BACTERIOLOGICAL CONTAMINATION OF FARM AND MARKET KALE IN NAIROBI AND ITS ENVIRONS; PATHOGENICITY AND ANTIBIOTIC SENSITIVITY OF ISOLATED SALMONELLAE

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A thesis submitted in partial fulfillment of requirements for Masters Degree of University of Nairobi (Clinical Pathology and Laboratory Diagnosis)

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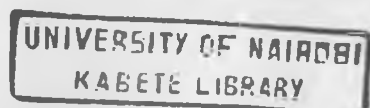

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This thesis is my original work and has not been presented for a degree in any other University

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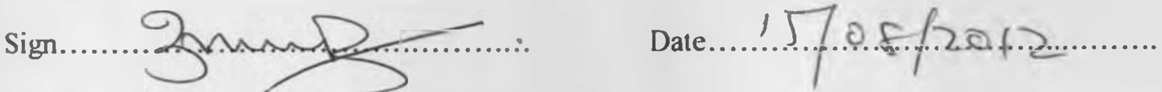

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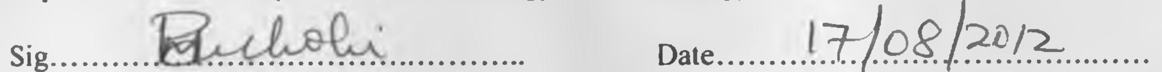

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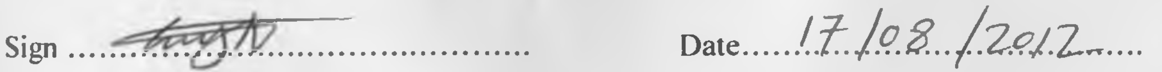

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DEDICATION

My wife and children

My parents, sisters and brothers

My friends

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TABLE OF CONTENTS

DECLARATION II

DEDICATION III

ACKNOWLEDGEMENTS IV

TABLE OF CONTENTS V

LIST OF TABLES X

LIST O FIGURES XII

LIST OF APPENDICES XV

LIST OF ABBREVIATIONS AND ACRONYMS XVII

ABSTRACT XIX

CHAPTER ONE 1

1.0INTRODUCTION 1

1.1 Null Hypotheses 4

1.2 Objectives 4

1.2.1 Overall objective 4

1.2.2 Specific objectives. 5

1.3 Justification for the study 5

CHAPTER TWO 6

2.0LITERATURE REVIEW 6

2.1 Contamination of vegetables by pathogenic bacteria 6

2.1.1 Pre-harvest contamination 7

2.1.2 Post-harvest contamination 8

2.2 Urban agriculture in Nairobi 8

2.3 Possible vegetable contaminants 9

2.3.1 Coliforms 9

2.3.2 *Escherichia coli* 10

2.3.3 *Salmonella* species 11

2.4 *Salmonella* and *Escherichia* species in non-host environment 11

2.5 Pathogenicity of *Salmonella* isolates in experimental mice 13

2.6 Resistance of *Salmonella* isolates to antibiotics 13

CHAPTER THREE 15

3.0MATERIALS AND METHODS 15

3.1 Sampling sites 15

3.2 Sample size 19

3.3 Sampling design for trader and farmer survey 21

3.4 Kale and water sampling for bacteriological analysis 22

3.5 Kale sample preparation 24

3.6 Isolation and characterization of isolates 24

3.6.1 Coliform count on kale samples 24

3.6.2 Confirmation of *Escherichia coli* 25

3.6.3 Fecal coliform count in water samples 25

3.6.4 Isolation of *Salmonella* organisms 26

3.7 Mice used for *Salmonella* experiments 27

3.8 Virulence testing (calculation of ID_{50}) of *Salmonella* isolated from kale and in-contact water, and from human clinical samples (for comparison sake): using mice 29

3.8.1 *Salmonella* organisms studied 29

3.8.2 Preparation of the stock suspension 29

3.8.3 Viable counting of the stock cultures 29

3.8.4 Mice inoculations and monitoring for clinical signs 30

3.8.5 Determination of infectious dose fifty (ID_{50}) 31

3.9 Pathogenicity testing for selected *Salmonella* isolates using mice 31

3.9.1 *Salmonella* serotypes used to inoculate mice 31

3.9.2 Bacterial dosage used to inoculate mice 32

3.9.3 Mice inoculation and observation 32

3.9.4 Liver bacterial counts 35

3.10 Antimicrobial susceptibility testing of the *Salmonella* isolates 35

3.11 Statistical analysis 36

CHAPTER FOUR 37

4.0RESULTS 37

4.1 Kale production practices in peri-urban areas of Nairobi 37

4.2 Practices of kale traders in the study area 45

4.3 Bacteria on kale samples and water used for irrigation obtained from farms 50

4.4 Contamination of kale and washing water with *E.coli* and *Salmonella* 53

4.5 Comparison of coliform counts between farm and market kale, respectively. 57

4.6 Summary on the *Salmonella* isolates from kale and contact water obtained from farms and market sites 59

4.7 Fifty percent infectious dose of *Salmonella* isolates 60

4.8 Pathogenicity of *Salmonella* isolates in mice 62

4.8.1 Observed clinical signs in mice 62

4.8.2 Haematological values 62

4.8.3 Biochemical values 63

4.8.4 Bacterial viable cell count in the liver 78

4.8.5 Gross pathology observed in inoculated mice 78

4.9 Antibiotic susceptibility testing of *Salmonella* isolates 79

CHAPTER FIVE 84

5.0DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS 84

5.1 Discussion 84

5.2 Conclusions 89

5.3 Recommendations 90

CHAPTER SIX 91

6.0REFERENCES 91

CHAPTER SEVEN 105

APPENDICES 105

LIST OF TABLES

Table 3.1: Ninety five percent confidence level for sample size to detect presence of organisms....20

Table 4.1: Sources of water for irrigation used for kale production in the peri-urban farms of Nairobi 38

Table 4.2: Sources of manure used for kale production in Nairobi peri-urban farms 43

Table 4.3: Methods of manure application in kale farming and the number of farmers practicing them in Nairobi peri- urban areas....44

Table 4.4: Mode of kale transport used by traders from the wet markets of Nairobi 47

Table 4.5: Methods of packaging kale used by traders in the wet markets of Nairobi....48

Table 4.6: Mean coliform counts and prevalence of *Salmonella* and *Escherichia*....51

Table 4.7: Faecal coliform counts of water samples used for irrigation at the peri-urban farms, Nairobi....52

Table 4.8: Mean coliform counts and prevalence of *Salmonella* and *Escherichia coli* on kale purchased from the market sites in Nairobi 55

Table 4.9: Mean fecal coliform counts (per 100 milliliters) of water samples used for washing kale at the wet markets, Nairobi 56

Table 4.10: Fifty percent infectious dose of *Salmonella* isolates monitored in 3-week old mice 61

Table 4.11: Haematological and biochemical parameters of mice inoculated with *Salmonella* isolate from human clinical source (CL1) 64

Table 4.12: Haematological and biochemical parameters of mice inoculated with *Salmonella* isolate from water source (KNG 14) 65

Table 4.14: *P* values obtained by comparing mean values of the parameters of *Salmonella* infected and control mice 67

Table 4.15: Sensitivity of *Salmonella* isolates to nine commonly used antibiotics 79

LIST O FIGURES

Figure 3.1: Farm and market sites 18

Figure 3.2: Sampling frame utilized for vegetable sampling 23

Figure 3.3: Mice housed in polypropylene plastic cages, with mice pellets (top view).......28

Figure 3.4: Investigator collecting blood sample from a nipped tail of a mouse using a capillary tube 34

Figure 4.1: River Athi waters used for irrigation by Athi river farmers 39

Figure 4.2: Waste water pond from a slaughter house used to irrigate vegetables by Athi River farmers 40

Figure 4.3: Peri-urban kale produced using animal manure in Ngong 42

Figure 4.4: Kale transported using open bags to wet market in Githurai 49

Figure 4.5: Coliform levels on kale samples from farms and markets 58

Figure: 4.6 Mean percentage packed cell volume in *Salmonella* infected and control mice. 69

Figure 4.7: Mean total erythrocyte count in *Salmonella* infected and control mice 70

Figure 4.8: Mean haemoglobin concentration in *Salmonella* infected and control mice.....71

Figure 4.9: Mean total leukocyte count in *Salmonella* infected and control mice 72

Figure 4.10: Mean percent neutrophil count in *Salmonella* infected and control mice.......73

Figure 4.11: Mean percent lymphocyte count in *Salmonella* infected and control mice.....74

Figure 4.12: Mean total protein content in *Salmonella* infected and control mice 75

Figure 4.13: Mean albumin content in *Salmonella* infected and control mice......................76

Figure 4.14: Mean globulin content in *Salmonella* infected and control mice....................77

Figure 4.15: Sensitivity of *Salmonella* isolates to nine commonly-used antibiotics............81

Figure 4.16: Frequency of multiple resistance to tested antibiotics among *Salmonella* isolates 82

Figure 4.17. Antibiotic sensitivity profiles for *Salmonella* Enteritidis from kale showing diverse sensitivity reactions to various antibiotic disks on Mueller Hinton agar...........83

LIST OF APPENDICES

APPENDIX 1: Interview questionnaire 105

APPENDIX 2: Coliform count (colony forming units per gram) on kale from the peri-urban farms 108

APPENDIX 3: Coliform count (per hundred millilitre) in irrigation water and washing water samples from farms and markets 109

APPENDIX 4: Coliform count (colony forming units per gram) on kale from the wet markets 110

APPENDIX 5: Coliform count (colony forming units per gram) on kale from the supermarket and high- end specialty store 111

APPENDIX 6: Percentage packed cell volume of *Salmonella* infected and control mice.112

APPENDIX 7: Total Erythrocyte count of *Salmonella* infected and control mice 114

APPENDIX 8: Haemoglobin content (grams per decilitre) in *Salmonella* infected and control mice 116

APPENDIX 9: Total leukocyte count of *Salmonella* infected and control mice 118

APPENDIX 10: Percent leukocyte differential count of *Salmonella* infected and control mice 120

APPENDIX 11: Total protein, albumin and globulin (grams per decilitre) of *Salmonella* infected and control mice 123

APPENDIX 12: Bacterial viable count (colony forming units per gram) in the livers of *Salmonella* infected and control mice 126

APPENDIX 13: Antibiotic sensitivities of the *Salmonella* isolates 127

Appendix 14: Inhibition zone diameter size interpretive standards for selected antimicrobial disks appropriate for *Salmonella* 128

Appendix 15: Names of the manufacturers of the media and reagents used 129

LIST OF ABBREVIATIONS AND ACRONYMS

ATCC- American type culture collection

BGA- Brilliant green agar

BO890:- code for *Salmonella* isolate from kale

BO973:- code for *Salmonella* from kale

CAC- Codex alimentarious commission

CDC-Centre for disease control and prevention

CFIA - Canadian food inspection agency

CL1- Code for *Salmonella* isolate from human clinical source

CL2- Code for *Salmonella* isolate from human clinical source

CL3 - Code for *Salmonella* isolate from human clinical source

DAEC- Diarrheagenic *Escherichia coli*

DLC-Differential leukocyte count

EaggEC- Entero aggregative *Escherichia coli*

EHEC- Enterohemorrhagic *Escherichia coli*

EIEC- Entero invasive *Escherichia coli*

EPEC- Enteropathogenic *Escherichia coli*

ETEC- Entero toxigenic *Escherichia coli*

FAO- Food and agriculture organization of the United Nations

FC- Fecal coliforms

FDA-Food and drug administration of the United States of America

Hb- Haemoglobin

HC- Haemolytic colitis

HUS- Haemolytic uremic syndrome

ICMSF-International commission on microbiological safety of foods

KEMRI- Kenya Medical Research Institute

KNG14- Code for *Salmonella* from water source

MKTNB- Mueller Kauffman tetrathionate novobiocin base

NACMCF- National advisory committee on microbiological criteria for foods

NCCLS- National committee for clinical laboratory standards

PCV- Packed cell volume

RVS- Rappaport vassiliadis soya broth

SPSS- Statistical package for social scientists

TEC- Total erythrocyte count

TLC- Total leukocyte count

VRBA- Violet red bile agar

VT- Verotoxin

WHO-World health organization

XLD- Xylene lysine deoxycholate

Abstract

This study aimed at determining the microbiological safety of kale (*Brassica oleracea Acephala*), a green leafy vegetable produced and sold in Nairobi. The assessment was carried out on kale and water (used for irrigation on farms and washing/refreshing of kale at the markets); the water being assessed as one of the sources of contamination for the kale. This was a cross-sectional study in which kale samples were collected from randomly selected farmers and traders. Data on farming and trading practices was collected using questionnaires administered through personal interview.

Samples were collected from selected peri-urban farms in Athi River, Ngong, and Wangige. They were also collected from traders from wet markets in Kawangware, Kangemi and Githurai, a supermarket and a high-end specialty store all within Nairobi city. Coliform counts, plus *Escherichia coli* and *Salmonella* species isolation and characterization were done. Isolated *Salmonella* were further analyzed for virulence, pathogenicity and antibiotic sensitivity. This study was necessary since there have been public health concerns over the poor practices in production and distribution of leafy vegetables in Nairobi and its environs.

Mean coliform counts on kale leaves from farms ranged from $1.6 \times 10^5 \pm 9.1 \times 10^4$ to $4.0 \times 10^5 \pm 1.3 \times 10^5$ colony forming units per gram (cfu/g) while those from the wet markets ranged from $1.1 \times 10^6 \pm 6.7 \times 10^5$ to $1.1 \times 10^7 \pm 3.0 \times 10^6$ cfu/g. Kale samples from supermarkets had a mean coliform count of $2.7 \times 10^6 \pm 5.5 \times 10^5$ cfu/g while those from high-end specialty store were $4.7 \times 10^5 \pm 2.3 \times 10^5$ cfu/g. Coliform numbers obtained on kale samples from the wet markets and the supermarket were significantly higher ($p<0.05$) compared to those from

farms. Kale samples purchased from high- end market had similar levels of coliform loads as those from the farms ($p > 0.05$). *Escherichia coli* prevalences in samples from farms ranged from 37.7% (6/16) to 81.1% (18/22). Those from the wet markets ranged from 33.3% (6/18) to 62.5% (10/16) while those from supermarkets and high-end specialty store, were 20% (5/25 and 3/15, respectively). *Salmonella* organisms were detected on 4.5% (1/22) and 6.3% (1/16) of samples collected from farms in Wangige and a market in Kawangware, respectively. It was also detected in 12.5% (1/8) of water samples used for washing/refreshing kale from a market in Kangemi. Fecal coliforms in water used on farms (for irrigation) and in the markets (for washing the vegetables) exceeded levels recommended by World Health Organization (WHO) counts of 10^3 per 100 milliliters.

Salmonella strains isolated from kale and water were virulent and pathogenic to mice though they exhibited significantly lower ($p<0.05$) pathogenic characteristics when compared to those from human clinical sources. They also showed resistance to only Penicillin, out of the 9 antibiotics that they were screened for; unlike those from clinical sources which showed multi - drug resistance.

This study has, therefore, demonstrated low bacteriological quality of kale and associated water in Nairobi and its environs. These may be carriers of pathogenic organisms which could cause disease to human consumers. The isolation of *Salmonella* organisms has further emphasized this, more so since the *Salmonella* isolates demonstrated resistance to some antibiotics. It is therefore recommended that good farming and handling practices be undertaken to increase the safety of leafy vegetables. Consumers are also advised to cook their vegetables well before consumption.

CHAPTER ONE

1.0 INTRODUCTION

Vegetables play a significant role in human nutrition as good sources of vitamins, minerals and roughage. They also contribute to the economy of many countries (FAO and WHO. 2008). As many as eight hundred million people in cities and towns world-wide are already cultivating crops in vacant plots, marginal lands, and in small private plots (Hussain *et al.*, 2001). The reasons for increase in production of vegetables particularly in and around cities in developing countries are: increased rate of urbanization, population growth and increase in demand for vegetables. Studies in nine African cities (Foeken and Mwangi, 2000) revealed that, on average, 35% of households engage in some form of agriculture; this could rise to over 70% depending on their location along the peri-urban to urban transect. It is, however estimated that at least 20 million hectares in developing countries are irrigated with low quality water (Dreschel *et al.*, 2002). This low quality water contains pathogens that can be a source of contamination for the vegetables (Karanja *et al.*, 2010).

In Kenya, vegetables are consumed regularly by nearly every household in rural and urban areas (Ayieko *et al.*, 2003). There is therefore a commercial vegetable sector that has developed both within Nairobi city and in surrounding areas, taking advantage of the availability of ready and rapid access to urban city markets (Prain *et al.*, 2007). About 3700 farmers within a 20 km radius of Nairobi centre practice irrigation agriculture and 36% of them use low quality water (Hide *et al.*, 2001) that could contain pathogens.

Contamination of leafy vegetables could also occur along the vegetable value chain; post-harvest processing can amplify contamination (CAC, 2006). These vegetables are exposed

to potential microbial contamination at every step including cultivation, harvesting, transporting, packaging, storage and retailing (FDA, 2004); water used to wash or refresh the vegetables at retail markets could also be source of harmful bacterial contamination. Compared to non-leafy vegetables, leafy vegetables are taken to be commodities of higher microbiological safety-concern since they have a higher potential to cause large and widespread food borne disease outbreaks. Kale is a green leafy vegetable that is largely produced and consumed by Nairobi city population; this is why it was chosen for this study. It is a fast growing crop, high yielding with high nutritional value. Its local name is *sukuma wiki* meaning "to push the week", referring to its importance to many poor people at times of financial hardship (Foeken and Mwangi, 1998).

Pathogenic bacteria of major concern on fresh vegetables are *Salmonella*, *Shigella*, *Escherichia coli* and *Klebsiella* species (Olayemi, 1997). The most commonly isolated bacteria have been *Salmonella* and *Escherichia* species (Olayemi, 1997; Beuchat, 1996). Total coliform count (including *E.coli*, *Klebsiella* and *Enterobacter* species) is normally used to indicate the level of gross bacterial contamination. *Escherichia coli* which is mainly excreted from an infected animal through faeces and urine consists non- pathogenic and pathogenic serotypes: - the worst serotypes being O157:H7 (Doyle *et al.*, 2006) and the recently emerging serotype O104:H4 (Woo *et al.*, 2006). Contamination of fresh produce by *Salmonella* organisms has also been documented (Wagner and McLaugh, 1986). Studies in some African cities show a high pathogen contamination on vegetables that occur during production and distribution (Amponsah *et al.*, 2010; Obaigeli *et al.*, 2010; Serani *et al.*, 2008). However, few studies have been done in Kenya to determine the vegetable safety

despite the public health concerns that have been raised over the poor urban and peri-urban production and distribution practices. This study has attempted to do so.

This study further focused on recovered serotypes of *Salmonella enterica,* one of the pathogenic bacteria that have been documented to contaminate vegetables. *Salmonella enterica.* comprises more than 2000 serotypes that are capable of causing a range of intestinal and extraintestinal infections in humans (Brenner and Murlin, 1998). In addition, the diverse *Salmonella* serotypes vary in their host adaptation and virulence (Baumler *et al.*, 1998);.the virulence genes being distributed on large genomic regions of 10-200 kb known as *Salmonella* pathogenicity islands (SPIs) (Hensen, 2004). Many *Salmonella* serotypes harbor large plasmids of varying sizes that carry genes responsible for their virulence (Gulig, 1990; Gulig *et al.*, 1993). Since plasmids are associated with pathogenicity (Hensen 2004), loss of respective plasmids would lead to reduced virulence; a feature that was assessed in this study as comparison was made between clinical *Salmonella* and *Salmonella* recovered from kale and in-contact water.

When antibiotic treatment is desired in severe cases of infections, the drugs of choice are usually ampicillin, third-generation cephalosporins (ceftriaxone) or fluoroquinolones (ciprofloxacin). Lately, emergence of *Salmonella enterica* with decreased susceptibilities to both classes of the fluoroquinolones and the cephalosporins has complicated treatment of salmonellosis (Gupta *et al.*, 2003; Harish *et al.*, 2006; Parvathi *et al.*, 2011). It was therefore important to establishe the antibiotic sensitivity/resistance of the isolated salmonellae.

The present study was designed to compare *Salmonella* isolates from kale/water environment with those from clinical cases, focusing on virulence/pathogenicity and antimicrobial sensitivity. Salmonellosis is endemic in Kenya yet the contribution of kale and associated water to the disease burden is unknown. Such a study will help to understand the infection potentials, virulence/pathogenicity and antimicrobial sensitivity exhibited by *Salmonella enterica* introduced into the environment. This, plus identification of other bacteria isolated in this study, will contribute towards formulation of preventive strategies for the disease- causing organisms.

