative, analytical cross sectional study design in order to determine the prevalence and risk factors associated with *Salmonella and* selected enterobacteria in quails in Lusaka.

3.2 Study Setting

The study was conducted in Lusaka, the capital city of Zambia. Lusaka city lies on a plateau 1280 metres above sea level covering an estimated area of 360km2 and is located at 15º30' latitude south and 28º17' longitude east. It was conducted among small holder poultry farms that keep quails in production for consumption and sale. A total of 15 farms from across the city were sampled. They had the required age for birds going into the market and also were farmers that consented to answer the questionnaires.

Selection of participants (quail farmers)

Fifteen quail farms in Lusaka were identified and the consented farmers given questionnaires to answer.

Quails.

Inclusion criteria- farms with quails that enter the market for sale at 6 to 8 weeks and farmers that had consented.

Exclusion criteria-all poultry species except for quails, birds on recent antimicrobial therapy in last 2-3 weeks and farmers that have not consented.

Study Variables

Dependent Variable- *Salmonella, E. coli* and *Proteus* positive on culture

Independent Variable- Age of birds, veterinary services, other poultry species, recent use of antimicrobials and educational attainment of farmer, .

3.3 Sample size determination (Selection of participants)

The prevalence of *Salmonella and* other enteropathogenic isolates in quails in Zambia is unknown. However this study took the prevalence rate of Salmonella in chickens as an estimate at 16.9% (Ulaya *et al.,* 2015). This was considered this way because most poultry farmers may still keep chicken species on the same farm premises as quails. Then prevalence was set at 16.9% to yield the maximum value of sample size (n). Assuming that we would require the estimate to be within 5% of the true value in either direction at 95% CI, using a sample size formula by Kish Leslie for cross sectional studies, sample size was given by

$$ss = \frac{Z^2 * (p) * (1 - p)}{d^2}$$

This was done at confidence Level of 0.95 and desired precision of 0.05

Where:

ss= Sample size

Z = Z value for 95% confidence level (1.96)

p = 0.17

d = confidence interval (0.05)

Sample size will be given at 217 quails.

Probability Proportional to size sampling was then used for number of samples taken at each farm. The probability proportional to size sampling frame was used to arrive at the total number of 217 samples collected. This Sampling method was used to take into account the varying sample sizes in account. The total number of quails was recorded at each individual farm and the total sum arrived at for all fifteen farms. Thereafter, the number of quails sampled was derived from dividing the individual farm number of quails by the total number of fifteen farms multiplied by sample required. This study took the prevalence rate of *Salmonella* in chickens as an estimate at 16.9% cite to yield a maximum sample size of 217 The birds from individual farms were then selected through systematic sampling method at set intervals of ten.

3.4 Data Collection

Faecal samples were collected from anal openings of the birds at the farms. The samples were the subjected to standard laboratory diagnostic culture and biochemical tests. This was supplemented with questionnaires administered to the quail farmers. The questionnaire

included questions about management, use of antimicrobials, flock size and from which breeding farms they acquire quails. To reduce bias and enhance performance of the questionnaires, questions on *Salmonella* and other entero-pathogenic isolates were disguised among other health related conditions.

3.4.1 Data analysis

These were interview guided questionnaires. The factors included the socio-economic status and environmental conditions around the birds and the slaughtering process of birds going into the markets. The questionnaire had epidemiological questions which included the age of birds at point of sale, any history of sick birds. Others were husbandry practices such as vaccination, any medication used in birds, veterinary services sought and any diseases present or previously seen in birds. The information was used to generate knowledge, altitudes and practices of quail farming. Information from questionnaire was cleaned, checked for accuracy, consistency and completeness and then put on data base created in excel which was exported into STATA Version 12 for analysis. Summary statistics were then calculated. Association between possible factors such as socio-economic and environmental characteristics with the positive outcome of isolates in quails from the quail farms were analysed. All variables were included in the initial multiple logistic regression model and using the backward elimination method, variables which showed independent association at a significant level of p-value <0.05 were retained in the model.

3.4.2 Sample Collection

Faecal samples were collected aseptically from the anal regions of the birds using Amies sterile swabs. The faecal samples were kept cool at 4˚C in cooler boxes with ice. Double packaging of faecal swabs in sealable containers and later within sealable plastics was done to prevent cross-contamination of samples and associated packaging materials (OIE Terrestrial Manual, 2013). *Salmonella* may be isolated using various techniques that may include pre enrichment to resuscitate sub-lethally damaged *Salmonella*, enrichment media that may contain inhibitory substances to suppress competing organisms, and selective plating agars to differentiate *Salmonella* from other enter bacteria (OIE Terrestrial Manual, 2013) Then various biochemical and molecular tests were applied to the pure culture to provide a definitive confirmation of the isolated strain. Genetic technique such as PCR analysis was used to identify specific resistant genes as well as providing additional

information on the virulence of the isolates (Herrera-Leon *et al*., 2004; Porwollik *et al.,* 2004; Batchelor et al., 2008; Wattiau *et al.,* 2008).

3.4.2.1 Sample Preparation and *Salmonella* Isolation

The collected swabs were placed in Buffered Peptone Water as pre-enrichment medium. The samples were then incubated at 37°C for 24hrs. One ml of each pre-enriched culture were transferred into Tetrathionate (TT) Enrichment Broth (Oxoid Basingstoke, UK) and incubated aerobically at 42˚C for 48 hrs. Then two to three loopfuls of each enriched broth culture were streaked onto the surface of a selective medium Xylose Lysine Deoxycholate Agar (XLD) (Merck 2005), and then all plates were incubated at 37˚C for 24 to 48 hrs.

3.4.2.2 *E.coli* and *Proteus* Isolation and PCR

The swabs were labelled appropriately and collection of the swab samples was aseptically done. This involved the use of sterile swab sticks (Oxoid, Basingstoke, UK) which were placed in tubes containing a Carry-Blair transport medium (Oxoid, Basingstoke, UK). The poultry samples were inoculated on MacConkey agar (Oxoid, Basingstoke, UK) containing 2mg/L of cefotaxime (Sigma-Aldrich, Munich, Germany) for preliminary screening of ESBL producing bacteria (Rayamajhi *et a*l.,2008). The plates were later incubated at 37°C for 24 hours.

Colonies that grew on MacConkey agar were identified as lactose fermenters or non-lactose fermenters. Identification of *E. coli* lactose-fermenting positive colonies was done using phenotypic characteristics and confirmed by the Triple Sugar Iron (TSI) and IMViC tests as described by Rayamajhi et al.,2008 and Batchelor et al.,2005. For genetic detection, *E. coli* isolates were cultured on brain-heart-infusion broth (Nissui, Tokyo, Japan) at 37°C for 24 hours. After incubation, DNA was extracted by boiling methods (Reich and Klein 2013). The *E. coli* isolates were subjected to PCR for confirmation of resistance genes TEM (Temoniera), SHV (Sulphydryl Variable) and CTX-M (Cefotaxime –Munich) using primers previously used by other workers (Batchelor *et al*.,2005 and Ranjbar *et al*.,2008). The PCR (Finnzymes Oy, Finland) was performed in a total reaction volume of 10µl consisting of 5µl Phusion master mix, 2µl sterile distilled water, 2µl primers (forward and reverse) and 1µl bacterial DNA template. The PCR was performed using the rapid cycle DNA amplification method comprising of an initial denaturation step at 98ºC for 30 seconds, followed by 35 cycles of template denaturation at 98ºC for 1 second, primer annealing at 60ºC for 5 seconds and 72ºC for 1 second with final extension at 72ºC for 10 seconds. The PCR products were

later viewed with ethidium bromide after electrophoresis through 1.5% agarose gel (Clinical and Laboratory Standards Institute, 2009).

3.4.2.3 Antimicrobial Sensitivity Identification

The antimicrobial susceptibility testing was done using the Kirby-Bauer disc diffusion method on Mueller Hinton Agar (Becton, Dickinson and Company, MD, USA) based on the Clinical Laboratory Standard Institute (CLSI) guidelines, 2009. The antibiotic discs (Becton, Dickinson and Company, MD, USA) used included sulfamethoxazole/trimethoprim (1.25/23.75µg), ciprofloxacin (5µg), tetracycline (30µg), gentamicin (10µg), chloramphenicol (30µg), ceftazidime (30µg), norfloxacin (10µg) and cefotaxime (30µg). The phenotypic confirmation of ESBL isolates was done by the combination of disc approximation method using either ceftazidime (30µg) or cefotaxime (30µg) alone followed by over- night incubation at 37°C for 18 – 24 hrs. Interpretation of susceptibility patterns on other anti-microbial discs was done using guidelines laid down in the CLSI, which provides break points corresponding to zone of inhibition diameter. An increase in antibiotic zone diameter (5 – 12 mm) for either ceftazidime or cefotaxime indicated ESBL production (Clinical and Laboratory Standards Institute, 2009). Quality control standard laboratory procedures were strictly adhered to avoid contamination. *Escherichia coli* ATCC 25922 were used as a quality control organism

CHAPTER FOUR

RESULTS

4.1 Clinical Bacterial Strains

During the study period, no species of *Salmonella* isolates were recovered from the 217 birds sampled. However, 23 *E. coli* species and 30 *Proteus* species were isolated from the birds (Table 1). The overall proportions of isolates observed in the study were 10.6% *E. coli* and *Proteus* 13.8% as shown below

TABLE 1: Number of Bacterial Isolates and percentages

Bacterial isolates n(217)	Number of isolates	Percentage %	95% CI
Salmonella	0	0	
E.coli	23	10.6	7-15
Proteus	30	13.8	9-19
Total	53	24.4	

TABLE 2: *E. coli* isolates producing ESBL

Resistant Genes	*E. coli* n(23)	
	ESBL	Non-ESBL
	19	4
CTX-M	6	-
SHV	4	-
TEM	1	-

From the table 2, nineteen isolates were shown to produce extended spectrum beta lactamase. Six isolates were CTX-M positive while four were having SHV gene and one isolate was positive for TEM gene.

4.2 Antimicrobial Susceptibility

The positive isolates were then subjected to different antimicrobial agents for sensitivity using the disc diffusion method (Table 3).

E. coli species showed 86.9% and 100% sensitivity to Norfloxacin and Ciprofloxacin respectively while showing resistance to 91.3%, 100% and 86.9% to Co-trimoxazole, Cefotaxime and Ceftazidime respectively. There was 78% resistance to Tetracycline. All *E. coli* were sensitive to Gentamicin and 82% sensitive to Chloramphenicol.

Proteus species were 100% sensitive to Norfloxacin, Ciprofloxacin and Cefotaxime. However, sensitivities to Co-trimoxazole and Ceftazidime were 70% and 93% respectively. *Proteus* species showed more sensitivity to the antibiotics although 30% of the isolates showed resistance to Co-trimoxazole and 6 % resistant to Ceftazidime. All *Proteus* species were resistant to Tetracyline. *Proteus* were 90% sensitive to both Gentamicin and Chloramphenicol.