1.1 Null Hypotheses

1. There are no bacterial contaminants in urban-farmed and market kale and associated water in Nairobi and its environs
2. Urban- farmed and market kale and associated water do not contain pathogenic, antibiotic resistant *Salmonella*

1.2 Objectives

1.2.1 Overall objective

To determine the bacteriological contamination of farm and market kale in Nairobi and its environs, and establish pathogenicity and antibiotic sensitivity of the isolated salmonellae.

1.2.2 Specific objectives.

1. To determine the bacteriological contamination of kale and water used for growing and washing/refreshing of kale at farm and market levels, respectively, with focus on *Salmonella* organisms.
2. To determine virulence of recovered *Salmonella* organisms using mouse model.
3. To establish pathogenicity of the *Salmonella* organisms in mice.
4. To determine the antibiotic sensitivity profiles of the *Salmonella* isolates.

1.3 Justification for the study

Rapid population growth in Nairobi city has led to high demand for vegetables such as kale. In trying to meet this demand, farmers have resorted to production of these vegetables in the urban/peri-urban farms under informal irrigation. This practice, coupled with poor hygiene practices amongst those concerned along the vegetable distribution chain, pose a great risk of contamination by pathogenic microbes and subsequent infection to farmers and consumers. However, scanty information on contamination level of vegetables by pathogens such as *Salmonella* and *Escherichia* exists. Determining this contamination level, as well as biological and pathogenic characteristics of the isolated *Salmonella* organisms, will contribute positively towards vegetable -safety and will help public health policy makers come up with relevant control measures.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Contamination of vegetables by pathogenic bacteria

Traditionally, vegetables have been considered low risk foods in terms of causing food borne illnesses as compared to food of animal origin. The assumption is that the vegetable pH is too low to support the growth of human disease-causing pathogens. The natural barriers of vegetables have also been known to prevent microorganisms from growing in them (Beuchat, 1996). However, currently, there seems to be change of pattern and one of the reasons for increased disease outbreaks is evolution of more pathogenic forms of bacteria that can now survive acidic conditions and refrigeration (FAO, 2002).

The Center for Disease Control and Prevention in the United States (CDC) has shown that food borne illness due to contaminated vegetables is on the rise (FDA, 2004). Investigations into these illnesses have identified issues such as agricultural water quality, use of manure as fertilizers, presence of animals in fields or packing areas, and the health and hygiene of workers handling the fresh produce during production, packing, processing, transportation, distribution or preparation (FDA, 2004). These sources of contamination can be grouped into pre-harvest and post-harvest sources (Beuchat and Jee-Hoon, 2005). Vegetable contamination can also arise from entry of pathogen through scar tissues, natural uptake of pathogens through root system, surface contamination and transfer of surface contaminants onto edible plant tissues during slicing (Beuchat and Doyle, 2007).

2.1.1 Pre-harvest contamination

Some of the sources of pre-harvest contamination include faeces, soil, irrigation water, water used to apply fungicides, insecticides, green or inadequately composted manure, wild and domestic animals, insects and human handling. Farm workers may be sources or vehicles for contamination of produce in the growing field. Machinery and equipment have also been considered to have the potential to transfer microbial hazards from contaminated areas to growing fields (FAO, 2002).

Organic wastes play an important role in providing nutrients to crops and improving overall soil quality and their use also provides a means of managing animal, human and plant wastes. However, pathogens associated with these manures may survive for extended periods thus contaminating the vegetables (FAO, 2002).

Irrigation water can also be a source of contamination of leafy vegetables. Microbiological quality of water derived from surface or subsurface sources is highly variable and can pose a risk when used for vegetable production. Potable supplies or rainwater stored in closed containment systems are considered safest for the production of leafy vegetables when they are delivered to crops through well maintained distribution systems. However, this is not practiced in most cities in developing countries (FAO, 2002).

2.1.2 Post-harvest contamination

Post-harvest operations can be very varied, from simple open-air packing to more sophisticated washing, drying and processing steps. Post- harvest contamination can thus be through faeces, human handling (workers, consumers), harvesting equipment, transport containers (field to packing shed), wild and domestic animals, insects, wash and rinse water, sorting, packing, cutting and further processing equipment. Other sources of contamination are transport vehicles, improper packaging, cross-contamination (foods in storage, preparation, and display areas), vermin and insects and improper handling after wholesale or retail purchase (FAO, 2002). Particular concerns have been raised regarding post-harvest processes where there is potential for altering leaf structure and forcing pathogens into the plant cells (infiltration or internalization), for example as a result of mechanical injuries during harvest or washing (FAO, 2002)

2.2 Urban agriculture in Nairobi

Use of wastewater and human faeces (known as "night soil") in peri-urban agriculture in Nairobi is both extensive and unregulated and so over a thousand farmers use untreated wastewater to grow vegetables and have been reported to block sewers to get the water (Kang'ethe *et al.*, 2007; Muneri, 2011). An assessment of surface water quality used for irrigation in informal irrigation in the peri-urban zone of Nairobi showed that the number of total and faecal coliforms exceeds the recommended levels set by WHO, which are 10^3 counts per100ml for unrestricted irrigation (Hide *et al.*, 2001; Karanja *et al.*, 2010; Muneri, 2011). Farmers in Nairobi consider use of waste water for crop production beneficial especially for the purposes of food security. However, few of them are ready to recognize this practice as having serious health implications (Muneri, 2011). Municipal authorities and

government ministries, along with the local population and media, have raised concern regarding the potential health threats posed by use of polluted waters to crop irrigators and consumers. However, frequently, there is lack of information on which sound judgments can be based (Hide *et al.*, 2001) and therefore the need for this study.

2.3 Possible vegetable contaminants

2.3.1 Coliforms

Coliforms are aerobic and facultatively anaerobic, Gram negative, non-spore forming, rod shaped bacteria that ferment lactose with acid and gas production within 48 hours at 37°C.They are differentiated from other microorganisms by their ability to grow in media containing bile salts and their use of carbon source to produce acid and gas (Yousef and Carlstrom, 2003). They include: *Escherichia, Klebsiella and Enterobacter* species.

Although their presence does not necessarily indicate that pathogens are present, coliforms are a good indicator of faecal contamination and poor hygiene. The World Health Organization (WHO, 2006) has recommended that when crops are likely to be eaten raw, water or effluent used for their irrigation should have been disinfected to achieve a coliform level of not more than 1000 coliforms per 100 ml in eighty percent of the samples. However, international guidelines or standards for the microbiological quality of irrigation water used on particular crops are not available, as crop-specific contamination data and disease transmission or infection data are not often collected (Cole *et al.*, 2008).

2.3.2 *Escherichia coli*

Escherichia coli (*E.coli*), a coliform, is commonly found in the lower intestine of warm-blooded organisms (endotherms). Cells are typically rod-shaped, and are about 2.0 micrometres (μm) long and 0.5 μm in diameter, with a cell volume of 0.6 – 0.7 $(\mu m)^3$ (Kubitschek,1990). It can live on a wide variety of substrates and uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Optimal growth of *E. coli* occurs at 37°C but some laboratory strains can multiply at temperatures of up to 49°C (Fotadar *et al.*, 2005).

Escherichia coli levels have been used since 1890 as nonpathogenic indicators of enteric pathogens, such as *Salmonella.* However, as knowledge of enteric diseases increased, investigators began isolating strains of *E. coli* that had acquired virulence characteristics that caused pathogenicity to humans and/or animals. Six classes of diarrheagenic *E. coli* are therefore recognized to date: Enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EaggEC), enteropathogenic (EPEC), and diffusely adherent (DAEC).The worst of these pathogenic strains are EHEC, symbolized by the serotypes O157:H7 (Buchanan and Doyle, 1997) and the recently emerging serotype O104:H4 (Woo *et al.*, 2006).

Escherichia coli serotype O157:H7 are characterized by their inability to ferment sorbitol. They are emerging food pathogens that produce Shiga toxins, potent cytotoxins which are lethal to humans at low doses. They are the causative agents of hemorrhagic colitis (HC), a severe bloody diarrhea which may progress to potentially fatal disease "hemolytic uremic syndrome (HUS)" (Robinson *et al.*, 2006). This study did not look into the presence of these

pathogenic strains. Though *Escherichia coli* are bacteria of animal origin, raw fruits and vegetables can become contaminated by improperly treated manure, contaminated soil and irrigation water, wildlife, and poor hygienic practices (CFIA, 2009).

2.3.3 *Salmonella* species

Salmonella are facultatively anaerobic Gram negative bacteria belonging to the family *Enterobacteriaceae*. The first strain of *Salmonella* was discovered and reported in 1885 by D.E. Salmon, a veterinary surgeon. Today there are more than 2400 known serotypes, grouped in the two species *S. bongori* and *S. enteric* (Petria and Angeli, 2008).

While *Salmonella* organisms have been associated with animal products including poultry meat, eggs and manure (Wagner and McLaugh, 1986), their significance as vegetable born bacteria is well documented (Ercolani, 1976). *Salmonellae* have been isolated from many types of vegetables (Beuchat, 1996). An example is its isolation from leaf surface of Dodo (*Amaranthus dubius*) from a contaminated site in Kampala (Cole *et al.*, 2008).The human health risk is increased further by the propensity of *Salmonella* to grow in precut salad mixes and on fresh produce moisturized during retail display at ambient temperatures (Beuchat and Doyle, 2007). However, there is limited data available on level of vegetable contamination by this pathogen in Kenya.

2.4 *Salmonella* and *Escherichia* species in non-host environment

Both *Salmonella* and *Escherichia* organisms spend a good part of their lives as residents of animal hosts (Mollie and Eduardo, 2003). The animal host is believed to be the primary habitat of these two enteric species. The organisms are thus endowed to do well in this environment which provides them with a warm constant temperature, as well as high

concentrations of free amino acids and sugars, which are conducive to bacterial growth (Savageau, 1974). Once excreted from an animal host, the organisms find themselves battling for survival, facing limited nutrient availability, osmotic stress, large variations in temperature and pH, and predation (Marshall, 1980).

Raw sewage is often discharged into low-temperature marine and aquatic environments, raising the question of whether human enteric pathogens like *Salmonella* and indicator organisms such as *E. coli* are able to adapt to and persist in these extreme environments. The exposure of these bacteria to extremely cold conditions of Antarctica has been shown to lead to reduction in their physiologic function (Mollie and Eduardo, 2003).

Non- host environment has been shown to lower the virulence and pathogenicity of these bacteria. For example *Salmonella* isolates from various sources revealed a correlation between invasion level in mice and the isolate source (Shoba *et al.*, 1996). Almost 100% of the isolates from human blood and animal organs were positive for virulence plasmid and were invasive while the waste water isolates contained no virulence plasmid and therefore not invasive in mice (Shoba *et al.*, 1996). However, some studies have reported that *Salmonella* isolates from environmental sources have similar and comparable virulence and pathogenicity to *Salmonella* from clinical sources (Parvathi *et al.*, 2011). Despite these findings, there is little documentation on virulence and pathogenic characteristics of isolates from non clinical sources. There was therefore, need to compare organisms isolated from kale/water used for irrigation or washing the kale with those from human origin, with respect to their virulence and pathogenicity.

2.5 Pathogenicity of *Salmonella* isolates in experimental mice

Various animal models have been used for pathogenicity study of human pathogens, including *Salmonella*, *Yersinia*, *Helicobacter* and *E.coli* (Suzana *et al.*, 2005). These include rats, mice, rabbits, guinea pigs, hamsters, calves and birds. In mice, *Salmonella* penetrates the intestinal epithelial barrier and spreads to the liver and spleen, and the respective mice eventually succumb to systemic infection (Hurley and McCormic, 2003; Suza *et al.*, 2005).

Salmonellosis in mice may exist as a latent or subclinical disease and animal death may occur due to flare-ups following stress (Donald and Holmes, 1984). Signs of acute and sub acute infections include diarrhea, anorexia, weight loss and a rough hair coat, while sub acute to chronic infection is characterized by splenomegaly and the presence of yellow/white military foci in the liver, spleen and lymph nodes (Donald and Holmes, 1984).

2.6 Resistance of *Salmonella* isolates to antibiotics

Antibiotic resistance in bacteria is a contemporary global public health problem (Levy and Marshall, 2004), Kenya not excluded (Bebora and Nyaga, 1989; Bebora *et al.*, 1994; Njagi *et al.*, 2004; Mapenay *et al.*, 2006; Kariuki *et al.*, 2004). Most reports suggest that the main force behind emergence of drug resistance is the use, misuse, and abuse of antimicrobial agents during the past decades, but there is also evidence that epidemic spread of drug-resistant bacteria could be a contributing factor (Livermore, 2003; Bebora *et al.*, 1994). Indeed, the rate of antibiotic resistance emergence is related to the total consumption of antibiotics, regardless of whether adequately used or not. However, antibiotic-resistant bacteria have been found in hosts and environments apparently free from any antibiotic pressure imposed by man (Caprioli and Donelli, 1991). In fact, as resistance is studied in

bacteria isolated from presumably nonselective environments unrelated to clinical ones, the emergence and spread of antibiotic-resistant bacteria have also been reported (Ash *et al.*, 2002).

When antibiotic treatment is desired in severe cases of *Salmonella* infections, the drugs of choice are usually ampicillin, third-generation cephalosporins (ceftriaxone) or fluoroquinolones (ciprofloxacin). Lately, emergence of *Salmonella enteric* with decreased susceptibilities to both classes of the fluoroquinolones and the cephalosporins has complicated treatment of salmonellosis (Gupta 2003; Harish *et al.*, 2006; Parvathi *et al*, 2011; Kariuki *et al.*, 2004). This is the reason why this study screened the *Salmonella* organisms isolated from kale and water used for irrigation and washing of the kale for antibiotic sensitivity and compared antibiotic sensitivity patterns with those shown by salmonellae from human clinical sources (reference strains code numbers, CL1, CL2 and CL3).

CHAPTER THREE

3.0MATERIALS AND METHODS

3.1 Sampling sites

The area covered by this study is Nairobi and its environs. Nairobi is located at latitude 1° 17' 0" South and longitude 36° 49' 0" East. It is at an elevation of 1670 metres above sea level and covers an area of 700 kilometre square (Google map, tracks for Africa, 2011. http://www.google.co.ke/#hl=en&cp=10&gs_id=2d&xhr=t&q=map+of+nairobi&pf=p&sclient=psyab&biw=1280&bih=503&source=hp&pbx=1&oq=map+of+nai&aq=0&aqi=g4&aql=&gs_sm=&gs_upl=&bav=on.2,or.r_gc.r_pw.,cf.osb&fp=1b2eb014a49ab5d9). The city and its environs receive 1,050 millimeters of rainfall which is bimodal, with the long rains falling between March and May and short rains between October and December. The mean annual temperature is 17°C, while the mean daily maximum and minimum temperatures are 23°C and 12°C. respectively. Irrigation activity is expected to occur during the dry months between June and September. .However, for more than 90% of urban and peri-urban farmers, irrigation is a year round activity (Hide *et al.*, 2001).

This study was conducted in the main vegetable growing peri-urban areas around Nairobi, namely Athi River, Wangige and Ngong. The three areas were selected on the basis of production practices and history of industrial activities. Athi River town is situated South of Nairobi city and its geographical coordinates are latitude1° 27' 0" South and longitude 36° 59' 0" East (Google map, tracks for Africa, 2011. http://www.google.co.ke/#hl=en&cp=10&gs_id=2d&xhr=t&q=map+of+nairobi&pf=p&sclient=psyab&biw=1280&bih=503&source=hp&pbx=1&oq=map+of+nai&aq=0&aqi=g4&aql

=&gs_sm=&gs_upl=&bav=on.2,or.r_gc.r_pw.,cf.osb&fp=1b2eb014a49ab5d9). It is host to several industries that discharge their wastes into the River Athi that passes through the town. Athi River farmers depend on the heavily contaminated river for supply of irrigation water, while a group of farmers use untreated effluent from the nearby slaughterhouse and meat processing factory (Hide *et al.*, 2001).

Ngong is located to the South West of Nairobi city at 1° 22' 0" South and 36° 39' 0" East, while Wangige is located at 1.3° South and 36.7° East, (Google map, tracks for Africa, 2011. http://www.google.co.ke/#hl=en&cp=10&gs_id=2d&xhr=t&q=map+of+nairobi&pf=p&sclient=psy-ab&biw=1280&bih=503&source=hp&pbx=1&oq=map+of+nai&aq=0&aqi=g4&aql=&gs_sm=&gs_upl=&bav=on.2,or.r_gc.r_pw.,cf.osb&fp=1b2eb014a49ab5d9). They both represent typical peri-urban farming communities whose enterprises are being impacted by rapid urbanization of Nairobi. Farmers in the peri-urban Ngong and Wangige sites depend on streams, shallow wells and boreholes for their irrigation water. These farm units are characterized by intensive use of animal manure (Hide *et al.*, 2001).

Markets were selected based on perceived sanitation; they represented market segments frequented by high income consumers (high-end specialty store/market), middle income consumers (supermarkets), and low income consumers (wet markets). Wet markets are normally characterized by poor sanitation while supermarkets are perceived to have relatively good hygiene than the wet markets. High-end markets are perceived to be the best in cleanliness, and therefore serve the high income residential estates (Ayieko *et al.*, 2003).

All these markets sell a variety of vegetables. Most of the markets were located in Nairobi while Githurai market was in the outskirts of Nairobi (Figure 3.1).

Figure 3.1: Farm and market sites (Source: Google map, tracks for Africa http://www.google.co.ke/#hl=en&cp=10&gs_id=2d&xhr=t&q=map+of+nairobi&pf=p&sclient=psy-ab&biw=1280&bih=503&source=hp&pbx=1&oq=map+of+nai&aq=0&aqi=g4&aql=&gs_sm=&gs_upl=&bav=on.2,or.r_gc.r_pw.,cf.osb&fp=1b2eb014a49ab5d9).

3.2 Sample size

Sample size was calculated according to the method described by Pfeiffer (2002).

$$N= [\log (1-\beta)] / [\log (1- d /N)]$$

(n=sample size, β = level of confidence in this case 95%, d= number of samples contaminated, N=population size, in this case infinite population). A simplified form of the formulae above by Pfeiffer (2002) is shown in Table 3.1. Since the same sample were being used for the analysis of both *Escherichia coli* and *Salmonella*, and *Escherichia coli* was expected to have a higher prevalence rate on kale than *Salmonella*, *Salmonella* prevalence was used to determine the least sample size required to detect the presence of both pathogens. An expected prevalence rate of 2% for *Salmonella* organisms was used to estimate the sample size in this study since similar studies (Mukherjee, *et al*., 2004; Serani *et al*., 2008) reported a prevalence rate of 0% and 2%, respectively. Therefore at least 149 samples were required for the study to detect the presence of the bacterial organisms.

Table 3.1: Ninety five percent confidence level for sample size to detect presence of organisms

	Prevalence					
Population size	0.1%	1%	2%	5%	10%	20%
10	10	10	10	10	10	8
50	50	50	48	35	22	12
100	100	96	78	45	25	13
500	500	225	129	56	28	14
1000	950	258	138	57	29	14
10000	2588	294	148	59	29	14
infinite	2995	299	149	59	29	14

Source: (Pfeifer, 2002)

3.3 Sampling design for trader and farmer survey

The list of kale traders in the wet markets (Kangemi, Kawangware and Githurai) were developed with the help of licensed local city council management. It included traders who traded during market and non-market days. The sampling list was developed through randomization, using a table of random numbers (Trek, 2010) http://stattrek.com/tables/random.aspx#examples). Randomization was also used to develop the replacement list in case a trader was not available for interview. Number of respondents interviewed in each market was based on number of traders in a targeted market. That is, probability proportionate to size sampling was used. The survey was conducted from early in the morning to late in the evening to enable large coverage of traders who trade at different times of the day. The questionanaire (Appendix 1) was pre-tested in one wet market (Kibera) before it was administered. A total of eighty traders were targeted (Githurai n=30, Kangemi n=24, Kawangware n=26).

The list of kale farmers was done with the help of agricultural officers and farmers' group leaders in all study sites and list verification was done prior to the survey. The sampling list was developed through randomization using a table of random numbers (Trek, 2010 http://stattrek.com/tables/random.aspx#examples). Randomization was also used to develop the replacement list in case a kale farmer was not available for interview. The number of respondents interviewed in each study site was based on size of the kale farmer population. That is, probability proportionate to size sampling was used. The questionnaire (Appendix 1) was pre-tested in farms in Kibera before it was administered. A total of one hundred and twenty kale farmers were targeted (Athi River n=24, Ngong=48 and Wangige=48).

3.4 Kale and water sampling for bacteriological analysis

The list of farmers and traders interviewed were again randomized to give the list of traders and farmers from whose farms/market-stalls samples were to be collected. A total of sixty samples (one sample per farm) were collected (Athi River n=16, Ngong=22 and Wangige=22). From each farm, kale leaves were picked randomly from various locations; moving in a zigzag pattern (Figure 3.2) after every four rows across the plot. Leaves were picked from lower, middle and upper part of each selected plant. Samples from each plot were then pooled, mixed and a respective sub sample of five hundred grams taken for analysis. The samples were pooled and mixed to ensure that the subsamples being taken for analysis were representative.