TABLE 3: Bacterial Sensitivities

Bacterial Isolates		Norfloxacin 10 µg	Sulfamethoxazole-trimethroprim 1.25/23.75 µg	Ciprofloxacin 5 µg	Tetracyline 30 µg	Cefotaxime 30 µg	Ceftazidime 30 µg	Gentamicin 10 µg	Chloramphenicol 30 µg
E coli (n=23)									
sensitive		20	2	23	5	-	3	-	19
resistant		3	21		18	23	20	23	4
Proteus (n=30)									
sensitive		30	21	30	-	30	28	27	28
resistant		-	9	-	30	-	2	3	2

4.3 PCR Findings

PCR amplification of target resistance genes was also investigated. Six isolates were found to have the epidemiologic plasmid encoding gene for the CTX-M of extended beta lactamases. While four were found to have SHV and one isolate for the TEM plasmids that encode for antibiotic resistance.

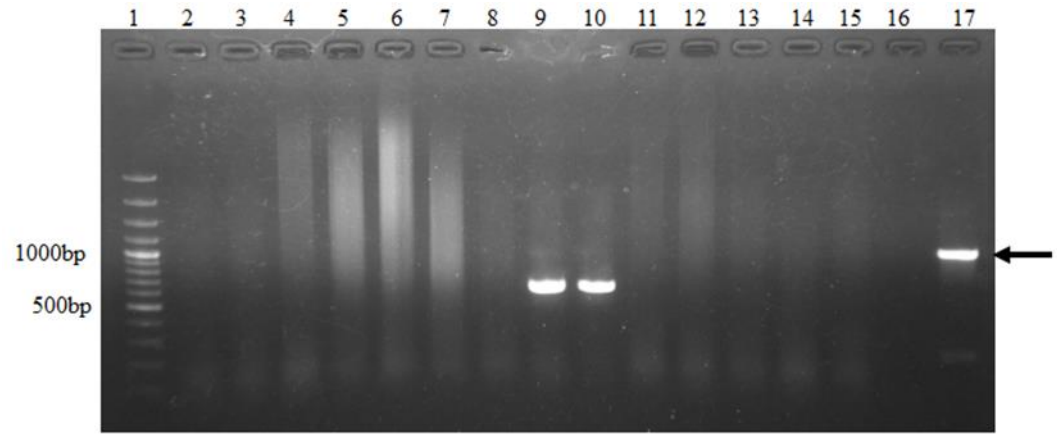

Figure 3: Electrophoresis pattern of isolates after PCR using CTX genes. Isolate No 9 and 10 were positive isolates for the CTX gene, while No 17 is a positive control. Lane 16 is a negative control and Lane 1 is a molecular weight marker. The positive amplicon is indicated by the arrow.

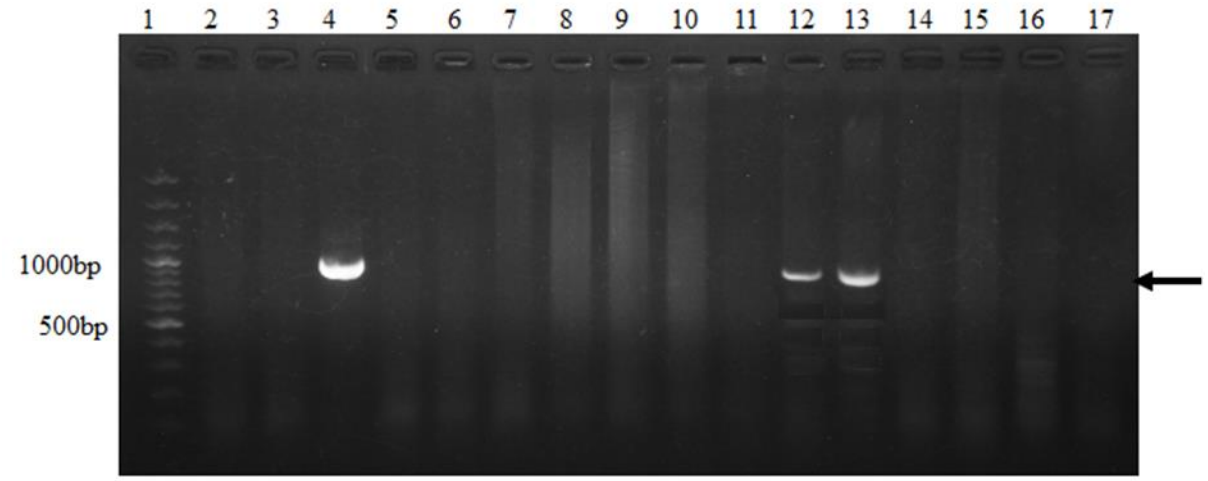

Figure 4: Electrophoresis pattern of isolates after PCR using SHV genes. Isolate No 12 and 13 were positive isolates for the SHV gene, while number 4 is a positive control. Lane 5 is a negative control and lane 1 is a molecular weight marker. The positive isolates are indicated by the arrow.

4.4 Farm Level Characteristics Influencing Bacterial Contamination

To identify the farm-level characteristics that were influencing the bacterial contamination/infection of quail meat in quail farms around Lusaka the study investigated the association between having a contamination/infection with a particular species of bacteria of public health significance and five variables for bacterial contamination/infection risk factors were assessed**.** These were age of the birds, use of veterinary services, other poultry species, employment status and education attainment.

4.4.1 Descriptive Statistics of Variables

TABLE 4: Study farms and sample sizes

Farm Id	N	% of Sample
1	18	8.3
2	11	5.1
3	15	6.9
4	21	9.7
5	13	6
6	8	3.7
7	25	11.5
8	12	5.5
9	10	4.6
10	13	6
11	10	4.6
12	8	3.7
13	10	4.6
14	13	6
15	30	13.8

1. **Measuring the Effects of the use of Veterinary Services on likelihood of *Proteus***

After adjusting for the confounding effects of all the hypothesised risk factors, we found that compared to quails on farms which did not use a veterinarians service, quail on farms which used a veterinarian's services were 85% less likely to be infected with *Proteus* spp. of bacteria [Adjusted Odds Ratio: 0.15 (95% C l, 0.04 – 0.56, $p<0.004$].