From each of the selected trader/market, sampling was done by picking kale bunches from the top, middle and lower part of the display unit/shelves. They were then pooled (to get a representative sample) and sub-sample of respective five hundred grams weighed and put into sterile paper bags without the sampler touching the vegetables, to avoid contamination. Permission was sought from the owners and payment made for the kale sample(s) collected.

Irrigation water (100 ml) samples were taken from the source where the farmer got water for irrigation. Samples from the streams were taken at the point where the farmers drew the water. Water used for washing/refreshing the vegetables at the markets were also collected from the containers in which traders put them. The respective water samples (100 ml) were collected into sterile plastic bottles and transported immediately to the laboratory in a cool box for bacteriological analysis.

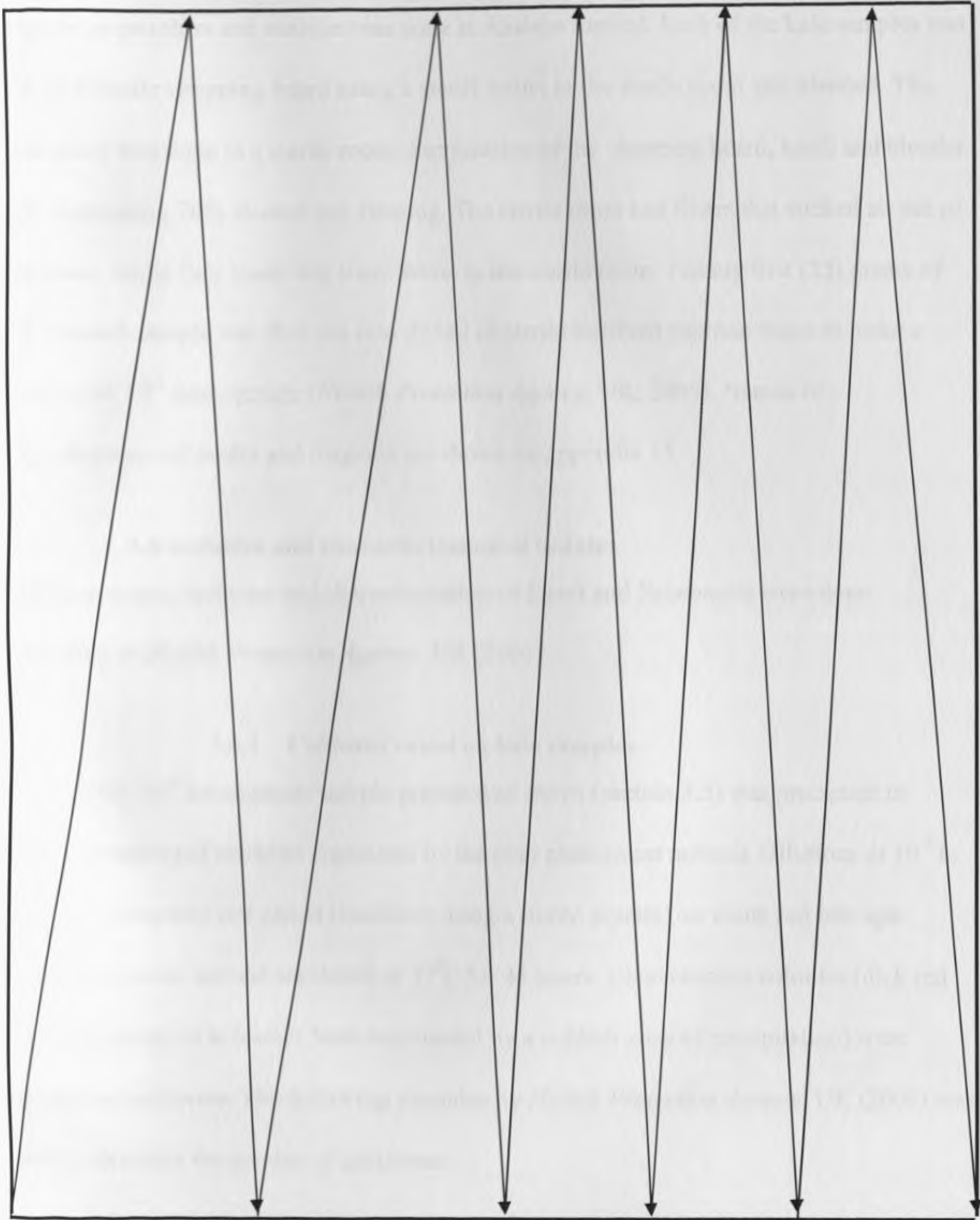

Figure 3.2: Sampling frame utilized for vegetable sampling (Roepstortff and Nansen 1998).

3.5 Kale sample preparation

Sample preparation and analysis was done at Analabs limited. Each of the kale samples was cut on a sterile chopping board using a sterile knife; in the sterile room and blended. The procedure was done in a sterile room. Sterilization of the chopping board, knife and blender was done using 70% alcohol and flaming. The sterile room had filters that sucked air out of the room, while face mask was worn while in the sterile room. Twenty five (25) grams of the blended sample was then put into 225ml of sterile buffered peptone water to make a dilution of 10^{-1} homogenate (*Health Protection Agency*, UK, 2005). Names of manufacturers of media and reagents are shown on appendix 15.

3.6 Isolation and characterization of isolates

Coliform count, isolation and characterization of *E.coli* and *Salmonella* were done according to *Health Protection Agency*, UK (2005).

3.6.1 Coliform count on kale samples

Each of the 10^{-1} homogenate sample prepared as above (section 3.5) was processed to enable counting of coliform organisms by the pour plate count method. Dilutions of 10^{-2} to 10^{-5} were prepared and plated (1mililitre using a sterile pipette) on violet red bile agar (VRBA) (Oxoid ltd) and incubated at 37^{0}C for 48 hours. Characteristic colonies (dark red with a diameter of at least 0.5mm surrounded by a reddish zone of precipitation) were counted as coliforms. The following formulae by *Health Protection Agency*, UK (2005) was used to calculate the number of coliforms:

$$N= \sum C [V (n_1 + 0.1n_2)]d$$

Where, N = number of colony forming units per milliliter

$\sum C$ = sum of all colonies on all petri dishes counted

n_1 = number of petri dishes in the first dilution counted

n_2= number of petri dishes in the second dilution counted

d = dilution for which the first count were obtained

V = volume applied to each petri dish

3.6.2 Confirmation of *Escherichia coli*

Five (5) different colonies were picked from each of the VRBA plates, inoculated onto MacConkey plates and incubated at 37°C to get pure cultures. One colony from each of the purity plates were picked and inoculated into tryptone water (for indole test), incubated at 44°C for 24 hours, Kovacs solution added and shaken gently. Those that were indole positive (formation of a red ring at the top of the broth) were taken as confirmed *E.coli* organisms. Only indole test was done because at this stage, only coliforms (*E.coli, Klebsiella and Enterobacter*) were being dealt with.

3.6.3 Fecal coliform count in water samples

Faecal coliforms in water were counted using the standard most probable number technique according to Bergey's manual of 2005. Each water sample was inoculated into MacConkey purple broth (Oxoid ltd) as follows: 50 ml of water sample was added to the bottle containing 50ml (double strength) of MacConkey broth, 10 ml of water sample was added to each of the five universal bottle containing 10 ml (double strength of broth) and 1ml of water sample to each of the five tubes containing 5 ml of (single strength) broth. Each tube

had inverted Durham tube in it. The contents of each bottle or tube were mixed and incubated at 44°C for 48 hours in a water bath. A positive test was indicated by gas produced (collected in the durham tubes), yellow colour and turbidity of the contents. To confirm the presence of *E.coli*, tubes of trypton water were inoculated with samples from positive bottles or tubes and incubated at 44°C for 24 hours. Kovacs reagent was added to each tube and checked for formation of a red ring on top. A standard Most Probable Number was used to show an estimate of faecal coliforms (Bergey, 2005).

3.6.4 Isolation of *Salmonella* organisms

The buffered peptone water used to prepare the homogenate of the blended kale (done in section 3.5) was used as the primary pre-enrichment media. The homogenate was incubated at 37°C overnight. Secondary pre-enrichment was then done by transferring 1ml of the primary pre-enrichment culture to 10ml of Mueller Kauffman tetrathionate novobiocin broth base and another 0.1ml transferred to Rappaport-Vassiliadis soya peptone broth (RVS) and both incubated at 37°C for 24 hours. Loopfuls from each of the secondary pre-enrichment cultures (MKTNB and RVS) were used to streak onto plates of brilliant green agar (BGA) and xylose lysine deoxycholate agar (XLD) (Oxoid ltd), respectively, and both incubated at 37°C for 24hours. *Salmonella* organisms on XLD were recognized as red colonies with black centres or isolated colonies which appeared yellow with black centres. On BGA *Salmonella* organisms appeared as red colonies surrounded by a bright red medium. The two secondary pre-enrichment media and two selective media were used to increase the chances of picking all *Salmonella* strains. Pure cultures of at least five suspect *Salmonella* colonies including one from each plate of the selection agar were prepared by subculturing, respectively, onto MacConkey agar plates and incubating at 37°C for 24hours.

They were then screened biochemically using triple sugar iron agar slope and urea agar. Those which showed positive reaction (yellow butt and deep pink slope with/without blackening) and urea negative were further processed for serotyping.

Final confirmation was done with analytical profile index 20E (API 20E) system from Biomerieux limited. Serotyping was carried out using the serological reagents from Statens Serum Institut (SSI) Diagnostica's *Salmonella* sero-quick ID kit *(Health Protection Agency*, UK, 2005). The same method was used in the isolation of *Salmonella* in water samples except that there was no blending of the sample.

3.7 Mice used for *Salmonella* experiments

Balb C mice aged three weeks, both male and female, were used. The mice were housed in polypropylene plastic base cages, with lids made of straight stainless steel wire (Figure 3.3). They were provided with feed and water *ad libitum* while wood shavings that were changed after every two days were provided as beddings. The animals were randomly grouped and identified by coded body marks using picric acid.

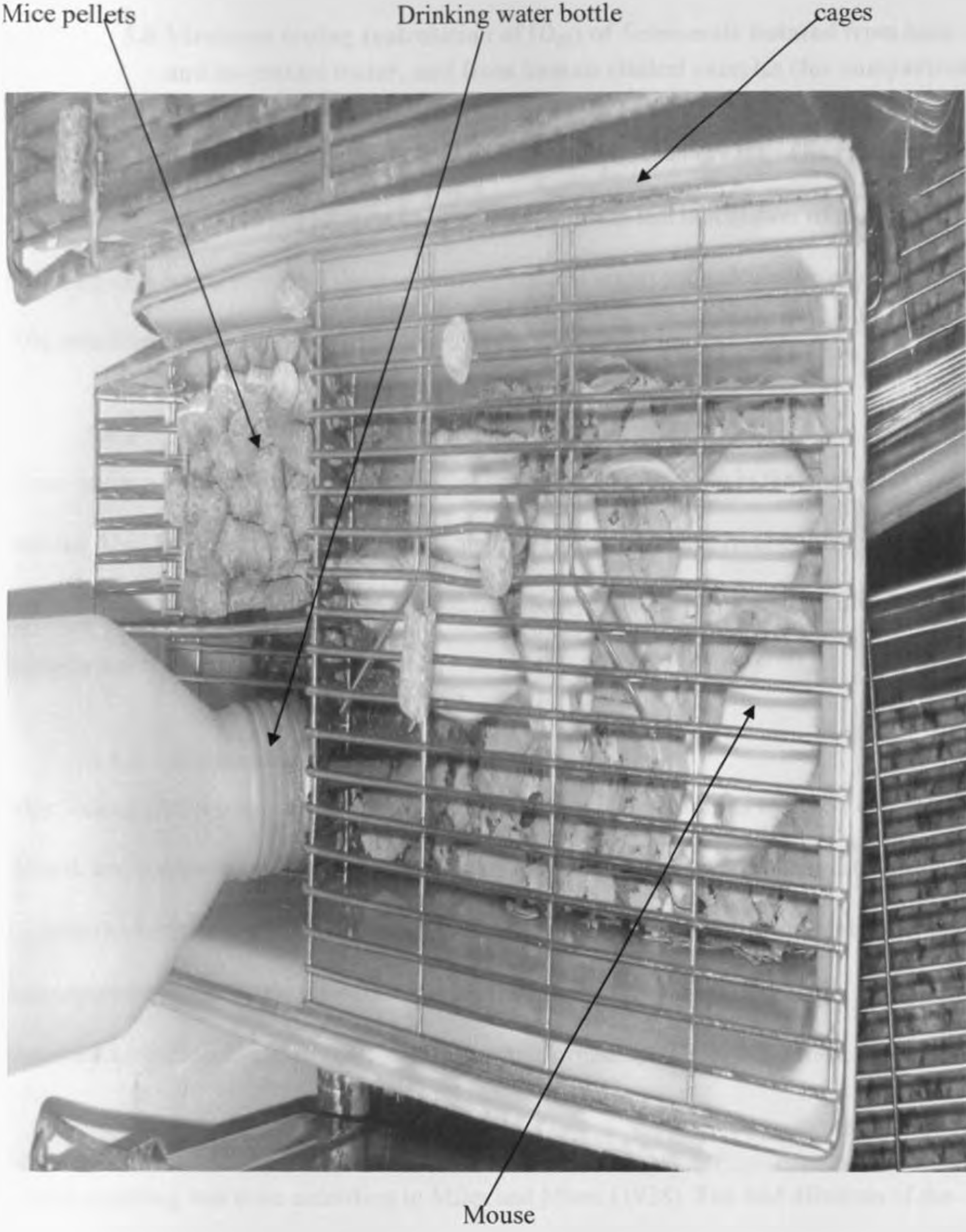

Figure 3.3: Mice housed in polypropylene plastic cages, with mice pellets (top view)

3.8 Virulence testing (calculation of ID_{50}) of *Salmonella* isolated from kale and in-contact water, and from human clinical samples (for comparison sake): using mice

This was done following the method given by Reed and Muench (1938). The procedure consisted purification of the bacteria to be studied; dilution and inoculation of mice; recording number of mice that die and/or show clinical signs; and calculation of respective 50% infectious dose (ID_{50}).

3.8.1 *Salmonella* organisms studied

Three environmental isolates (one from water used for washing kale in the market and two isolated from kale from farm and market) and three known isolates from human clinical sources (used as reference strains, acquired from Kenya Medical Research Institute) were used for the virulence study.

3.8.2 Preparation of the stock suspension

MacConkey plates were seeded with respective *Salmonella* organisms to give a confluent growth and incubated at 37°C for sixteen hours. The bacteria were then harvested using 4 milliliters of sterile physiological saline 0.85% w/v Sodium chloride, poured into each plate and scraped using a sterile glass rod. The harvest was then put into a sterile test tube and was used as the stock suspension for the respective organism (Miles and Misra, 1938).

3.8.3 Viable counting of the stock cultures

Viable counting was done according to Miles and Misra (1938). Ten fold dilutions of the harvest ranging from 10^{-2} to 10^{-10} were made. For each dilution, drops were made onto petri dishes that were demarcated into 4, containing MacConkey agar, using a micropipette capable of dropping 25 microliters per drop (equivalent to 40 drops per milliliter; volume

for each drop being 1/40th of a milliliter). Two separate drops were dispensed (one on separate quarter; labeled with the dilution factor); for each dilution the drop was allowed to dry before the plates were incubated aerobically at 37°C for 24 hours. The plates were then examined and counts of colony-forming-units made at the dilution giving up to 10 separate colonies. The concentration of the original harvest was calculated using the formula:

$$n \times 40 \times 10^{x}$$

Where n is the average number of colony-forming – units for the two drops, 40 is the number of drops that make one milliliter, and 10^{x} is the dilution factor. This was then used to estimate the concentrations of the other dilutions.

The set of dilutions were kept in the refrigerator until the next day (when the counting and calculation of concentrations were done). They were used in the next procedure (Section 3.8.4).

3.8.4 Mice inoculations and monitoring for clinical signs

The bacterial dilutions prepared for viable counting in Section 3.8.3 above (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10}) for each isolate were used to inoculate mice. Nine groups, each consisting five three-week-old mice; both male and female, were inoculated intraperitoneally with 0.4 ml of the respective bacterial suspension. Control group consisted five mice inoculated with sterile normal saline. Different groups were identified using different coloured dyes. The mice were monitored for 7 days for sign(s) of infection and/or death. The number of mice which showed clinical signs (rough hair coat, anorexia, reduced movement and dullness) and those which did not was recorded for each dilution used. Two

randomly-selected control mice were aseptically sacrificed before and after the experiment; their livers and spleens were cultured to ascertain that the uninoculated-mouse population was free from *Salmonella* organisms. Quality control (to check for possible contamination) was also done on the bacterial suspensions used for mice inoculations, before and after inoculation; this was done through inoculation of the diluted organisms onto MacConkey agar.

3.8.5 Determination of infectious dose fifty (ID_{50})

Infectious dose fifty calculations were done using the Reed and Muench (1938) mathematical formulae as shown below;

Index = (% infected at dilution immediately above 50% - 50%)/ % infected at dilution

immediately above 50% - % infected at dilution immediately below 50%

3.9 Pathogenicity testing for selected *Salmonella* isolates using mice

3.9.1 *Salmonella* serotypes used to inoculate mice

Two *Salmonella* serotypes were used for this experiment; they included one environmental isolate (KNG 14), that was shown to be the most virulent, and the human clinical isolate (CL1) acquired from Kenya Medical Research Institute (KEMRI). They both happened to be *Salmonella* Typhimurium. The CL1 isolate (referred to as *Salmonella* serotype from human clinical source) was used as reference strain.

3.9.2 Bacterial dosage used to inoculate mice

The dosage used for inoculation was 2 x respective ID_{50} values, determined earlier in the virulence study. The identified dose was calculated using the formula:

$$\frac{RV}{O} = \text{Volume to be diluted}$$

Where, R – required concentration, V = required volume, O. = Original concentration

The dose for CL1 (human strain) was 1.4 x10^4 viable cells per mouse; that for KNG 14 (environmental strain; from water) was 1.2 x10^6 viable cells per mouse.

3.9.3 Mice inoculation and observation

Twenty four, three-week old mice, both male and female, were put into three groups of eight each and caged separately. One group was inoculated intraperitoneally with CL1 (1.4 x10^4 organisms in 0.4 ml per mouse) and the other with KNG 14 (1.2 x10^6 organisms in 0.4 ml per mouse) isolate. The control group was inoculated with 0.4 ml of sterile physiological saline 0.85% Sodium chloride w/v (for comparison sake since normal saline were used to reconstitute the concentrations of the organisms) per mouse. Each animal was observed daily for development of any clinical signs (dullness, roughening of hair coat, reduced movement and anorexia). Blood parameters (packed cell volume, haemoglobin concentration, total leukocyte count, differential leukocyte count, total protein, albumin and globulin) evaluation and necropsy examination was done. Blood samples for haematology and biochemistry were collected by tail puncture and usage of capillary tube (Figure 3.4) on days 0, 3, 7 and 14 post inoculation. Smears for differential blood cell counts were prepared from fresh blood and stained with Giemsa. Packed cell volume (PCV), total erythrocyte

count (TEC), total leukocyte count (TLC) and haemoglobin (Hb) concentration were estimated as described by Benjamin (1978).

Post mortem examinations were done on mice that died during the experimental period while those which survived were randomly selected (two from each group) and sacrificed at intervals of 3, 7 and 14 days post inoculation. Mice were sacrificed by gently striking at the back of the mice head with a small rod. It was then dissected on dissecting board using sterile surgical blades. The dissection tools (surgical blades, forceps and dissection board) were sterilized using 70% methanol then flamed. Gross pathological examination for lesions was done. Samples of livers were collected aseptically into sterile petri dishes for bacterial culture and viable cell count using plate count method of Miles and Misra (1938).