In contrast, quail on farms that employed the use of a veterinarian's services where 5 times more likely to be infected with *E. coli* bacteria compared to those on farms which didn't use the service [Adjusted Odds Ratio: 4.85 (95% C l, 1.25 – 18.83, $p<0.022$].

Table 5: Results of the bi-variate and multivariate regression analysis investigating

Independent Predictors of Infection	Bivariate Analysis			Multivariate Analysis		
	Crude OR	*p* value	95% CI	Adjusted OR	*p* value	95% CI
1. Flock Age At Testing (Ref: 6 Weeks)						
8 Weeks Old	1.47	0.330	0.68 3.20	1.14	0.895	0.16 7.98
2. Used Veterinary Services (Ref: Don't Use)						
Uses Veterinary Services	0.21	0.005*	0.07 0.61	0.15	0.004*	0.04 0.56
3. Rearing of Other Poultry (Ref: Only Quail)						
Rears Other Poultry	0.47	0.059	0.22 1.03	0.80	0.812	0.13 5.07
4. Employment of Owner (Ref: No other Employment)						
Has formal Employment	0.45	0.057	0.20 1.02	0.66	0.485	0.20 2.15
5. Owner's Formal Education (Ref: Primary)						
Secondary School Education	1.12	0.812	0.43 2.93	2.00	0.314	0.51 7.86
6. E. coli Infection Status (Ref: Not Infected)						
E. coli infected	4.15	0.004*	1.58 10.89	5.99	0.002*	2.00 18.13

determinants of infection with Proteus spp. bacteria in farmed quail in Lusaka, Zambia.

*Predictors of *Proteus* spp. infection with statistically significant effects ($p<0.05$)

2. Measuring the Effects of E. coli on likelihood of Proteus

After adjusting for the confounding effects of all the hypothesised risk factors, we found that compared to quails which were not infected with *E. coli*, quail which were infected with *E. coli* were 4 time more likely to be infected with *Proteus* spp. of bacteria [Adjusted Odds Ratio: 4.15 (95% Cl, 2.0 – 18.13, $p<0.002$]. Similarly, After adjusting for the confounding effects of all the hypothesised risk factors, we found that compared to quails which were not infected with *Proteus* species,, quail which were infected with *Proteus* spp were 4 time more likely to be infected with *E. coli* bacteria. [Adjusted Odds Ratio: 4.15 (95% Cl, 2.0 – 18.13, $p<0.002$]

3. Measuring the Effects of farmers Education level on likelihood of E . coli

After adjusting for the confounding effects of all the hypothesised risk factors, we found that compared to quails on farms whose owners only had primary school-level formal education, quails on farms whose owners had secondary school-level education were 84% less likely to

be infected with *E. coli* bacteria [Adjusted Odds Ratio: 0.16 (p<0.001, 95% Cl 0.03 – 0.49,]. In contrast, education was not a factor for *Proteus* infection

Table 6: Results of the bi-variate and multivariate regression analysis investigating determinants of infection with *E. coli* bacteria in farmed quail in Lusaka, Zambia.

*Predictors of *E. coli* infection with statistically significant effects (p<0.05)

Independent Predictors of Infection	Bivariate Analysis			Multivariate Analysis		
	Crude OR	*p* value	95% CI	Adjusted OR	*p* value	95% CI
1. Flock Age At Testing (Ref: 6 Weeks)						
8 Weeks Old	0.79	0.603	0.33 1.90	0.83	0.847	2.20 21.23
2. Used Veterinary Services (Ref: Don't Use)						
Received Training	1.26	0.599	0.53 3.03	4.85	0.022*	1.25 18.83
3. Rearing of Other Poultry (Ref: Only Quail)						
Rears Other Poultry	1.00	0.995	0.40 2.47	0.85	0.874	0.12 6.29
4. Employment of Owner (Ref: No other Employment)						
Has formal Employment	0.75	0.509	0.31 1.78	0.43	0.181	0.13 1.47
5. Owner's Formal Education (Ref: Primary)						
Secondary School Education	0.59	0.290	0.23 1.55	0.16	0.024*	0.03 0.49
6. E. coli Infection Status (Ref: Not Infected)						
Proteus infected	4.15	0.004*	1.58 10.89	6.82	0.001*	2.20 21.23

CHAPTER FIVE

DISCUSSION

As an emerging problem worldwide, gram negative bacterial organisms are increasingly becoming resistant to antimicrobials, in particular the extended-spectrum beta-lactamase (ESBL)-producing enterobacteriaceae. Several studies have documented organisms isolated from poultry in chickens, pathogens that have been associated with foodborne illness and that the occurrence of antimicrobial resistant strains of zoonotic bacteria constitutes a public health risk, increasing the risk of treatment failures as reported by Middleton and Ambrose, 2005. Quails previously considered as wild birds and a known delicacy in middle-eastern parts of the world but now we see this source of protein farmed for commercial production increasingly on menus in food outlets and indeed hotels sold out to the public in Zambia. The findings of this study reveal the occurrence of *Proteus* and *E. coli* species. Out of the 217 samples examined, the prevalence rate of *Proteus* organisms was found to be slightly higher at 13.8% than E. coli spp. at 10% from the domestic quails examined.

5.1 Determination of *Salmonella*

No *Salmonella* spp. were recovered from the 217 birds sampled as test results were consistently negative. In this cross sectional study, results revealed that despite none of tested quails showed characteristic clinical symptoms or pathological lesions, there were of some zoonotic bacterial species that were detected among them indicating asymptomatic or carrier infections. Bacterial strains of *E. coli* and *Proteus* species were isolated from the faecal swabs taken from quails however no *Salmonella* strains were recovered from the birds. This agrees with Teixeira *et al*., 2013 whose studies had shown also that no *Salmonella* isolates were seen but recovered other enterobacteria such as *E. coli* and *Proteus* among others. Other studies such Matankari (2014) have shown bacterial isolates of *Escherichia coli, Salmonella* species and *Pasteurella* species were highly prevalent in the investigated quail egg shells. For the *Salmonella* organisms alone, this is a remarkable result in the sense that it shows that it may not be a worrying danger for these food pathogens as a source of enteric infections associated consumption of protein contaminated at slaughter. Salmonellosis is an important disease worldwide and it remains important that surveillance of poultry species at farms. Monitoring of these zoonotic organisms, may not necessarily cause any health problems in animals but pose as a health challenge in humans through enteric infections.

5.2 *E. coli* and *Proteus* Isolates

Avian pathogenic *E. coli* strains that are responsible for Colibacillosis and is one of the most important economic loss through carcass rejection at slaughter facilities (FAO, 2002). However, commensal organisms such as faecal *E. coli* constitute a reservoir of resistance genes for (potentially) pathogenic bacteria. Resistant commensal bacteria of food animals might contaminate, like zoonotic bacteria, meat (products) and so reach the intestinal tract of humans (Bogaard and Stobberingh, 2000). From this study, 10.6% of *E. coli* isolates were accounted for. Antimicrobial susceptibility and PCR tests confirmed the isolates for having the antimicrobial resistance and the genes CTX-M (Cefoxatime-M). *E. coli* isolates were also subjected to PCR for confirmation of resistance genes TEM (Temoniera), SHV (Sulphydryl Variable). Six isolates were positive for CTX-M (Cefoxatime-M) genes, 4 isolates were confirmed having SHV (Sulphydryl Variable) genes and one isolate positive for TEM (Temoniera) gene. This TEM and SHV plasmids encode the genes responsible for antimicrobial resistance. This study did not establish how this six isolates had the CTX-M gene while other 13 did not have. This can be argued to imply a possible horizontal transmission of these resistant genes among the commensal bacteria in the gut (Ron, 2010).

5.3 Antibiotic Resistance of *E.coli and Proteus*

In avian species, antimicrobial agents are often continuously provided as antimicrobial growth promoters and this has resulted in increased antibiotic selection pressure for resistant bacteria, resulting in their faecal flora containing a relatively high proportion of resistant bacteria. The use of antibiotics has become the most important factor promoting the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (Bogaard *et al*., 2001)

E. coli species were resistant to this group of cephalosporins antibiotics and in particular cefoxatime at 100% and ceftazidime 86%. *Proteus* species isolates did not show ESBL properties but on antimicrobial tests there was 30% resistance to co-trimoxazole and 6% resistance to ceftazidime. All *Proteus* species were resistant to tetracylines while 78% of *E. coli* were resistant as were seen also in a study done by Roy *et al*., (2006). *Proteus* species or bacilli as there are well known now, are classified as opportunistic pathogens that cause illness in man. The results regarding the antimicrobial resistance pattern of *E. coli* isolates were in agreement with Roy *et al*., (2006) who found that the antimicrobial resistance pattern was 50% or more of that isolates were multi-drug resistant against oxtetracycline and

gentamicin. Sensitivity of bacterial strains to antimicrobials in the case of *E. coli* was higher for the third generation cephalosporins antibiotics cefotaxime and ceftazidime compared to *Proteus* species for as high as 100%. Use of cephalosporins in food producing animals could be a selective factor for the appearance of extended spectrum beta lactamases producing bacteria (Omoshaba et al., 2017). In addition, the bacterial organisms were highly resistant to broad spectrum antibiotic such as tetracycline favoured for its use in animal feeds to enhance growth and reduce disease incidence. It was further observed that *E. coli* was highly resistant to Sulfamethoxazole trimethoprim and Gentamicin as opposed to *Proteus* spp. These two antibiotics are fairly used more often for human treatments for urinary tract infections (Rodríguez-Ban˜o et al., 2004). The organisms were sensitive to other antibiotics such as chloramphenicol, norfloxacin and ciprofloxacin suggestive that they can actually be used in treatments to control for these bacterial infections. Prevalence rates of these organisms in this study are low compared to other studies; the findings of this research suggest that quails could emerge as new population reservoirs for antibiotic resistant pathogens in the case of *E. coli.*

There are three kinds of these opportunistic species *P. vulgaris, P. penneri and P. mirabilis.* It is now said that *Proteus mirabilis* has been implicated in urinary tract infections, wounds and infections of the gastrointestinal form arising from the consumption of meat products (Rozalski *et al*., 1997). Although the study did not identify which Proteus sub species they were and because of the potential virulence factors of *Proteus* bacilli, it still suggests that screening of these organisms and their potential for ESBL production should be routinely considered in quail species.

The significance of these findings is that, these organisms carry these enzymes to help in not breaking them down by these antimicrobials. These organisms become resistant to antimicrobial therapy hence the public health concern of resistant bacterial organisms that may find their way onto poultry carcasses at slaughter hence into the food chain thereby posing a health risk to mankind. Use of antimicrobial therapy in poultry as antimicrobial growth promoters in feed to enhance productivity and reduce infections may favour introduction of new resistant pathogens and if they find their way on meat products may present as a challenge for human treatments when consumed raw or undercooked.