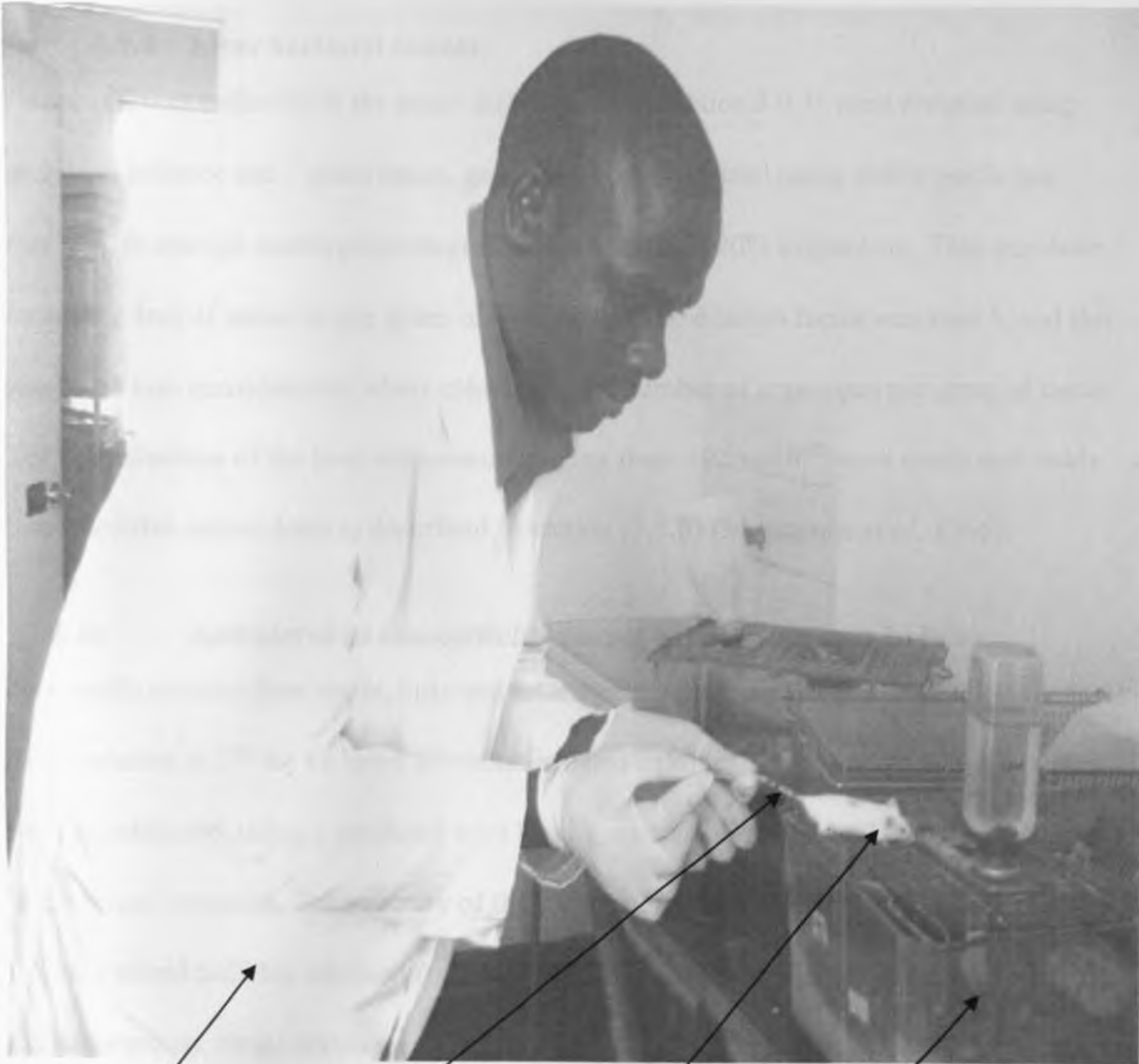

Figure 3.4: Investigator collecting blood sample from a nipped tail of a mouse using a capillary tube

3.9.4 Liver bacterial counts

Pieces of livers collected in the petri- dishes (done in section 3.9.3) were weighed using analytical balance and 1 gram taken, ground and homogenized using sterile pestle and mortar with enough sterile physiological saline to make a 20% suspension. This was done by adding 4ml of saline to one gram of liver tissue. The dilution factor was thus 5, and this was taken into consideration when calculating the number of organisms per gram of tissue. Ten fold dilutions of the liver suspension ranging from 10^{-2} to 10^{-10} were made and viable liver bacterial counts done as described in section (3.8.3) (Mackaness *et al.* 1966).

3.10 Antimicrobial susceptibility testing of the *Salmonella* isolates

Salmonella isolates from water, kale and reference strains were streaked onto blood agar and incubated at 37° for 18 hours to obtain isolated colonies. Three well-isolated colonies were selected and, using a sterilized wire loop, were transferred to 5 ml sterile saline in a test tube and vortexed. The turbidity of the suspension was then adjusted to match that of 0.5 McFarland turbidity standard (tube). Within 15 minutes after adjusting the turbidity of the suspensions, sterile cotton swabs were separately dipped into the suspensions, then, on withdrawal, pressed firmly against the wall of the respective tube to remove excess liquid. Each swab was then streaked over the entire surface of a Mueller Hinton agar plate three times while rotating the plate approximately 60 degrees, and finally swabbed (ringed) around the entire edge of the agar surface. Nine commonly used antibiotics in Kenya for treatment of *Salmonella* were chosen for study and they included: Ampicillin/Cloxacillin (10 micrograms), Cefuroxime (30 micrograms), Co-Trimoxazole/Trimethoprime (25 micrograms), Nalidixic acid (30 micrograms), Penicillin (10 micrograms), Ceftriazone (30

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micrograms). Chloramphenicol (50 micrograms), Ciprofloxacin (30 micrograms), Erythromicin (15 micrograms).

Antimicrobial (Oxoid, UK) disks were applied on the plates, which were then inverted and incubated at 37°C for 24 hours. After incubation diameters of the zones of complete inhibition were measured using a ruler. *Escherichia coli* ATCC 25922 was included as positive control organism (CDC and WHO, 2003). Interpretation of results was done according to performance standards as shown on appendix 14.

3.11 Statistical analysis

Statistical package for social scientists (SPSS) and analysis of variance methods (ANOVA) were used in interpretation of results. Student t-test was used in the evaluation of the significance between the groups. The significance between the values of the parameters were evaluated at $p < 0.05$ and confidence limits at 95%. Parameters evaluated included bacterial counts and haematological as well as biochemical parameters in mice.

CHAPTER FOUR

4.0RESULTS

4.1 Kale production practices in peri-urban areas of Nairobi

Farmers in peri-urban areas produced kale using irrigation. In Athi River 100% (24/24) of farmers irrigated kale, while in Ngong and Wangige, it was 96% (46/48) and 89% (43/48), respectively, giving an average irrigation rate of 95% (113/120). Most farmers in Wangige and Ngong [66.7% (32/48) and 62.5% (30/48), respectively] used water from shallow wells dug within the farms. Others (33.3% (16/48) in Ngong and 16.7% (8/48) in Wangige) used river water, while 4.2% (2/48) in Ngong and 16.7% (8/48) in Wangige used other methods (dam, borehole) or did not irrigate their kale. Those in Athi River, 50% (12/24) used river water and 45.5% (11/24) used effluent from the Kenya Meat Commission; only 4.2% (1/24) used water from a borehole (Table 4.1, Figures 4.1 and 4.2).

Table 4.1: Sources of water for irrigation used for kale production in the peri-urban farms of Nairobi

Farming area	Sources of water for irrigation				
	Well	River	Slaughter house waste water	Bore hole and/or dam	
	Number of farmers (%)	Number of farmers (%)	Number of farmers (%)	Number of farmers (%)	Total number of practitioners (%)
Athi River (n=24)	0 (0%)	12 (50%)	11 (45.8%)	1 (4.2%)	24 (20%)
Ngong (n=48)	30 (62.5%)	16 (33.3%)	0 (0%)	2 (4.2%)	48 (40%)
Wangige (n=48)	32 (66.7%)	8 (16.7%)	0 (0%)	8 (16.7%)	48 (40%)
Total (n=120)	62 (51.7%)	36 (30%)	11 (9.2%)	11 (9.2%)	120 (100%)

Legend:

n – number of water samples collected

%:- Percentage of farmers

Figure 4.1: River Athi waters used for irrigation by Athi river farmers

Figure 4.2: Waste water pond from a slaughter house used to irrigate vegetables by Athi River farmers

The leafy vegetables, mostly kale (Figure 4.3), were mostly produced using manure; this was particularly so in Wangige and Ngong where 95.8% (46/48) and 75 % (36/48) of farmers, respectively, reported that this was the source of the plant nutrient. Only 41.7% (10/24) of farmers in Athi River applied manure on kale crop. Thus, on average, 71.7% (86/120) of the vegetable farmers from peri-urban zone of Nairobi applied manure to the kale while 30% (36/120) did not apply manure. Generally, farmers used cattle manure: 91.7% (44/48) in Wangige, 56.3% (27/48) in Ngong and 25% (6/24) in Athi River. About 6.3% (3/48) and 2% (1/48) of farmers from Ngong and Wangige, respectively, used poultry manure; while another 8.3% (4/48) of the farmers from Ngong used pig manure. Compost from crop residues/ household wastes was also used by farmers in Ngong (14.5%; 7/48), Athi River (8.3%; 2/24) and Wangige (6.3%; 3/48) (Table 4.2). Animal manure was used at the rate of 74.2% while compost from crop residues/ household wastes were used at the rate of 9% by peri-urban farmers in Nairobi. Manure was mixed or incorporated into the soil by 40.8% (49/120) of the farmers. Other farmers spread the sludge from the zero grazing units onto the plots: 12.5% (6/48) in Wangige, 8.3% (2/48) in Athi River and 6.3% (3/48) in Ngong. Another 3.3% (4/120) of peri-urban farmers applied manure in furrows for planting leafy vegetables (Table 4.3).

Figure 4.3: Peri-urban kale produced using animal manure in Ngong

Table 4.2: Sources of manure used for kale production in Nairobi peri-urban farms

	Sources of manure						
	Cattle	Poultry	Sheep/goat	Pig	Compost*	Others*	
Farming area	Number of farmers (%)	Number of farmers (%)	Number of farmers (%)	Number of farmers (%)	Number of farmers (%)	Number of farmers (%)	Total number of practitioners (%)
Athi River (n=24)	6 (25%)	0 (0)%	1 (4.2%)	0 (0%)	2 (8.3%)	15 (62.5)	24 (20%)
Ngong (n=48)	27 (56.3%)	3 (6.3%)	2 (4.2%)	4 (8.3%)	7 (14.6%)	5 (10.4%)	48 (40%)
Wangige (n=48)	44 (91.7)	1 (2.1%)	0 (0%)	0 (0%)	3 (6.3%)	0 (0%)	48 (40%)
Total (n=120)	77 (64.2)	5 (4.2%)	3 (2.5%)	4 (3.3%)	11 (9.2%)	20 (16.7)	120 (100%)

Legend:

Compost*:- from crop residues/ household wastes

%: - Percentage of farmers

n:- number of kale farmers

Others*:- use inorganic fertilizers

Table 4.3: Methods of manure application in kale farming and the number of farmers practicing them in Nairobi peri- urban areas

	Method of manure application					
	Spread sludge	Spread compost	Ploughed into soil	Placed in furrow	Others*	
Farming area	Number of farmers (%)	Number of farmers (%)	Number of farmers (%)	Number of farmers (%)	Number of farmers (%)	Total number of practitioners (%)
Athi River (n=24)	2 (8.3%)	3 (12.5%)	5 (20.8%)	0 (0%)	14 (58.3%)	24 (20%)
Ngong (n=48)	3 (6.3%)	11 (22.9%)	23 (47.9%)	0 (0%)	11 (22.9%)	48 (40%)
Wangige (n=48)	6 (12.5%)	16 (33.3%)	21 (43.8%)	4 (8.3%)	1 (2.1%)	48 (40%)

Legend:

n- number of kale farmers

%- percentage of farmers

Others*- Farmers who used inorganic fertilizers

4.2 Practices of kale traders in the study area

Kale traders who operated at the wet markets used different modes of transportation of kale to the market. Traders in Kawangware transported their vegetables as follows: public transport (38.5%; 10/26), open trucks (19.2%; 5/26), human transport on back and closed trucks (each 15.4%; 4/26) and other modes (11.5%; 3/26). Traders in Kangemi market transported their vegetables in open trucks (37.7%; 9/24), human transport on back and wheelbarrow (each 16.7%; 4/24), closed trucks (12.5%; 3/24), public transport (8.3%; 2/8), push carts (*mkokoteni*) and other modes (4.2%; 1/24) each). Traders in Githurai market transported their vegetables in open trucks (50%; 15/30), human transport on back (26.6%; 8/30), closed trucks (13.3%; 4/30), public transport (6.7%; 2/30) and other modes (3.3%; 1/30). Thus, generally, most traders in the wet markets in Nairobi transported their vegetables using open trucks (36.3%; 29/80), while others transported as follows; human transport on back (20%; 16/80), public transport (17.5%; 14/80), closed trucks (13.8%; 11/80), wheelbarrow (5%; 4/80), push cart (*mkokoteni*) (1.3%; 1/80) and other modes (6.3%; 5/80) (Table 4.4).

Traders carried their vegetables to the market using various containers. Those in Kawangware market carried their vegetables in sealed (closed) bags (73.1%; 19/26), open bags (15.4%; 4/26); while 11.5% (3/26) did not pack their vegetables. Traders in Kangemi carried their vegetables using sealed bags (58.3%; 14/24), open bags (37.5%; 9/24); while only 4.2% (1/24) did not pack their vegetables. Traders in Githurai market carried their vegetables using sealed bags (80%; 24/30), open bags (16.7%; 5/30); while 3.3% (1/30) did not pack their vegetables. Thus, generally, traders in the wet markets in Nairobi carried their

vegetables as follows; sealed bags (71.3%; 57/80), open bags (22.5%; 19/80); while only 7.5% (6/80) did not pack their vegetables (Table 4.5, Figure 4.4).

Table 4.4: Mode of kale transport used by traders from the wet markets of Nairobi

Market type	Mode of kale transport							
	Open trucks	Closed trucks	Public transport	Human*	Wheel-burrow	Mkokoteni	Others*	
	Number of kale traders (%)	Number of kale traders (%)	Number of kale traders (%)	Number of kale traders (%)	Number of kale traders (%)	Number of kale traders (%)	Number of kale traders (%)	Total number traders (
Kawangware (n=26)	5 (19.2%)	4 (15.4%)	10 (38.5%)	4 (15.4%)	0 (0%)	0 (0%)	3 (11.5%)	26 (32.5
Kangemi (n=24)	9 (37.5%	3 (12.5%)	2 (8.3%)	4 (16.7%)	4 (16.7%)	1 (4.2%)	1 (4.2%)	24 (30
Githurai (n=30)	15 (50%)	4 (13.3%)	2 (6.7%)	8 (26.6%)	0 (0%)	0 (0%)	1 (3.3%)	30 (37.5
Total (n=80)	29 (36.3%)	11 (13.8%)	14 (17.5%)	16 (20%)	4 (5%)	1 (1.3%)	5 (6.3%)	80 (100

Legend:

Human*:- Human transport on back, n:- number of kale traders, Others*:- bicycle/ motorbike

Table 4.5: Methods of packaging kale used by traders in the wet markets of Nairobi

Market	Packaging method			
	Sealed bags	Open bags	Not packed	
	Number sampled (%)	Number sampled (%)	Number sampled (%)	Total number of traders (%)
Kawangware (n=26)	19 (73.1%)	4 (15.4%)	3 (11.5%)	26 (32.5%)
Kangemi (n=24)	14 (58.3%)	9 (37.5%)	1 (4.2%)	24 (30%)
Githurai (n=30)	24 (80%)	5 (16.7%)	1 (3.3%)	30 (37.5)
Total (n=80)	57 (71.3%)	19 (22.5%)	6 (7.5%)	80 (100%)

Legend:

%: - percentage of kale samples packed/unpacked

n:- number of kale samples collected

sealed bag- closed bag

Figure 4.4: Kale transported using open bags to wet market in Githurai

4.3 Bacteria on kale samples and water used for irrigation obtained from farms

Table 4.6 gives breakdown of mean coliform counts and prevalence rates of *E. coli* and *Salmonella* organisms on kale purchased from peri-urban farms in Nairobi, while Table 4.7 gives the mean faecal coliform counts of water used for irrigation in the respective farms. Mean coliform counts on kale samples from the three farming sites ranged from $1.6\times10^5 \pm 9.1\times10^4$ to $4.0\times10^5 \pm 1.3\times10^5$ colony forming units/gram (cfu/g). The difference in the mean coliform counts between the sites was not statistically significant ($p > 0.05$). Kale samples from Ngong had *E. coli* prevalence of 81.8% (18/22), while those from Wangige and Athi River had *E. coli* prevalences of 72.7% (16/22) and 37.7% (6/16), respectively. Of the three farming sites, only one sample from Wangige yielded *Salmonella* organisms; prevalence rate of 4.5% (1/22). On serotyping, it was found to be *S.* Enteritidis (serotype 1, 9, 12: g, m). Mean faecal coliform count in irrigation water from the three farming sites ranged from $3.9\times10^6 \pm 3.5\times10^6$ to $2.6\times10^8 \pm 2.2\times10^8$ counts/100 ml. Detailed results are given in Appendices 2 and 3.

Table 4.6: Mean coliform counts and prevalence of *Salmonella* and *Escherichia coli* on kale purchased from the peri-urban farms, Nairobi

	Coliforms cfu/g			Prevalence (%)	
Sources of kale samples	Mean	SD	SE	Number of *Escherichia coli* organisms (%)	Number of *Salmonella* organisms (%)
Athi River n=16	1.9×10^5	4.0×10^5	9.9×10^4	6 (37.7%)	0 (0%)
Ngong n=22	4.0×10^5	6.2×10^5	1.3×10^5	18 (81.8%)	0 (0%)
Wangige = 22	1.6×10^5	4.2×10^5	9.1×10^4	16 (72.7%)	1 (4.5%)

Legend:

SD: - Standard deviation

%:- percentage of kale samples contaminated by bacteria

n:- number of kale samples tested

cfu/g: – colony forming units per gram of kale

SE: - standard error

WHO recommended levels for coliforms is <1000 cfu/g

Table 4.7: Faecal coliform counts of water samples used for irrigation at the peri-urban farms, Nairobi

	Bacteriology MPN index in100ml		
Sources of samples	Mean	SD	SE
Athi River (n=7)	2.6×10^{8}	5.9×10^{8}	2.2×10^{8}
Ngong (n=10)	1.1×10^{7}	1.5×10^{7}	4.8×10^{6}
Wangige (n=10)	3.9×10^{6}	1.1×10^{7}	3.5×10^{6}

Legend:

SD: -Standard deviation

n:- number of water samples tested

SE: – Standard Error

ml: - milliliter

WHO. 2006 recommended faecal coliform concentration is < 1000 counts/100 ml of water

4.4 Contamination of kale and washing water with *E.coli* and *Salmonella*

Table 4.8 gives breakdown of mean coliform counts and prevalence rates for *E. coli* and *Salmonella* organisms on kale purchased from market sites in Nairobi, while Table 4.9 gives breakdown of mean coliform counts and prevalence rate of *Salmonella* organisms in water samples used for washing/refreshing kales at the wet markets, Nairobi. Mean coliform counts on kale samples from wet markets (Kawangware, Kangemi, Githurai) ranged from $1.1x10^6 \pm 6.7x10^5$ to $1.1x10^7 \pm 3.0 \, x10^6$ cfu/g of kale leaves. Those for supermarket and High-end specialty market were $2.6x10^6 \pm 5.5x10^5$ and $4.7x10^5 \pm 2.3x10^5$, respectively.

Kawangware market had significantly higher coliform count on kale samples when compared to those from Kangemi ($p= 0.0193$) and Githurai ($p= 0.002$). However, there was no significant difference ($p> 0.05$) in coliform numbers on kale samples from Kangemi market when compared to those from Githurai wet markets.

There was a significant difference ($p= 0.0027$) in mean coliform count on kale samples from wet markets when compared to those from high- end specialty store. Similarly, a significant difference ($p =0.008$) between the coliform numbers on kale samples from supermarkets and the high-end specialty store was noted. However, there was no significant difference ($p= >0.05$) in coliform numbers on kale samples from supermarket when compared to those from wet markets. Detailed results are given in Appendices 3, 4 and 5.

Escherichia coli prevalence of 64.1% at the wet markets was significantly higher (0.047) than that of supermarket and high- end specialty store which recorded prevalence of 20% each. Of the 5 market sites, only one sample in Kawangware market yielded *Salmonella*

organisms; prevalence of 6.3% (1/16). On serotyping, it was found to be *Salmonella* Enteritidis (serotype 1, 9, 12: g.m).

Mean faecal coliform counts in water samples used for washing kale at the wet markets ranged from $1.0x10^7 \pm 5.7x10^6$ to $8.4x10^6 \pm 4.2x10^6$ counts/100 ml (Table 4.9 and Appendix 3). Of the water samples taken from the 3 wet markets in Nairobi, only one sample in Kangemi yielded *Salmonella* organisms; prevalence of 12.5% (1/8). On serotyping, it was found to be *Salmonella* Typhimurium (serotype 1, 4, 12:1, 2).

Table 4.8: Mean coliform counts and prevalence of *Salmonella* and *Escherichia coli* on kale purchased from the market sites in Nairobi

Site	Mean coliform counts (cfu/g)			*E.coli* prevalence	*Salmonella* prevalence
	Mean	SD	SE	Number of *E.coli* organisms (%)	Number of *Salmonella* organisms (%)
Kawangware (n=16)	1.1×10^7	1.2×10^7	3.0×10^6	7 (43.8%)	1 (6.3%)
Kangemi (n= 16)	2.2×10^6	7.5×10^6	1.9×10^6	10 (62.5%)	0 (0%)
Githurai (n= 18)	1.1×10^6	2.8×10^6	6.7×10^5	6 (33.3%)	0 (0%)
Supermarket (n=25)	2.6×10^6	2.7×10^6	5.5×10^5	5 (20%)	0 (0%)
High end market (n= 15)	4.7×10^5	8.9×10^5	2.3×10^5	3 (20%)	0 (0%)

Legend:

SD: – standard deviation

SE: - standard error

Cfu/gm:- colony forming units per gram

E.coli:- Escherichia coli

%:- percentage of kale samples contaminated by bacteria

Kawangware, Kangemi and Githurai are wet markets

WHO recommended levels for coliforms is <1000 cfu/g

Table 4.9: Mean fecal coliform counts (per 100 milliliters) of water samples used for washing kale at the wet markets, Nairobi

Source	Bacteriology MPN index in 100 ml			*Salmonella* prevalence
	Mean	SD	SE	Number of *Salmonella* organisms (%)
Kawangware market (n=8)	1.0×10^{7}	1.6×10^{7}	5.7×10^{6}	0 (0%)
Kangemi market (n=8)	8.4×10^{6}	4.4×10^{6}	4.2×10^{6}	1 (12.5%)
Githurai market (n=7)	2.4×10^{6}	6.0×10^{6}	2.2×10^{6}	0 (0%)

Legend:

SD*-Standard deviation

% - Percentage of water samples contaminated by bacteria

n - number of water samples collected

WHO recommended faecal coliform concentration is < 1000 counts/100 ml of water

4.5 Comparison of coliform counts between farm and market kale, respectively.

This was as shown on Figure 4.5. There was significantly higher (n = 0.0015) mean coliform count on kale from the wet markets when compared with those from farms; particularly so for the Kawangware market. A significant difference was also noted on mean coliform counts from kale samples obtained from supermarket ($p = 0.0002$) when compared to those from farms. However, there was no significant difference in coliform counts on kale samples obtained from the farms and those from High-end specialty market ($p>0.05$). There was, thus, a general increase in coliform numbers on kale samples from farm sites to the various markets.

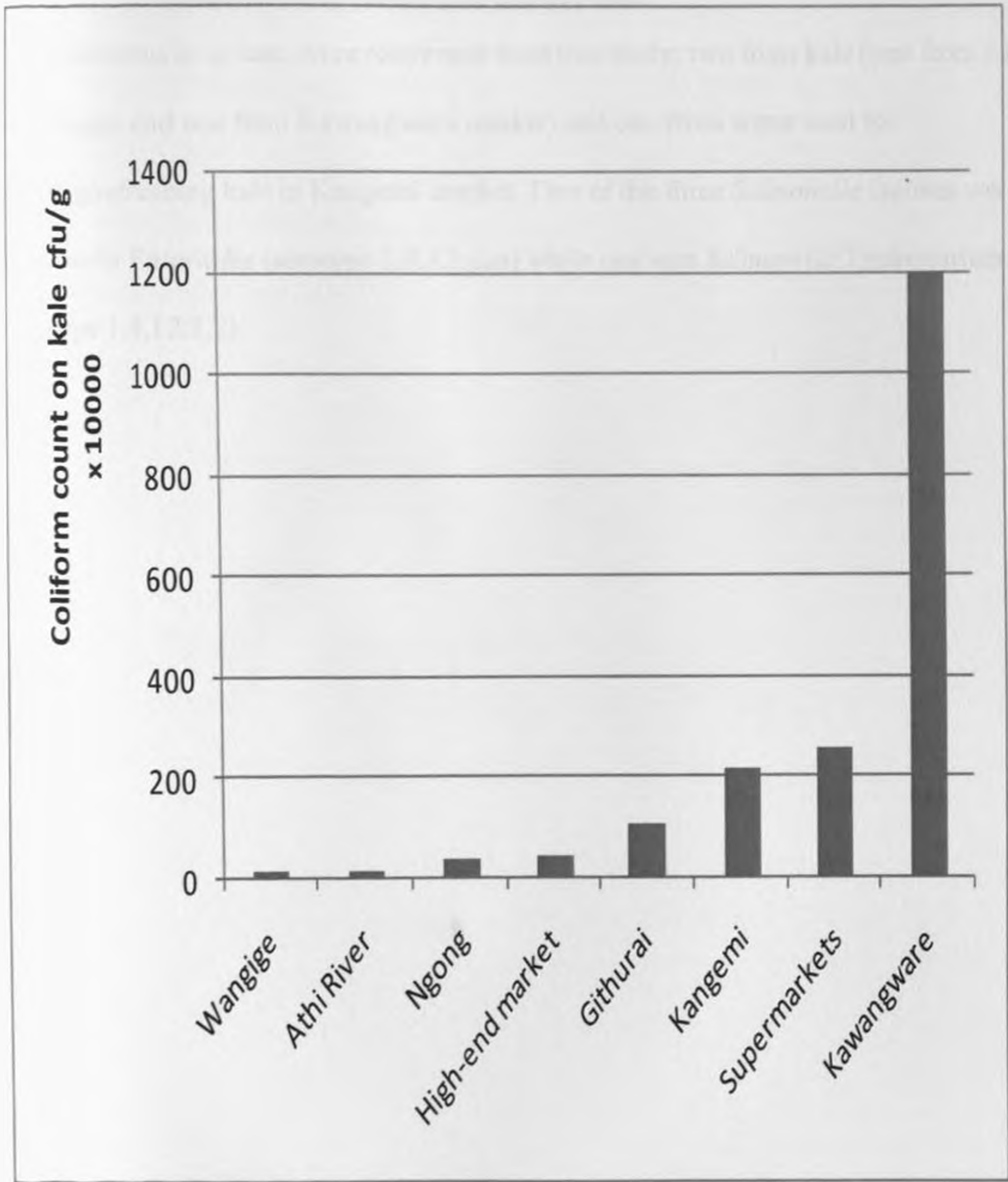


Figure 4.5: Coliform levels on kale samples from farms and markets

Legend:

Cfu/g – colony forming units per gram of kale

4.6 Summary on the *Salmonella* isolates from kale and contact water obtained from farms and market sites

Three *Salmonella* isolates were recovered from this study; two from kale (one from a farm in Wangige and one from Kawangware market) and one from water used for washing/refreshing kale in Kangemi market. Two of the three *Salmonella* isolates were *Salmonella* Enteritidis (serotype 1,9,12:g,m) while one was *Salmonella* Typhimurium (serotype 1,4,12:1,2).

4.7 Fifty percent infectious dose of *Salmonella* isolates

Results for the infectious dose fifty (ID_{50}) determinations were as shown in Table 4.10. Clinical isolates, CL1 and CL2, gave ID_{50}s of 7.0×10^3 and 3.6×10^4, respectively. The environmental isolates, KNG 14 and BO973, gave ID_{50}s of 6.0×10^5 and 7.2×10^7, respectively. Clinical isolates had significantly lower ID_{50} values when compared to environmental isolates (p =0.0416).

Table 4.10: Fifty percent infectious dose of *Salmonella* isolates monitored in 3-week old mice

Isolate code	Serotype	Source	ID_{50} = number in 0.4ml	Overall conc.
CL1	*S.*Typhimurium	Human clinical source	7.0×10^3	1.8×10^4 org/ml
CL2	*S.*Enteritidis	Human clinical source	3.6×10^4	9.0×10^4org/ml
KNG 14	*S.*Typhimurium	Washing water in the markets	6.0×10^5	1.5×10^6org/ml
BO973	*S.*Enteritidis	Kale	7.2×10^7	1.8×10^8org/ml

Legend:

$ID_{50:}$ - infectious dose fifty

S. Enteritidis: - *Salmonella* Enteritidis

S. Typhimurium:- *Salmonella* Typhimurium

Conc.: - concentration

Org/ml:-organisms per millilitre

CL1:- code for *Salmonella* isolate from clinical source

CL2 :- code for *Salmonella* isolate from clinical source

KNG14: – code for *Salmonella* isolate from water

BO973 - Code for *Salmonella* isolate from kale

4.8 Pathogenicity of *Salmonella* isolates in mice

4.8.1 Observed clinical signs in mice

All the mice infected with *Salmonella* organisms showed clinical signs six hours post inoculation. They had rough hair coat, dullness, anorexia, shivering and reduced movement. The control group did not show any clinical signs. Two mice infected with human clinical isolate (CL1) exhibited acute infection and died on day two while two others from the same infected group (CL1 infected group) died on day four post inoculation.

4.8.2 Haematological values

The haematological parameters exhibited by the 3 groups of experimental mice are given on Tables 4.11, 4.12 and 4.13. There were significant reductions ($p<0.05$) in PCV, TEC and Hb values in the infected groups as compared to the controls (Table 4.14). A decrease in percentage PCV, TEC and Hb was observed in the *Salmonella* infected group from inoculation to day fourteen. Figures 4.6, 4.7 and 4.8 illustrate the mean PCV, TEC and Hb, respectively during the experimental period. Detailed results are given in Appendices 6, 7 and 8.

Table 4.14 gives the comparison of mean values of various parameters of the *Salmonella* infected mice with the control mice, using unpaired student t-test. There were significant increases ($p<0.05$) in TLC and percentage neutrophil values in the infected groups as compared to the controls. However, there was significant decrease in mean percentage values of the lymphocytes. Increase in TLC and percent neutrophil counts (Figure 4.13) was observed in *Salmonella* infected mice from inoculation to day fourteen. However, a decrease in percent lymphocyte counts in the *Salmonella* infected mice was observed upto

fourteen days post inoculation. Figures 4.9, 4.10 and 4.11 illustrate the mean TLC, neutrophil and lymphocyte values, respectively, during the experimental period. Detailed results are given in Appendices 9 and10. No significant alteration was observed in the eosinophils, basophils and monocytes in all the groups when compared with the controls ($p<0.05$).

4.8.3 Biochemical values

Biochemical parameters exhibited by the 3 groups of the experimental mice are given on Tables 4:11, 4:12 and 4:13, respectively while Table 4.14 gives the comparison of mean values of the respective parameters of the infected groups with the control group, using unpaired student t-test. Detailed results are given in Appendix 11. There was a significant difference in the total plasma protein concentrations between the *Salmonella* infected mice and control mice ($p<0.05$). Significant alteration in distribution of the proteins was observed, with a decrease in albumin and an increase in globulin ($p<0.05$). There was a significant decrease in total protein and albumin in the *Salmonella* infected group from inoculation to day fourteen ($p<0.05$). However, a slight increase was observed in globulin concentration. Figures 4.12, 4.13 and 4.14 illustrate the mean total protein, albumin and globulin concentrations, respectively. Table 4.14 shows the *p* values of parameters of the infected groups when compared with those of the control group using unpaired student t-test. There was significant decrease ($p= 0.043$) in mean packed cell volume in CL1 infected mice when compared with those of KNG 14 infected group. However, there were no significant differences ($p>0.05$) in other haematological and biochemical parameters.

Table 4.11: Haematological and biochemical parameters of mice inoculated with *Salmonella* isolate from human clinical source (CL1)

	Days post inoculation with CL1 isolate		
	0	3	7
Mean PCV (%)	46.5± 0.5	40.25±0.31	38.5±0.5
Mean Hb (g/dl)	15.51±0.47	13.42±0.32	12.84±0.23
Mean TEC	$2.2\times 10^{7}\pm 1.1\times 10^{7}$	$5.5\times 10^{6}\pm 1.0\times 10^{6}$	$4.5\times 10^{6}\pm 3.0\times 10^{5}$
Mean TLC	$3.3\times 10^{3}\pm 1.3\times 10^{2}$	$3.8\times 10^{3}\pm 1.1\times 10^{2}$	$6.7\times 10^{3}\pm 5.0\times 10^{1}$
Neutrophils (%)	19.5±0.98	22.83±0.87	37±1
Lymphocytes (%)	78.13±1	75.17±1.38	61±1
Eosinophils (%)	1±0.27	1±0.26	1±1
Basophils (%)	1.13±0.16	0.5±0.34	0.5±0.5
Monocytes (%)	0.25±0.46	0.33±0.21	0.78±0.5
Total protein(g/dl)	7.51±0.05	6.76±0.09	4.45±0.23
Albumin(g/dl)	4.48±0.06	2.81±0.24	1.19±0.03
Globulin (g/dl)	3.01±0.09	3.97±0.18	3.38±0.07

Legend:

PCV: – packed cell volume

Hb:- hemoglobin

TEC: – total Erythrocyte count

TLC: – total leukocyte count

%: - percentage of parameters

g/dl: - grams per deciliter

± :- plus- minus sign

CL1:- code for *Salmonella* isolate from human clinical source

Table 4.12: Haematological and biochemical parameters of mice inoculated with *Salmonella* isolate from water source (KNG 14)

rmeter	Days post inoculation with KNG 14 isolate			
	0	3	7	14
n PVC (%)	46.25±0.41	44.13±0.58	41.83±0.60	41±0.41
n Hb (g/dl)	15.42±0.39	14.71±0.55	13.49±0.49	13.36±0.95
n TEC	$7.8 \times 10^6 \pm 1.0 \times 10^6$	$8.8 \times 10^6 \pm 2.1 \times 10^5$	$7.5 \times 10^6 \pm 3.5 \times 10^5$	$4.4 \times 10^6 \pm 1.4 \times 10^6$
n TLC	$3.4 \times 10^3 \pm 1.4 \times 10^2$	$3.4 \times 10^3 \pm 1.4 \times 10^2$	$4.4 \times 10^3 \pm 3.4 \times 10^2$	$5.4 \times 10^3 \pm 2.3 \times 10^2$
trophils (%)	17.88±0.69	24.13±1.5	31±1.18	36±0.82
phocytes (%)	79.88±0.74	74.13±1.55	67.67±0.95	62±1.630.82
nophils (%)	1±0.19	0.5±0.20	0.83±0.40	0.75±0.25
ophils (%)	2±0.27	0.5±0.30	0.33±0.21	1±0.41
nocytes (%)	0.63±0.26	0.57±0.30	0.33±0.10	0.5±0.1
al	7.44±0.08	7.01±0.13	6.25±0.08	5.27±0.25
umin(g/dl)	4.4±0.04	4.05±0.06	3.11±0.003	2.13±0.29
bulin(g/dl)	3.02±0.09	2.89±0.07	3.14±0.10	3.14±0.06

Legend:

PCV: – packed cell volume

Hb: - haemoglobin

TEC: – total erythrocyte count

TLC: – total leukocyte count

%: - percentage of parameter

g/dl: - grams per deciliter

±: - plus- minus sign

KNG 14:- code for *Salmonella* isolate from water source

Table 4.13: Haematological and biochemical parameters of the control mice

ameters	Days post inoculation with normal saline			
	0	3	7	14
an PCV	45.63±0.38	45.38±0.45	45.67±0.61	45.25±0.62
ean Hb	15.209±0.35	15.131±0.44	15.23±0.50	15.87±1.08
ean TEC	$9.3\text{x } 10^{6} \pm 1.4\text{x}10^{5}$	$9.2\text{x}10^{6} \pm 1.7\text{x}10^{5}$	$9.2\text{x}10^{6} \pm 1.1\text{x}10^{5}$	$9.1\text{x}10^{6} \pm 1.7\text{x}10^{5}$
ean TLC	$3.6\text{x}10^{3} \pm 7.1\text{x}10^{1}$	$3.6\text{x}10^{3} \pm 7.0\text{x}10^{1}$	$3.6\text{x}10^{3} \pm 6.0\text{x}10^{1}$	$3.7\text{x}10^{3} \pm 4.0\text{x}10^{1}$
utrophils	19.25±1.03	18.75±1.03	18.13±1.0	18.37±1.32
mphocyte	78.5±1.05	80.01±1.02	79.38±1.2	79.25±1.89
sinophils	1.38±0.26	0.75±0.25	1.5±0.23	1.25±0.41
sophils	0.75±0.25	0.5±0.27	0.88±0.64	1.25±0.25
onocytes	0.13±0.10	0.42±0.25	0.25±0.17	0.38±0.25
tal	7.42±0.08	7.5±0.06	7.52±0.06	7.28±0.12
bumin(g/d	4.63±0.04	4.58±0.05	4.57±0.06	4.59±0.03
obulin	2.80±0.08	2.92±0.10	2.94±0.10	2.69±0.15

Legend:

PCV: – packed cell volume

Hb: - haemoglobin

TEC: – total Erythrocyte count

TLC: – total leukocyte count

%: - percentage of parameter

g/dl: - grams per deciliter

±: - plus- minus sign

Table 4.14: *P* values obtained by comparing mean values of the parameters of *Salmonella* infected and control mice

Compared groups	Variable	Days post inoculation			
KNG 14 and control group		0	3	7	14
	Mean PCV (%)	0.282	0.1143	0.0016*	0.0015*
	Mean Hb (g/l)	0.60801	0.1092	0.0004*	0.0015*
	Mean TEC	0.192	0.1051	0.0017*	0.0284*
	Mean TLC	0.2064	0.1297	0.0663	0.00025*
	Neutrophils (%)	0.2889	0.0189	0.000*	0*
	Lymphocytes (%)	0.306	0.0092*	0.000*	0.0005*
	Eosinophils (%)	0.2690	0.5885	0.1892	0.6290
	Basophils (%)	0.7360	0.33	0.12	0.629
	Monocytes (%)	0.114	0.444	0.1	0.6753
	Total protein(g/dl)	0.9512	0.0053*	0.0006*	0.0011*
	Albumin(g/dl)	0.1085	0.000*	0.000*	0.0014*
	Globulin (g/dl)	0.1085	0.8202	0.2007	0.0389*
CL1 and control group					
	Mean PCV (%)	0.1868	0.000*	0.0005*	-
	Mean Hb (g/l)	0.1757	0.0012*	0.0005*	-
	Mean TEC	0.3636	0.0114*	0.000*	-
	Mean TLC	0.122	0.1753	0.000*	-
	Neutrophils (%)	0.8903	0.000*	0.0005*	-
	Lymphocytes (%)	0.8633	0.0116*	0.0005*	-
	Eosinophils (%)	0.3356	0.506	0.6776	-
	Basophils (%)	0.2863	0.1	0.3548	-
	Monocytes (%)	0.5537	0.7608	0.6032	-

Table 4.14 continues

	Total protein(g/dl)	0.411	0.0001*	0.0118*	-
	Albumin(g/dl)	0.0797	0.000*	0.000*	-
	Globulin (g/dl)	0.1072	0.0006*	0.0879*	-

Legend

*:- values that were significantly different from the control group ($p<0.05$)

PCV: – packed cell volume

Hb: - haemoglobin

TEC: – total erythrocyte count

TLC: – total leukocyte count

%:- percentage of parameter

g/dl: - grams per liter

±:- plus- minus sign

-:- no parameters taken because mice died before this day

CL1:- code for *Salmonella* isolate from human clinical source

KNG 14:- code for *Salmonella* isolate from water source

NB: The parameters compared were as shown on table 4.11, 412 and 4.13

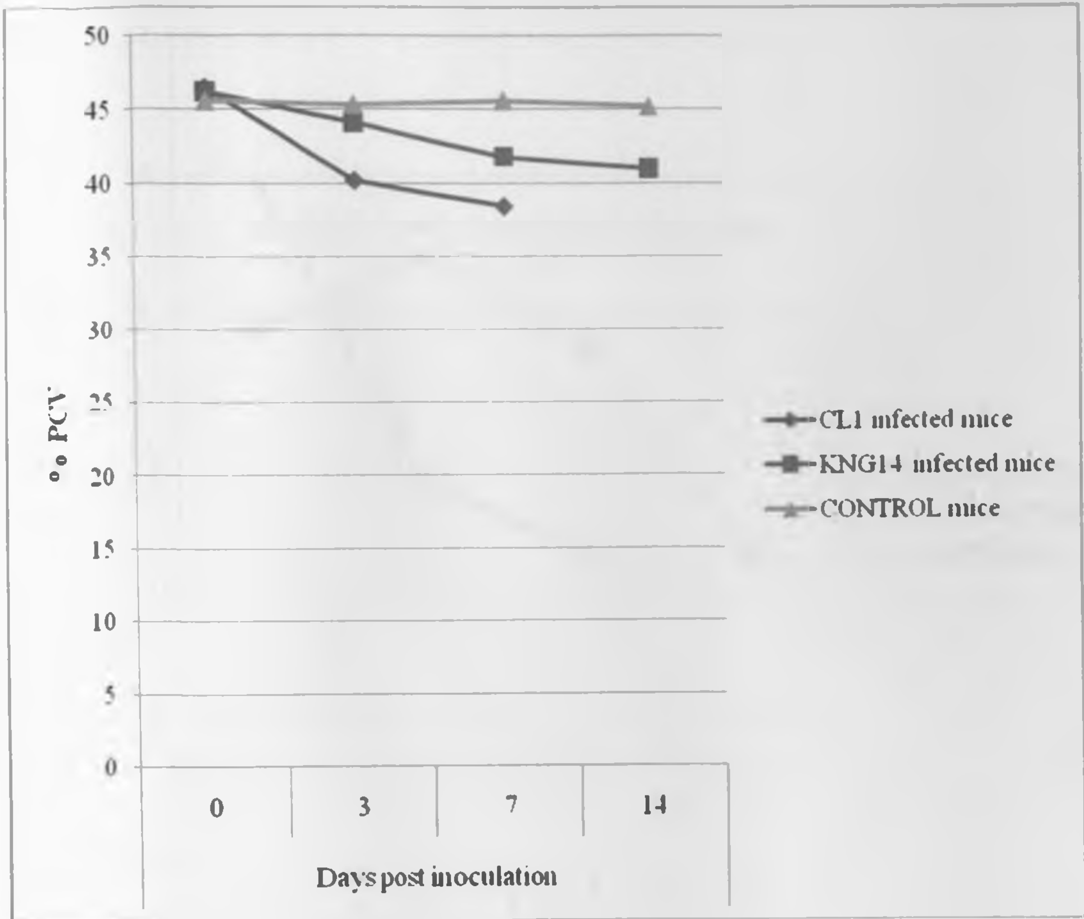


Figure: 4.6 Mean percentage packed cell volume in *Salmonella* infected and control mice.