5.4 Possible attributes and factors on the Public health significance of Identified bacteria

Several factors were hypothesized to assess whether certain attributes such veterinary service, age of birds, employment, education and the presence of other poultry species have on the risk of bacterial organisms isolated from the quails. Quails on farms which used a veterinarian's services were 85% less likely to be infected with *Proteus* spp. of bacteria adjusted for age of the birds, other poultry flock, employment and education status [Adjusted Odds Ratio: 0.15 ($p<0.004$ 95% Cl, 0.04 – 0.56]. This shows a significant correlation between use of veterinary services and *Proteus* status in the birds. A farmer who sought veterinary assistance was more likely to use therapeutic methods to reduce enteric organisms in the birds and reduce bacterial loads as seen in the isolates. This may also be supported by the correlation seen where education at secondary level reduced the likelihood of bacterial infection in the birds for *E. coli* but was not significant for *Proteus*. Several enteric organisms can be isolated from birds at different times and the interaction of these organisms may differ in proportions and the kind of sensitivity against antimicrobials. *Proteus* species was more sensitive to the antimicrobials as opposed to *E. coli* species.

In contrast, quail on farms that employed the use of a veterinarian's services where five times more likely to be infected with *E. coli* bacteria compared to those on farms which didn't use the service adjusted for other risk factors [Adjusted Odds Ratio: 4.85 ($p<0.022$ 95% C l, 1.25 – 18.83]. This does not tell as much about where the organisms are coming from except to show frequent use of veterinary services increased the *E. coli* count on the farms from the isolates recovered from the birds. It may also be that *E. coli* species increased in these birds because they were no longer sensitive to antimicrobials given through commercial feeds to enhance growth and hence flourish in the guts of these birds.

Veterinary services were then an important factor for less likelihood of *Proteus* infection which implies that farms that engaged veterinary input were less likely to suffer *Proteus* infection in birds. Therefore, this information emphasizes use of veterinary services help inform farmers on curbing infection through biosecurity measures but may be supported in that the species of *Proteus* isolated from these birds were sensitive to antimicrobials.

The results from this study may suggest interventions directed at the routine surveillance of these enteric organisms that may become opportunistic pathogens if meat products are contaminated. Secondly more studies that look at how quail keepers can use veterinary

services to help enhance production with the appropriate use of antimicrobials. The spread of zoonotic bacteria resistant to antibiotics is an important concern for the treatment of human infections, because it can compromise the effectiveness of the therapy (Kilonzo-Nthenge *et al.*, 2008). The study has shown that there are opportunities that exist for new health problems in quail species commercialized for meat or indeed their eggs emerging as new reservoirs of zoonotic entero bacteria.

Strengths and Limitations of the Study

The study only looked at quail farmers based in Lusaka and therefore generalizations of the study were limited to Lusaka town. However this the first time we have had a base line study on selected enteric microbes isolated from quail species and it would be important to screen this species, especially that they are now reared for commercial purposes, regularly to identify bacterial organisms that may carry resistant genes or even considered opportunistic pathogens. The results from this study provide useful information for surveillance purposes.

Methodological challenges

A lot of these quail farmers are small holder poultry keepers. This presents a challenge with keeping track of where the birds are sold to for consumption and also to the markets they go to such as supermarkets, restaurants, open markets and hotels as owners may not have written records. Written records for the purposes of tracing from which farms the birds are coming from is important in surveillance of zoonotic pathogens.

CHAPTER SIX

CONCLUSION

The aim of the study was to characterise and determine the public health significance of selected entero-pathogens in quails. The study findings revealed no *Salmonella* isolates. *E. coli* and *Proteus* species were isolated. The ESBL producing *E. coli* isolates were of significance in the role they play in resistance in poultry that may find their way into meat products on the market. This study has shown that multidrug resistance of *E. coli* species in poultry in particular quails may largely contribute to the wider and broad challenge of antimicrobial resistance and at the same time provide useful information for surveillance purposes. The use of antimicrobials does not necessarily lead to the selection of virulent strains but rather to the spread of resistance genes among commensal strains. It is also important to continue monitoring pathogens especially those of zoonotic nature such as *Salmonella* and maintain the status quo through effective surveillance.

CHAPTER SEVEN

RECOMMENDATIONS

There are research and policy implications for the study. This study has highlighted on the importance of conducting surveillance on enteric pathogens seen in other poultry species such as quails as opposed to chickens alone.

1. It is also important to investigate antimicrobial resistance seen in these poultry species and its impact on human health treatments.

2. There is need to look at prospective cohort studies and include all other quail farms in the Zambia.

3. The Ministry of Livestock and Fisheries at policy level, should control antimicrobial growth promoters in feed that are used to enhance productivity and their use monitored closely to discourage resistance of organisms that may find their way along the food chain.it is important to bring in veterinary services very close to the farmers that may help educate them on relevant use of antimicrobials.

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APPENDICES

Appendix 1: Participant Information sheet & Consent Forms

INFORMATION SHEET DOCUMENT FOR PARTICIPANTS

Study Title: isolation, Antimicrobial Susceptibility and Associated Risk Factors of *Salmonella* in Quails

Principal Investigator : Dr. Mukachikwikwi Hamakoko, 121 C Close Avondale, Lusaka. +260 977454945

Purpose of research project
This study is part of my research thesis in Masters in Public Health. The purpose of the research is to check the presence of bacteria *Salmonella* in faeces. The study aims at collecting fecal samples. After collection of samples, it is intended for the samples to undergo laboratory tests at the University Of Zambia. This is to confirm if the bacteria *Salmonella* are present or not. I also want to learn from the quail keepers if there is any more information that can help find out why the birds may have or may not have the bacterium by asking a set of questions.

Why you are being asked to participate?
Participants are farmers who are keeping quails and selling these quails on the market. You have been asked to participate because you fit these descriptions.

Procedures
If you agree to participate in the research:

- I will ask you to fill in a questionnaire. I will ask questions on what you know on health related topics on quails. Your name or your personal details will not be included in the typed documents. The questionnaire may take about 30 minutes to fill in.

Risks/discomforts
There are no physical risks to participating in the research. However, I recognize some information you may tell me or enter in the questionnaires may be personal. Some discomforts may be seen in the birds when taking fecal samples.

Benefits
There are no direct benefits to you.

Payment
There is no payment for participating in the research. However, I will inform you on the results.

Protecting data confidentiality

I have put up steps to protect the information I will get from you. First, I will make sure that your name is not included with any information. Only my research assistants and my supervisors at University of Zambia will have knowledge to the study information. The collected information will be locked in a secure place. I will keep copies of typed information on a flash disc in case i have a problem with the computer.

What happens if you do not want to participate?

You are free to decide whether you want to participate or not. Participants are free to withdraw from the study at any time and you may skip questions that you may not wish to answer.

Certificate of Consent

STUDY TITLE: **Isolation, Antimicrobial Susceptibility and Associated Risk Factors of Salmonella in Quails**

I have been asked to give consent to take part in the research study entitled "Prevalence, Antimicrobial Susceptibility, Molecular Characterization and Associated Risk Factors for *Salmonella* in Quails". This will involve taking part in answering questions from a questionnaire about knowledge, attitude and practices of quail keeping. I will also provide information on quail keeping. I have understood, read or it has been read to me and I fully understand all the information. I had the opportunity to ask questions and I was given satisfactory answers to my questions.

If the respondent is illiterate, a literate witness must sign and participants who are illiterate should include their thumb print as well. I have witnessed the accurate reading of the consent from potential participant and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

You are free to decide whether you want to participate or not. Participants are free to withdraw from the study at any time and you may skip questions that you may not wish to answer.

Who do I call if I have questions or problems?

- Call me, <<Dr. Mukachikwikwi Hamakoko, 121 C Close Avondale, Lusaka>>, at <<+260-977-454945>> if you have questions and complaints about the research.

- Call or contact ERES office for any ethical queries. The Ethics Committee contact information is:

  Address: 33 Joseph Mwilwa Road
  Rhodes Park, Lusaka

  Telephone : +260 955 155 633
  : + 260 955 155 634
  E-mail: eresconverge@yahoo.uk

What does your signature (or thumbprint/mark) on this consent form mean?

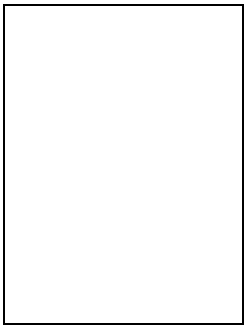
Your signature (or thumbprint/mark) on this form means:

- You have been informed about the research's purpose, procedures, possible benefits and risks.
- You have been given the chance to ask questions before you sign.
- You have voluntarily agreed to be in this research program

_____	_____	_____
Print name of Adult Participant	Signature of Adult Participant	Date

_____	_____	_____
Print name of Person/Witness Obtaining Consent	Signature of Person/Witness Obtaining	Date

Left Thumb Print below, if cannot put your signature



Translation to Chinyanja Kadunswa ka 3: Pepala ilangiza otenga mbali nakubvomekedza.

NKHANI YOLEMBEDWA YA OTENGA MBALI.

Mutu Wachobelenga: Kusankula,kugonja ku tudoyo todwalitsa matenda ndi chiopsezo cha Salmonella mu mbalani za nkwali.

Ofufudza: Dr. Mukachikwikwi Hamakoko, 121 Close, Avondale, Lusaka. +260 0977454945.

Cholinga chanchito yakufufudza.

Uku kubelenga nikwina kwambali yina yanga ya pepala ya masitala imene ninayanganapo zaulele za anthu. Cholinga chakubelenga kumene uku niku funa kuona ngati khungapedzeke kadoyo ka Samonella mamatubvi ya nkwali. Uku kufufudza kusayetsa-yetsa kusakila nakutenga matubvi ya nyoni zosiyana-siyana. Pambuyo potenga matubvi amenewa, yadzankhala yalikuikidwa mukupimiwa mumakina kusukulu lali khulu ya University of Zambia. Uku nikusimikidza kuona kuti ngati kadoyo ka Samonella kapedzekamo olo sikapedzekamo. Nidza funatso kufutsa kuli amene asunga nkwali ngati kuli nkhani yina inga tandidzile mukufufudza kwachifukwa nyoni zina zipedzeka nakadoyo aka kupyolela mukufutsa mafutso.

Chifukwa ninji mukupedwa kutenga mbali?

Otenga mbali ni alimi osunga nkwali ndipo ogulitsa kumutsika. Mwapedwa kutengako mbali chifukwa mwakwanilitsa zimene zilikulankhulindwa umu.

Mochitila.

Ngati mwabvomera kutengako mbali mukufufudza uku:

- Nidzakufutsani pali zamene mudziba pazaumoyo za nkwali. Khoma, dzindikilani kuti dzina lanu silidza lembedwapo pachipelala. Kuyankha mafudza amenewa kukatenga chabe nthawi itali monga pindi zili makhumi atathu (30 minutes).

Ziopezo/ Kusanzibvera bwino

Dzibani kuti kulibe ziopezo zilizonse mukuyankha mafutso. Khoma nidzindikila kuti nkhani yamene mudzalembamo kapena kuyika umu muchipepala chamafutso niya paumoyo wanu noka. Dzina zache zoipitsa dzidzaokela mutunyoni pa nthawi yotenga matubvi yachitsadzo.

Phindu.

Munchito imeneyi, kulibe phindu yomwe mudzapedzamo.

Malipilo.

Kulibe malipilo mukutenga mbali muku fufudza uku. Ngankhale telo, nidza ku udzani zimene zidzapedzeka pambuyo kwakusila kwakufufudza kumene uku.

Kuchingilidzidwa kwa nkhani yachitsitsi.