Legend:

%: - percentage

PCV: – packed cell volume

CL1:- code for *Salmonella* isolate from human clinical source

KNG 14:- code for *Salmonella* isolate from water source

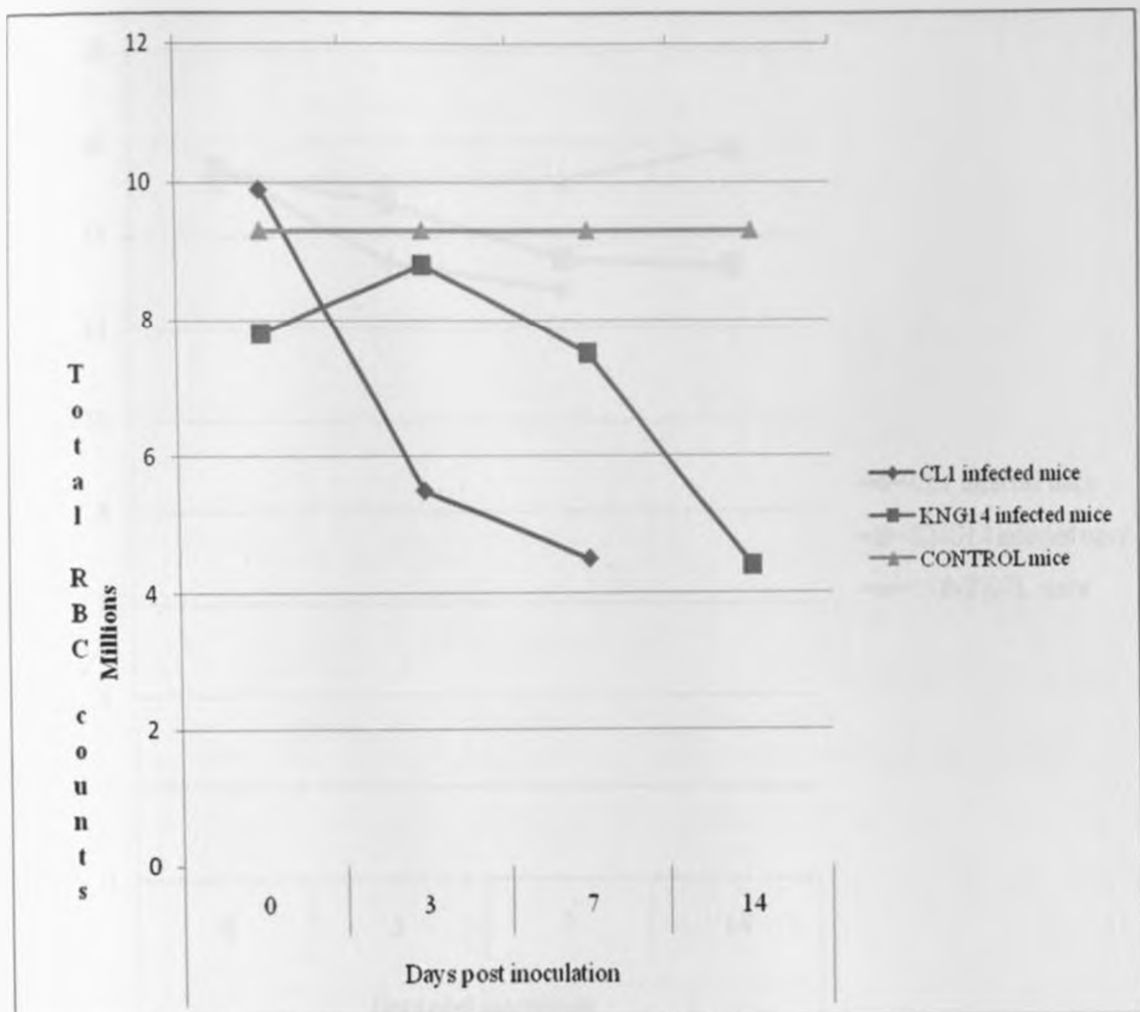


Figure 4.7: Mean total erythrocyte count in *Salmonella* infected and control mice

Legend:

CL1:- Code for *Salmonella* isolate from human clinical source

KNG 14:- Code for *Salmonella* isolate from water source

RBC:- Red blood cells (Erythrocytes)

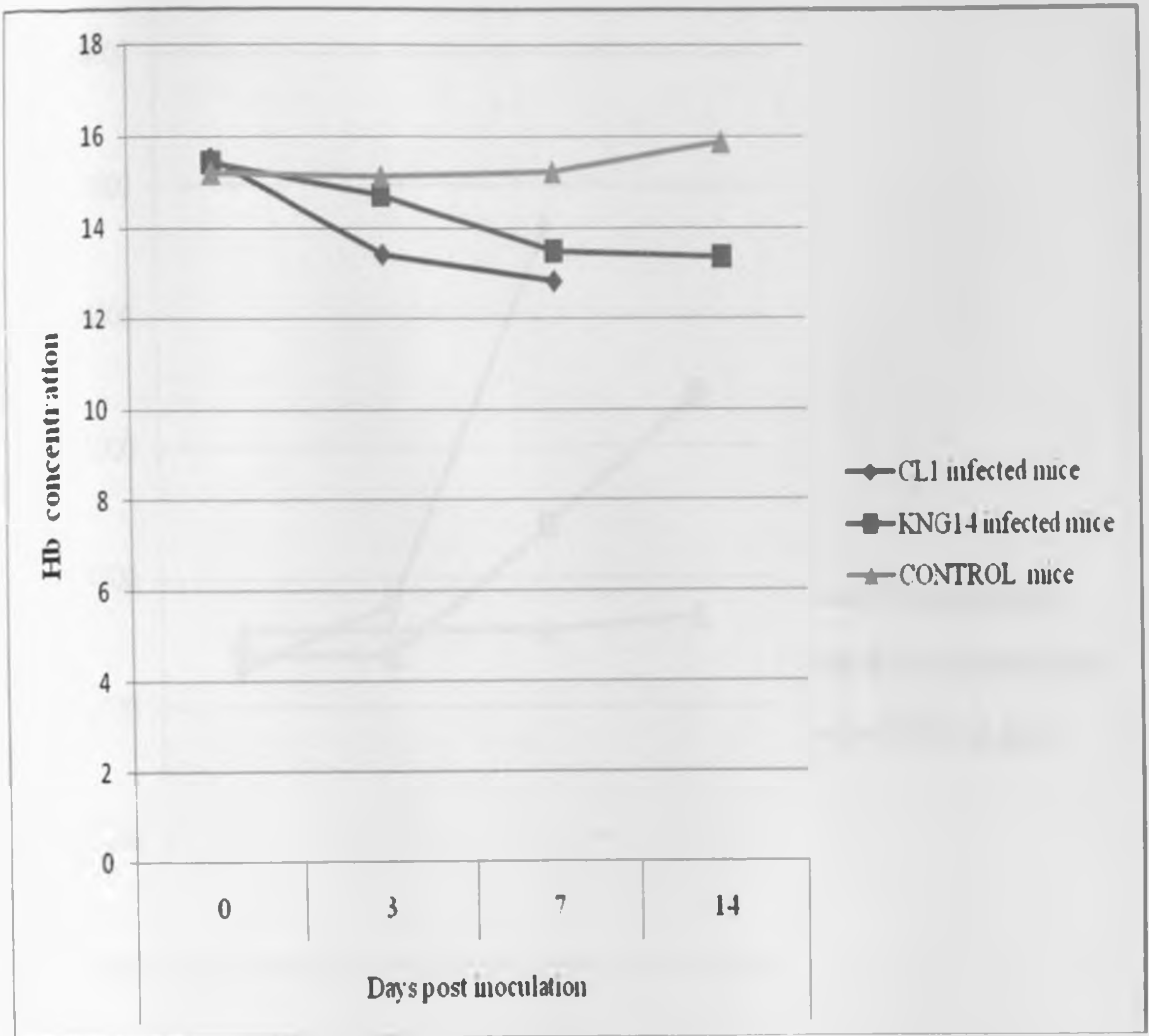


Figure 4.8: Mean haemoglobin concentration in *Salmonella* infected and control mice

Legend:

Hb:- haemoglobin

CL1:- code for *Salmonella* isolate from human clinical source

KNG 14:- code for *Salmonella* isolate from water source

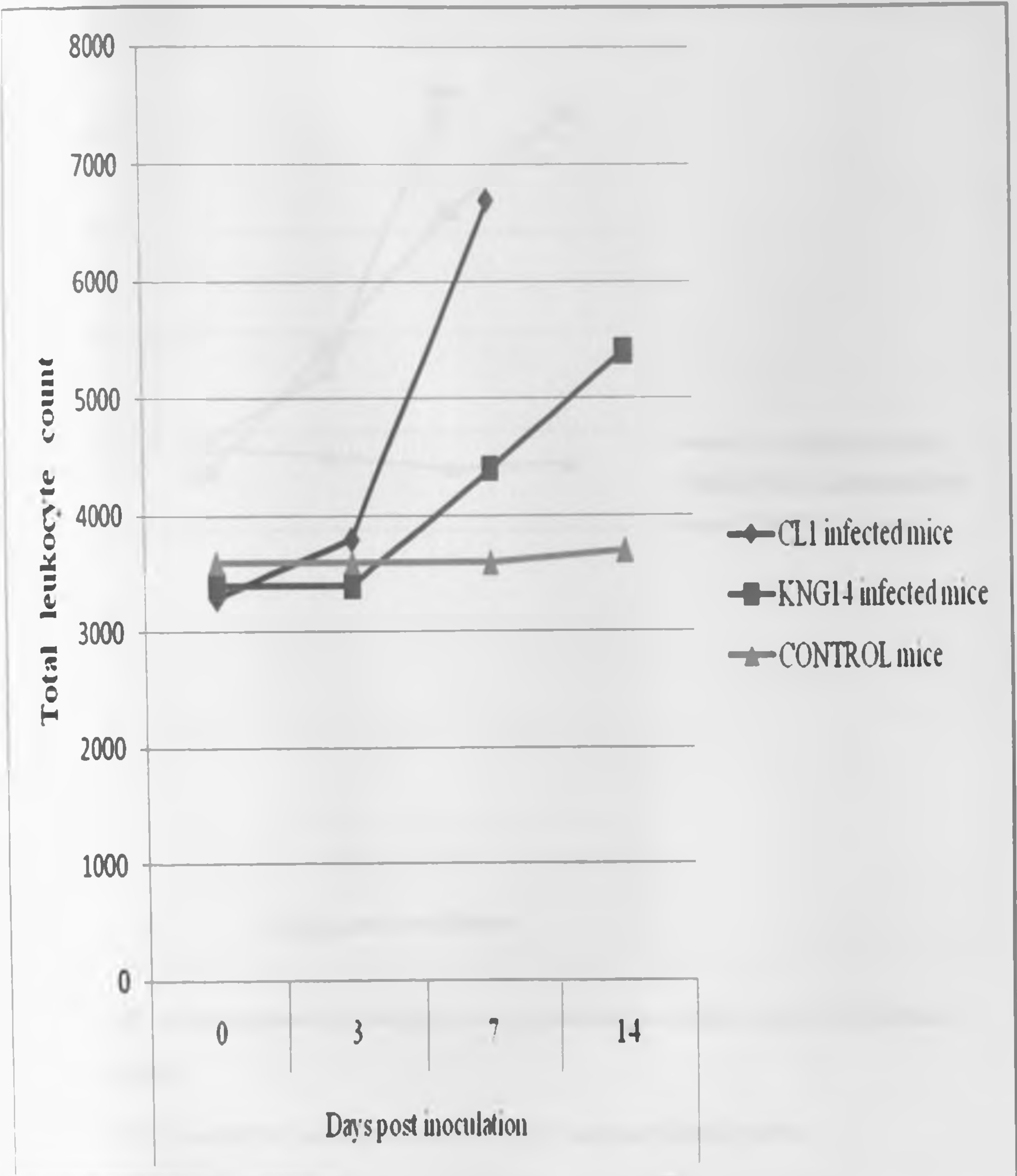


Figure 4.9: Mean total leukocyte count in *Salmonella* infected and control mice

Legend:

CL1:- code for *Salmonella* isolate from human clinical source

KNG 14:- code for *Salmonella* isolate from water source

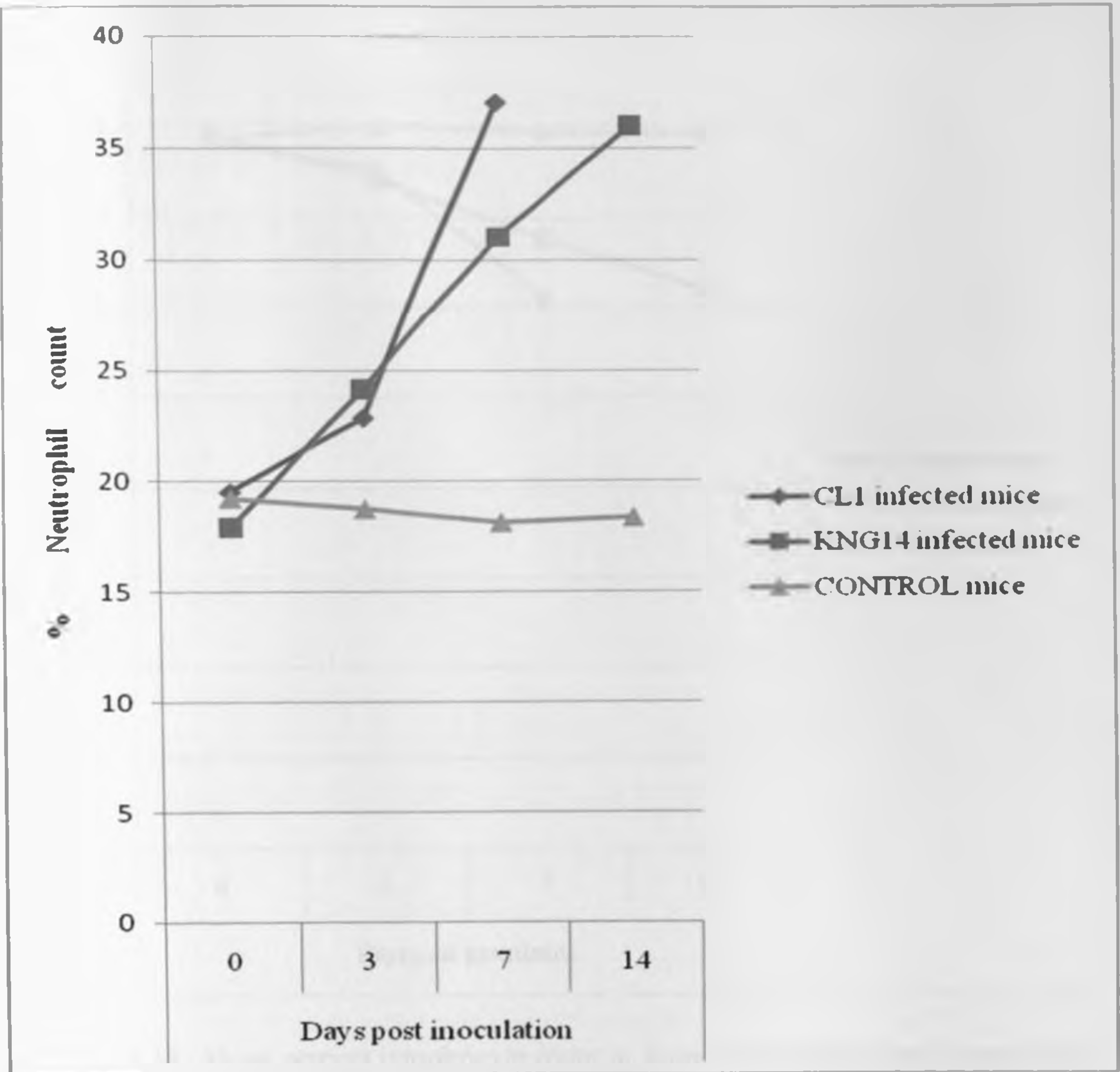


Figure 4.10: Mean percent neutrophil count in *Salmonella* infected and control mice

Legend:

CL1:- code for *Salmonella* isolate from human clinical source

KNG 14:- code for *Salmonella* isolate from water source

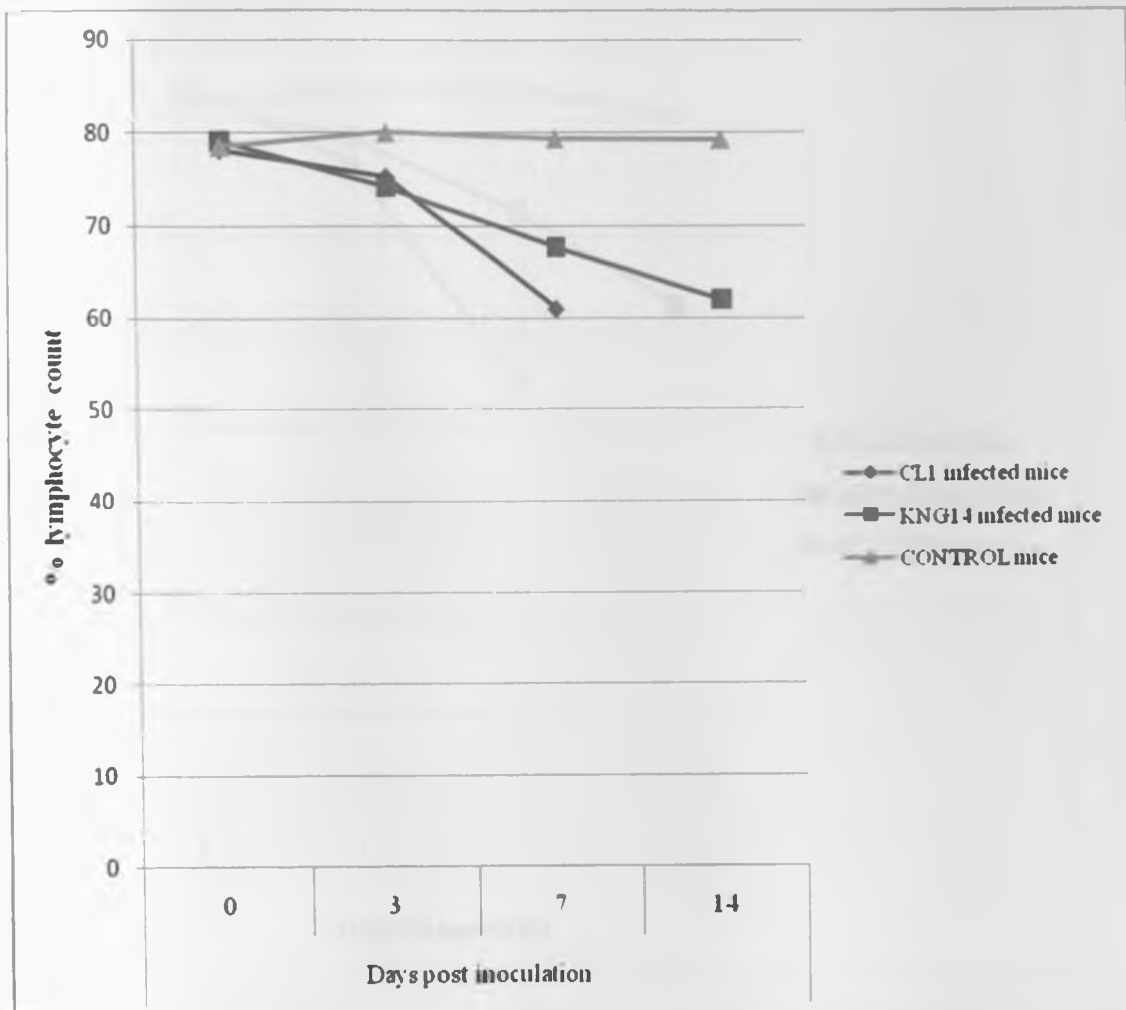


Figure 4.11: Mean percent lymphocyte count in *Salmonella* infected and control mice