Nayika ndondomeko yo chingilidza nkhani yamene nidzatenga kwa inu. Choyamba nichakuti sinidza ika dzina lanu papepala na nkhani ili yonse. Oka adzadziba nkhani iyi ni aja otandidzila mukufufudza kuyikilapo na aja akulu-akulu akumupando oyatiya ganila mukubelenga uku ku Universitt of Zambia. Nkhani iza tengedwa idza nkhala yokomewa nakusungiwa apo pamene munthu wina sanga iwone. Nidzasungatso zina zipepala dza nkhani yamene iyi kuchitila kuti mwina mwache napedzeka nabvuto na computer, sininga sobe pogwila.

Nichani chingachitike ngati simufuna kutenga mbali?

Muli na ufulu obvomela kutengako mbali olo kusatenga. Otenga mbali ali na ufulu kuchokamo mukubelenga uku pa nthawi ili yonse. Mafutso amene simukwanisa kuyanka munga yasiye yosayankhiwa.

CHIGAWO CHACHIWILI

Pepala limene lilangidza kubvomekedza.

Mutu Wachobelenga: Kusankula,kugonja ku tudoyo todwalitsa matenda ndi chiopsezo cha Salmonella mu mbalani za nkwali.

Napempedwa kuti nisonyedze kubvomekedza kwanga kwa kutenga mbali mukubelenga uku ko fufudza na mutu uyu: “kuchuluka kwakadoyo, kaonekedwe kakadoyo nadzina zoipitsa za kadoyo ka Samonella mu nkwali.” Ichi chidzachitika kupyolela mukuyankha mafutso kuchokela muchipepala cholembewamo mafutso pali kudziba, maganidzo namachitidwe ya osunga nkwali. Nidzakudzani futi nkhani yo sunga nkwali. Nabvetsa-tsa, nabelenga kapena kuti anibelengela nkhani iyi ndipo nabvela mokwana zonse izi zili umu. Ninali nayo danga lofutsa mafutso ndipo ananiyankha mafutso yonse mokwana.

Ngati boyankha sa ziba kubelenga, opedzekako odziba kubelenga ayenela kudinda chidindo ndipo amene zadziba kubelenga ayenela kudinda chidindo nachiikumo chawo. Naonetsa-tsa kubelenga kwabwino kwakubvomekedza kuchoka kuli otenga mbali ndipo munthu uyu anali na danga lo futsa mafutso. Nasimikidza kuti munthu uyu abvomela kutenga mbali. Muli na ufulu otengako mbali olo kusatenga mbali. Otenga mbali ali na ufulu kuchoka mukubelenga uku pa nthawi ili yonse ndipo mafutso amene si akwanilitsa kuyankha anga asiye kulibe kuyankha.

Nindani ningatumile lamya ngati napedzeka mumabvuto?

- Tumani: Dr. Mukachikwikwi Hamakoko, 121, C Close, Avondale, Lusaka @ 260 0977454945. Munga futse ngati mwapedzeka namafutso.

- Tumani olo bwelani ku ERES Office ngati mwapedzeka mamafutso monga pali zamalamulo. Aka kabungwe ka malamulo kapedzeka ku: 33 Joseph Mwilwa Road, Rhodes Park, Lusaka. Telephone: 260 955155 633. 260 955 155634

Email: eresconverge@yahoo.uk

Kodi chidindo chachikumo chanu nakudinda kwanu pachipepala chobvomekedza kutandiza nji?

Ichi chidindo chako pachipepala chilangidza kuti:

- Waudzidwa cholinga chakufudza uku, mochitila, phindu nazoipitsa.
- Wapadzidwa nthawi yo futsa mafutso musane yike chidindo
- Mwabvomela nakudzipeleka mwa ufulu kutenga mbali mundondomeko yaku fufudza uku.

Lemba dzina/ opedzekako:

Chidindo cha otenga mbali mukulu:

Siku:

Lemba dzina/opedzekako obvomekedza:

Chidindo/opedzekako:

Appendix 2: Data Collection tools

Questionnaire

Dear respondent,

My name is Mukachikwikwi Hamakoko; I am a Masters student at the University of Zambia studying Masters in Public Health. I am carrying out a research on the Prevalence, Antimicrobial Susceptibility, Molecular Characterisation and Associated Risk Factors for *Salmonella* in Quail species in Lusaka. You have been randomly selected to participate in this research. The findings of this research are purely for academic purposes. Be assured that your responses will be treated with utmost confidentiality. Nothing that can reveal your identity will be published. Respond as sincere as possible. Please write, circle or tick appropriate responses in the spaces provided. For any concerns on this questionnaire, please contact myself on this number: **0977454945**.

Farm Id number:

Location:

Questionnaire #

Demographic Information

Sex female or Male

Age

Level of education: a. primary b. secondary c. tertiary

Employment status: Employed or Self employed

General information

1. How many quail birds do you have on your premises?
2. Do you have any other poultry species on the farm?
3. List the poultry species……
4. Where do you get the quails from?
5. What age are the quails taken for sale?
6. Do you slaughter the quails yourself? Yes or No
7. If not who does the slaughter of the quails?

Farm management

1. Do you have assistants who help you with the quails? Yes or No
2. Do you buy feed commercially or make your own?
3. How often do you disinfect the housing units?
4. What disinfectants do you use?
5. Do you have challenges with rodents? Yes or No
6. How do you control them?
7. Have you had any specific training with poultry management? Yes or No

Vaccinations

1. Do you vaccinate your birds? Yes or No
2. How often do you vaccinate your birds?
3. Who does the vaccinations?
4. What vaccines have you used?

Drugs

1. Do you have any disease challenges?
2. What disease has affected your quails?

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3. Do you have access to veterinary services?.....
4. What drugs do you use?.....
5. Are you guided by the vets or other specialists on how to use the drugs? Yes or No
6. Do you have the syringes and needles needed for use? Yes or