Legend:

%:- percentage of lymphocyte

CL1:- Code for *Salmonella* isolate from human clinical source

KNG 14:- Code for *Salmonella* isolate from water source

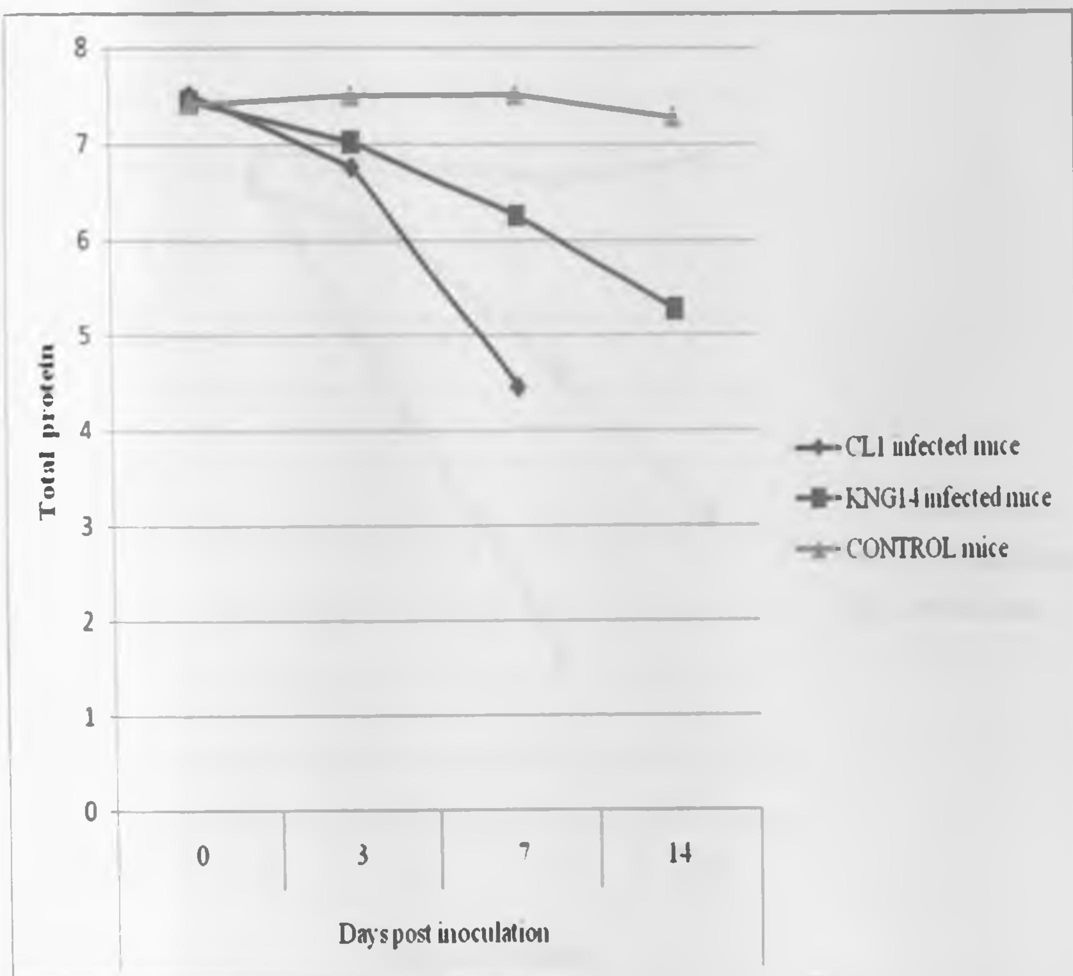


Figure 4.12: Mean total protein content in *Salmonella* infected and control mice

Legend:

CL1:- Code for *Salmonella* isolate from human clinical source

KNG 14:- Code for *Salmonella* isolate from water source

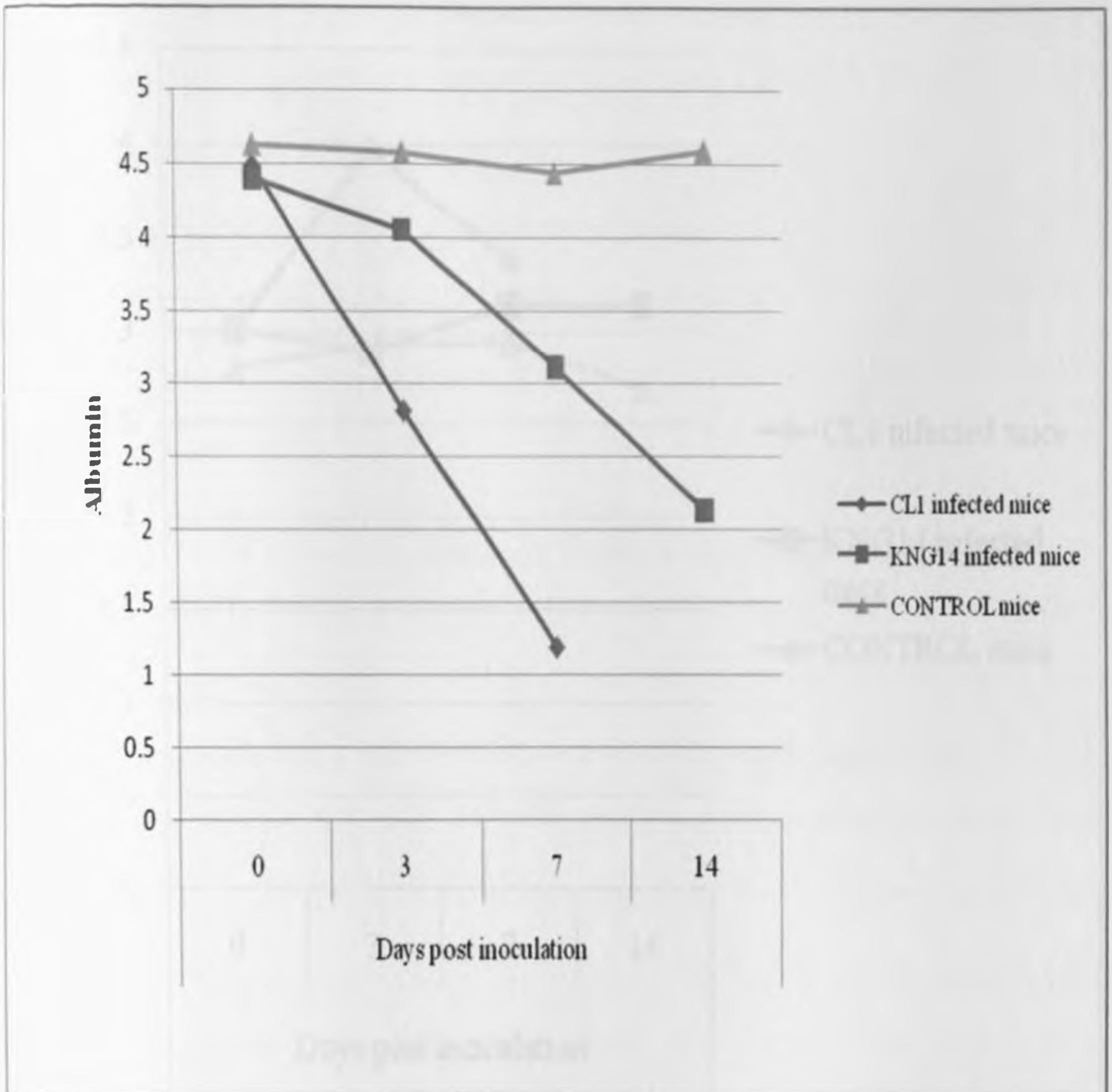


Figure 4.13: Mean albumin content in *Salmonella* infected and control mice

Legend:

CL1:- Code for *Salmonella* isolate from human clinical source

KNG 14:- Code for *Salmonella* isolate from water source

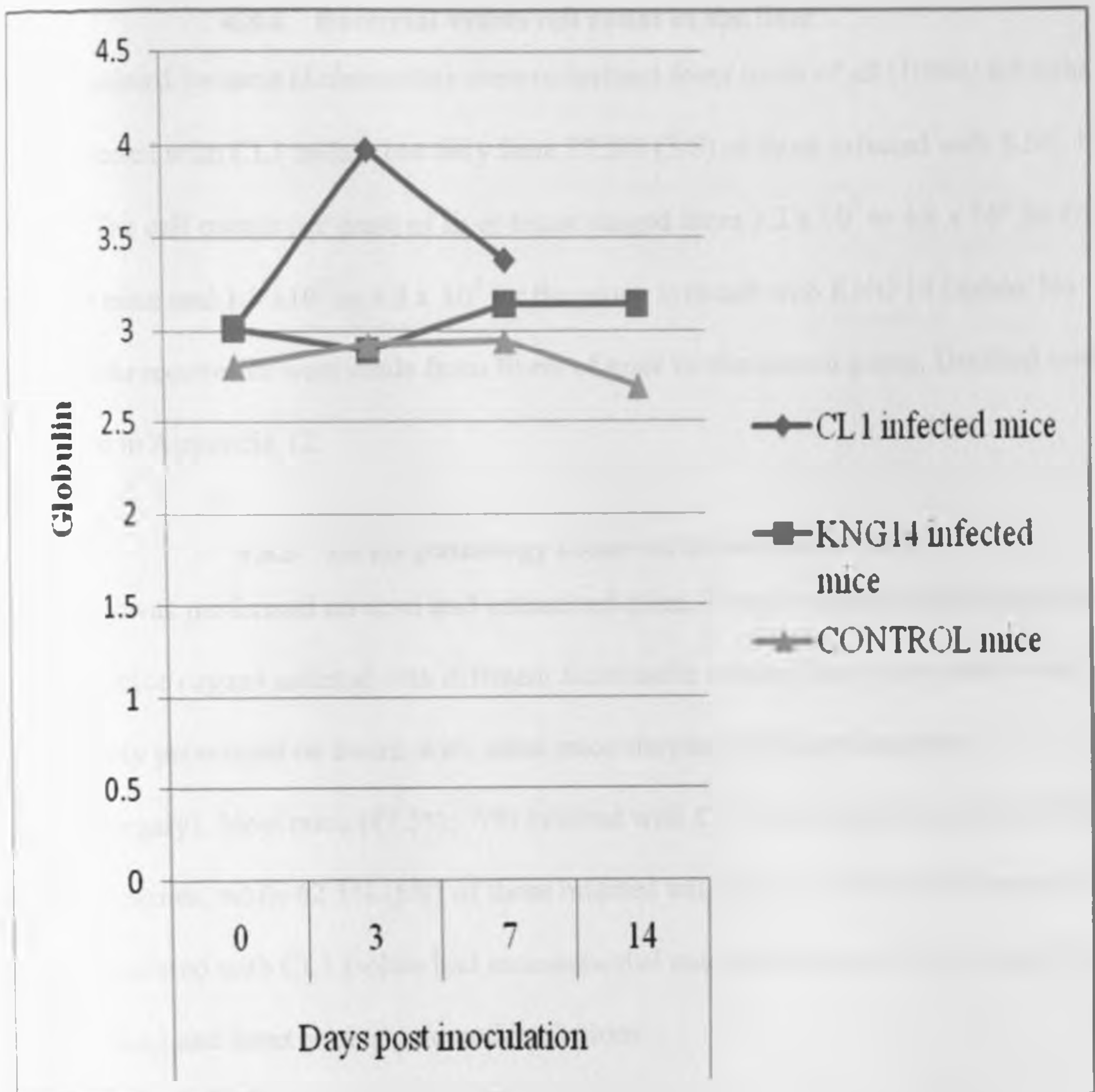


Figure 4.14: Mean globulin content in *Salmonella* infected and control mice

Legend:

CL1:- Code for *Salmonella* isolate from human clinical source

KNG 14:- Code for *Salmonella* isolate from water source

4.8.4 Bacterial viable cell count in the liver

The inoculated bacteria (*Salmonella*) were re-isolated from livers of all [100%; 8/8)] the mice infected with CL1 isolate but only from 37.5% (3/8) of those infected with KNG 14 isolate. The cell counts per gram of liver tissue ranged from 3.2×10^3 to 4.8×10^6 for CL1 infected mice and 1.8×10^2 to 4.8×10^3 for the group infected with KNG 14 isolate. No *Salmonella* recoveries were made from livers of mice in the control group. Detailed results are given in Appendix 12.

4.8.5 Gross pathology observed in inoculated mice

Necropsy was performed on dead and euthanized mice. There was generalized congestion on most mice organs infected with different *Salmonella* strains. Heavy congestion was particularly prominent on livers, with some mice showing spleen enlargement (splenomegaly). Most mice (87.5%; 7/8) infected with CL1 strain showed enteritis of the small intestines, while 62.5% (5/8) of those infected with KNG 14 isolate had enteritis. Two mice inoculated with CL1 isolate had mucocatarrhal enteritis and one showed grayish liver foci; its lung and heart having pale nodular lesions.

4.9 Antibiotic susceptibility testing of *Salmonella* isolates

The results of the antibiotic sensitivity testing are as shown on Table 4.15 and Appendix 13.

Table 4.15: Sensitivity of *Salmonella* isolates to nine commonly used antibiotics

Antibiotic	Number of *Salmonella* susceptible	Number of *Salmonella* resistant
Ampicillin/Cloxacillin^{10}	4	2
Cefuroxime^{30}	5	1
Co-Trimoxazole/Trimethoprime^{25}	4	2
Erythromicin^{15}	3	3
Ceftriazone^{30}	5	1
Chloramphenicol^{50}	4	2
Ciprofloxacin^{30}	6	0
Nalidixic acid^{30}	6	0
Penicillin^{10}	0	6

NB: The superscript figures denote the respective antibiotic concentrations contained in each disc in milligrams.

Two antibiotics Ciprofloxacin and Nalidixic acid were most effective, killing 100% of the isolates (no resistance). The susceptibility to the other antibiotics varied as follows:- Ceftriazone and Cefuroxime (83.3%); Ampicillin/Cloxacillin, Chloramphenicol and Co-Trimoxazole/Trimethoprime (66.7%); Erythromycin (50%) and Penicillin (0%). There was multiple resistance to antibiotics (Figures 4.16 and 4.17); this was particularly exhibited by human clinical isolates CL1, CL3 and CL2. The three environmental isolates were sensitive to all the tested antibiotics except Penicillin. Figure 4.18 illustrates sensitivity profile of one of the *Salmonella* isolates.

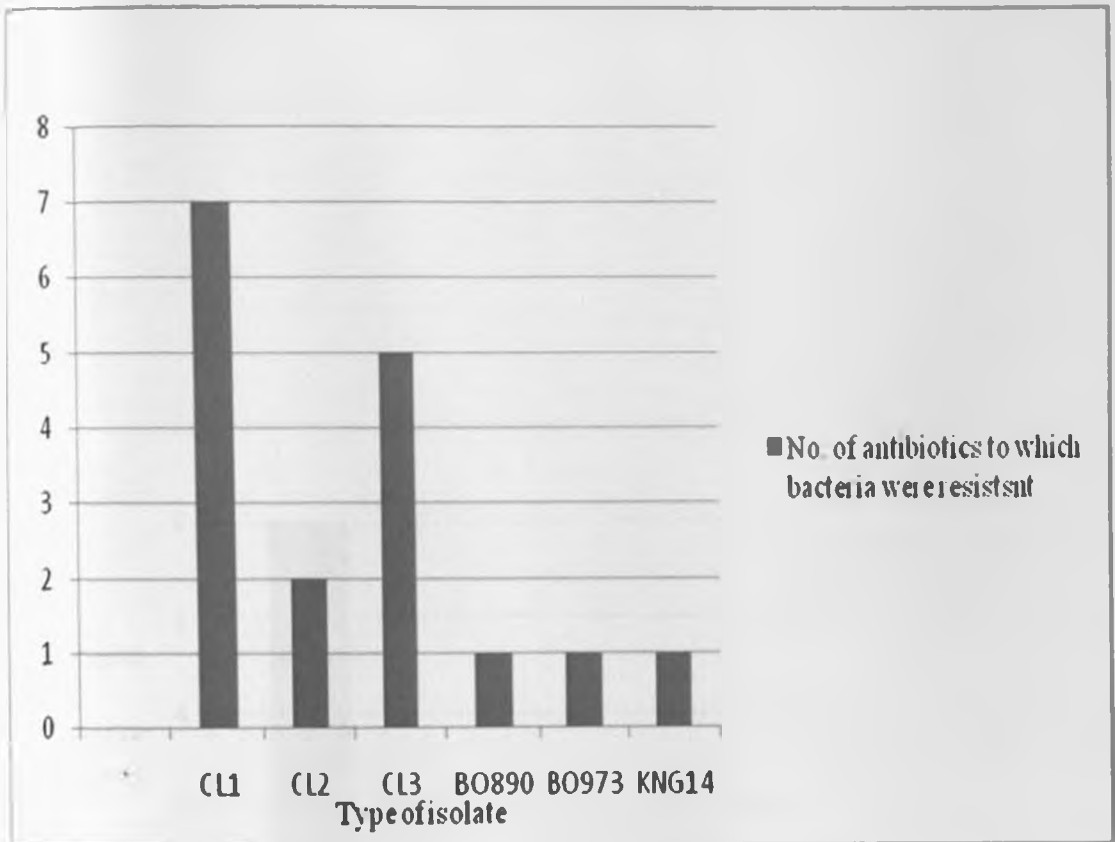


Figure 4.15: Sensitivity of *Salmonella* isolates to nine commonly-used antibiotics

Legend:

CL1: – code for *Salmonella* isolate from human clinical source

CL2:- code for *Salmonella* isolate from human clinical source

CL3 :- code for *Salmonella* isolate from human clinical source

BO890:- code for *Salmonella* isolate from kale

BO973:- code for *Salmonella* from kale

KNG 14:- code for *Salmonella* isolate from water

No.: - Number

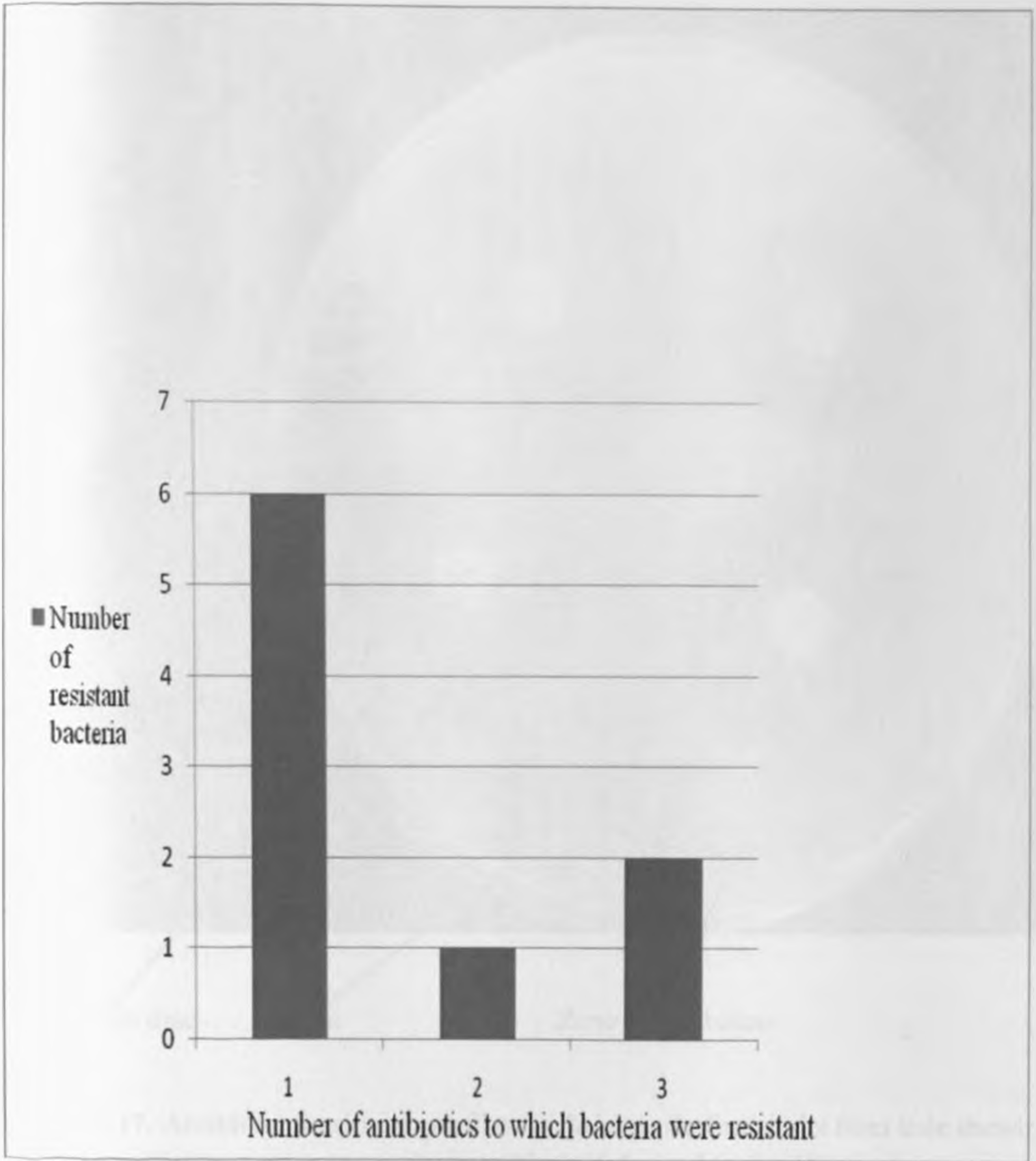
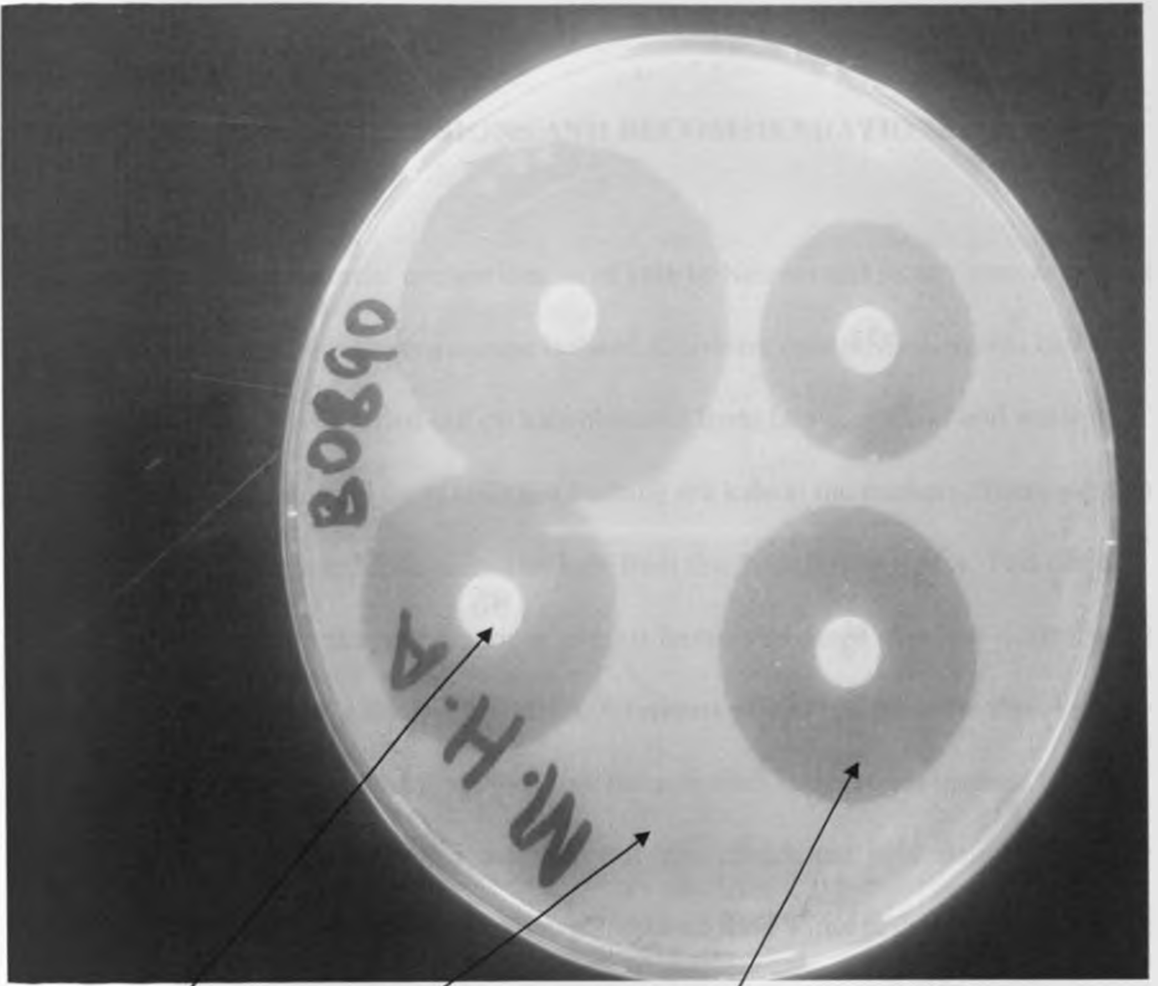


Figure 4.16: Frequency of multiple resistance to tested antibiotics among *Salmonella* isolates



Antibitiotic disc Media Zone of inhibition

Figure 4.17. Antibiotic sensitivity profiles for *Salmonella* Enteritidis from kale showing diverse sensitivity reactions to various antibiotic disks on Mueller Hinton agar.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This study established bacterial contamination of kale in Nairobi and its environs as well as biological characteristics of the organisms isolated. Coliform counts, *Escherichia coli* and *Salmonella* isolation were carried out on kale obtained from farms, markets and water used for irrigation at the farms and for washing/refreshing the kale at the markets. There were no significant differences in coliform count on kale from the three farming sites. This can be attributed to similarity in farming practices as most farmers irrigated with low quality water {above the recommended levels by WHO, (2006) counts of 10^3/100 ml in the three sites}. Majority of the farmers (74.2%) from the three farming sites used animal manure for cultivation of kale while a few (9%) used compost from crop/house-hold residues. Uncomposted animal manure is a well known source of food borne pathogenic bacteria and its inappropriate use in vegetable crops contributes a risk to consumer health (Lau and Ingham, 2001; Wachtel *et al.*, 2002). Cattle and other farm animal manure are known to be the predominant reservoir of *E.coli;* of importance is serotype O157:H7 which produces vero-toxin (VT) (Doyle *et al.*, 2006; Westcot, 1997). Other *E.coli* strains/serotypes that are pathogenic include those that produce heat-stable (ST) and heat-labile (LT) toxins, and those that cause septicemia (those that are entero-invasive). This study isolated *Escherichia coli* in 70% of samples from the farms. This frequency of isolation was higher than that reported by Mukherjee *et al.* (2004) who found *E. coli* in 10.7% (9/84) of field samples of leafy vegetables.

Salmonella was isolated from 4.5% of kale samples from Wangige farms and this could either be from low quality water used for irrigation or animal manure (used by 93.8% of farmers from this site), more so due to spreading as sludge on the plots. *Salmonella* has been isolated from many types of raw fruits and vegetables (Beuchat, 1996; Ercolani, 1976). One example is its isolation from leaf surface of Dodo (*Amaranthus dubius*) from a contaminated site in Kampala (Serani *et al.*, 2008).

While sources of kale in the markets might be different from those obtained from the farms sampled in this study, it is likely that handling increased bacterial loads along the value chain. Coliform numbers were significantly higher ($p< 0.05$) on kale from wet markets and supermarket than those from farms, indicating that post-harvest contamination increased bacterial loads along the value chain. This can be attributed to the use of low quality water (faecal coliform counts ranging from $1.0x10^7\pm 5.7x10^6$ to $8.4x10^6\pm4.2x10^6$ counts/100 ml) by traders in the wet markets for washing the vegetables, as well as poor hygiene and sanitation conditions in these markets. These observations were similar to those reported by Drechsel *et al.* (2000) who found that microbial contamination of vegetables increase as it moves from farms to markets. Other possible sources of post harvest contamination included poor handling and packaging, as well as transportation systems. Increase of contamination of kale as it is being handled along the transport chain is not surprising as there is a report on isolation of toxin-producing *E. coli* from food-handlers in some tourist-class hotels in Nairobi (Bebora *et al.*, 2005), and one on isolation of *Salmonella, E. coli* and other bacteria from commonly-handled coins (currency) in Kenya (Kuria *et al.*, 2009).

Traders normally handle the coins (currency) continuously while also handling the vegetables (as they refresh or sell them).

The way the kales are transported and displayed in the open-air markets needs to be emphasized as a major possible source of contamination. Most traders were found to transport their vegetables in open trucks, wheel-burrows, "mkokoteni", (push carts) open bags and public transport. The vegetables are also kept open at the market, so as to attract buyers. They are, thus, constantly exposed to dust, which is one of the carriers of bacteria. Mbaka *et al.* (2004) isolated *E. coli* from roof-collected rain-water. The source of the contamination might have been dust that was deposited on the roofs or faeces deposited by birds and animal as well as the containers used.

Vegetable suppliers to the supermarkets do outsource vegetables from farmers to meet the contracted quantities; some of these farmers wash the vegetables with low quality water. This market segment did not regulate the production and transport of vegetables and did not wash the vegetables supplied to them. However, their display units were relatively clean compared to those in the wet markets. Similar studies by Karanja *et al.* (2010) reported a significantly higher mean coliform bacterial count in vegetable samples purchased from informal markets (Korogocho and Kibera) in Nairobi compared to those produced by waste water farmers in Kibera. It, therefore, seems that post-harvest contamination is a major risk, in event of presence of pathogenic bacteria.

The high-end specialty market has inspectorate service department that advises farmers on safe production and handling practices, such as safe use of water for irrigation, proper use of

manure, as well as proper packing (closed sacks) and transport systems such as closed trucks.The lower coliform count reported on this market segment appears to reflect these conditions. Mehmet and Aydin (2008) attributed the difference in bacterial loads between vegetable samples from Green grocer and Bazaar to be due to cultivation, transportation conditions and personal application (such as washing vegetables and hand contacts). In general, bacterial loads recorded in this study were above the international commission on microbiological safety of foods (ICMSF, 1998) limit of 10^3 to 10^5 coliforms 100 g^{-1} wet weight of vegetables. This, however, can be reduced by adopting the guidelines for appropriate agricultural and post-harvest handling practices (WHO, 2006) so as to enhance consumer safety.

Salmonella isolates from the environmental sources (kale and water) exhibited lower virulence when compared to those from the human clinical sources as shown by the differences in the ID_{50} values. Similar observations were made by Olsen *et al.* (2001) who reported a lower virulence in *Salmonella* isolates from environment than those from human and animal clinical sources.

Other studies have shown that, genes present within *Salmonella* organisms enhance the survival of the pathogen outside the host environment at the expense of virulence; it is as if, when stressed, these organisms shed off unnecessary buggage –whatever is not essential for the organism's survival, including the plasmids coding for virulence. This however, does not imply that *Salmonella* isolates from the environment represent a low risk. This is because the evolution of virulence of pathogenic bacteria takes place by horizontal acquisition of genes (Parvathi *et al.*, 2011). *Salmonella* isolates from the environment (kale

and water), in this study, were pathogenic to mice; it thus shows that they pose a risk to humans.

The clinical isolate killed 50% of the experimental mice before termination of the experiment, showing a more pathogenic characteristic than the environmental isolate. This was also evidenced by the higher *Salmonella* recoveries made in the livers of the mice inoculated with this isolate. However, both the *Salmonella* infected mouse groups showed similar alterations in the haematological and biochemical parameters compared to the control group.

Results for the total leukocyte count (TLC) and differential leukocyte count in this study are similar to findings of Williams and Newberne (1970), who reported that dogs challenged with *S.* Typhimurium had leukocytosis due to neutrophilia and lymphocytosis. The hypoalbuminaemia observed due to salmonellosis in the present study may be attributed to the liver damage which resulted in less synthesis of albumin. It can also be due to accelerated protein catabolism because of the stress of the infection and fever and/or may be secondary to the increase in globulin concentration since colloid osmotic pressure has to be maintained within normal limits by a regulatory mechanism (Benjamin, 1978). Hepatic foci which were observed in one of the mice, might also have contributed to the hypoalbuminaemia since liver diseases have been reported to inhibit albumin synthesis (Kirsch, 1983; Hsu, 1989; Rai *et al.*, 1993). On the other hand, the hyperglobulinaemia might have been due to an increase in gamma-globulin production as a result of the immunological response (antibody production) to the infection, and excessive release of alpha- and beta-globulins from the liver as a result of hepatic necrosis (Putman, 1975).

Antibiotic resistance of the *Salmonella* organisms isolated from the human clinical source were higher than those from environmental sources. This could be due to the selective pressure imposed by the use of antimicrobials in human medicine which promotes the spread of multiple antimicrobial resistances (Alessandra, 2003). The resistance to penicillin in environmental strains may be due to human antibiotic use through the spread of resistant strains or their genes from human and agricultural systems. It can also be due to evolution and selection of new resistant strains or the amplification of pre-existing resistant strains in the environment (Alessandra, 2003). Antibiotic resistance among bacteria is a common and worrying trend, which limits effect of treatment of diseases caused by respective organisms. In Kenya, resistances have been documented in *E. coli* (Bebora *et al.*, 1994; Mapenay *et al.*, 2006), *Listeria* (Njagi *et al.*, 2004), *Salmonella* (Bebora and Nyaga, 1989; Kariuki *et al.*, 2004).

5.2 Conclusions

1. The study has shown that coliform numbers on kale from peri- urban farms and markets in Nairobi and its environs are high and exceed the recommended levels by WHO.
2. *Escherichia coli* and *Salmonella* organisms contaminate kale produced from the peri- urban farms and those sold at the markets, posing a public health risk.
3. Kale production practices such as use of low quality water for irrigation, use of animal manure/, inappropriate use as well as poor handling of kale at the market level are sources of bacterial contamination.

4. *Salmonella* organisms isolated from kale and associated water are pathogenic.to mice, although they tend to exhibit lower virulence when compared with isolates from human clinical sources. They, thus, pose a health risk to the consumers of kale.
5. *Salmonella* organisms from kale and associated water are resistant to some antibiotics and therefore a challenge to their treatment.

5.3 Recommendations

1. Application of multiple barrier approach involving practices such as good irrigation practices and vegetable washing using potable water at the market and before food preparation at the household should be encouraged to reduce health risks.
2. Consumers are encouraged to cook vegetables well before consumption.
3. More advanced molecular techniques need to be utilized for more accurate and faster disease diagnosis (detection of presence of pathogen) and virulence testing (detection of virulence genes). This is necessary for prompt and effective treatment.

CHAPTER SIX

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CHAPTER SEVEN

APPENDICES

APPENDIX 1: Interview questionnaire

KALE PRODUCTION PRACTICES AT PERI- URBAN FARMS OF NAIROBI

Part I: Respondent and site identification

1. Respondent name (in full)... 2. Phone number ....

3. Respondent ID... 4. Name of farming area.............................

Part 2: Details of the farm and production practices

1. On how many hectares do you grow leafy vegetables?...................

2. Do you deliver/sell directly to a market place? 1.Yes 1.No (only through other transporters/traders)

3. If YES to Q2 to which markets (List in order of magnitude of your delivery):

a)..................................

b)..................................

c)..................................

Appendix 1 (continues)

4. PLEASE COMPLETE THE TABLE BELOW FOR THE KALE YOU GROW

1. Area of plot in acres	2. Was this plot irrigated? 1.Yes 0.No	3. If YES to Q2 what was source of water? 1. Well 2.Tap 3.Furrow (not river) 4.River 5. Other (specify)	4. Did you apply manure on this plot? 1.Yes 0.No	5. IF YES to Q 4, how did you? 1=Spread sludge 2=Spread compost 3=Plowed into soil 4. Other (specify)	What is the source of your manure 1.Cattle 2.Poultry 3.Sheep/goat 4.Pig 5.Compost 6.Other…
Acres	Code	Code	Code	Code	

Appendix 1 (continues)

TRADER MARKETING PRACTICES IN THE WET MARKETS OF NAIROBI

Part I: Respondent and site identification

1. Respondent name (in full)... 2. Phone number ....

3. Respondent ID.. 4. Name of trading area.............................

Part 2: Respondent retailing practices

1.What mode of transport do you use? 1. Open truck 2. Closed truck 3. Public transport 4.Human (on back) 5. Wheelbarrow 6.*Mkokoteni* 7.Other............

2. How do you pack the vegetables you transport?

   1. Packed in sealed bags 2. Packed in open bags 3. Not packed 4.Other........

3. Where do you source the kale you transport/ sell (list first 3)

   a)............b)........................c)..............

APPENDIX 2: Coliform count (colony forming units per gram) on kale from the peri-urban farms

Serial No.	ARTHI RIVER	NGONG	WANGIGE
1	110000	1700000	190000
2	31000	1900000	18000
3	150000	270000	28000
4	170000	1100000	190000
5	140000	94000	2000000
6	10000	130000	31000
7	11000	180000	440
8	1600000	670000	350
9	80000	520000	390000
10	9000	1700000	130000
11	600	47000	12000
12	500	7000	24000
13	12000	30000	1900
14	86000	75000	1000
15	520000	38000	480
16	66000	23000	380000
17		4400	79000
18		150000	2000
19		29000	22000
20		24000	26000
21		170000	25000
22		600	3800

APPENDIX 3: Coliform count (per hundred millilitre) in irrigation water and washing water samples from farms and markets

Sample number	Farms			Wet markets		
	ATHI RIVER	NGONG	WANGIGE	KAWANGWARE	KANGEMI	GITHURAI
1	2000000	80000	800000	48000	8000000	16000000
2	16000000	32000000	1600000	44000000	400000	72000
3	800000	4000000	24000	400000	6800000	60000
4	120000000	36000000	40000	560000	7600000	200000
5	12000000	4000	4000	12000000	12000000	440000
6	1600000000	12000000	36000000	24000000	16000000	84000
7	52000000	200000	120000	160000	8000000	280000
8		290000	280000	80000	8000000	
9		32000000	40000			
10		80000	3200			

APPENDIX 4: Coliform count (colony forming units per gram) on kale from the wet markets

Sample number	Kawangware	Githurai	Kangemi
1	2600000	21000	30000000
2	6300000	460000	7500
3	30000000	91000	24000
4	30000000	200000	2600
5	460000	1100	3800000
6	490000	390000	390000
7	4700000	5400	53000
8	6400000	420000	21000
9	30000000	7300	600
10	4700000	8100000	44000
11	30000000	800	200
12	8700000	23000	24000
13	15000000	530000	700
14	510000	2100	610000
15	4100000	390000	24000
16	610000	10000	28000
17		2800	
18		9700000	

APPENDIX 5: Coliform count (colony forming units per gram) on kale from the supermarket and high- end specialty store

Sample number	Supermarket	High- end market
1	1200000	140000
2	81000	52000
3	6400000	130000
4	1100000	33000
5	290000	3500000
6	150000	530000
7	410000	390000
8	8200000	870000
9	2500000	60000
10	52000	12000
11	3100000	39000
12	430000	13000
13	33000	40000
14	27000	770000
15	60000	410000
16	8600000	
17	44000	
18	4100000	
19	3700000	
20	4100000	
21	4200000	
22	5200000	
23	4800000	
24	1700000	
25	5500000	

APPENDIX 6: Percentage packed cell volume of *Salmonella* infected and control mice

		Days post inoculation			
Test group (KNG 14)	Mice number	0	3	7	14
	1	48	46	-	-
	2	47	45	-	-
	3	45	45	42	-
	4	45	43	43	-
	5	46	42	44	41
	6	47	44	41	42
	7	45	42	40	40
	8	47	46	41	41
Test group (CL 1)					
	1	49	-	-	-
	2	46	40	-	-
	3	45	41	38	-
	4	48	41	-	-
	5	47	39	-	-
	6	46	40	-	-
	7	45	-	-	-
	8	46	40	39	-

Appendix 6 continues

Control group					
	1	44	44	-	-
	2	45	44	-	-
	3	47	46	47	-
	4	46	47	47	-
	5	45	45	44	45
	6	45	44	44	44
	7	46	47	47	47
	8	47	46	45	45

Legend:

CL1:-code for salmonella isolate from human clinical source

KNG14:- code for salmonella isolate from water source

APPENDIX 7: Total Erythrocyte count of *Salmonella* infected and control mice

		Days post inoculation			
Test group (KNG 14)	Mice number	0	3	7	14
	1	9500000	9400000	-	-
	2	9700000	9600000	-	-
	3	9800000	9000000	8600000	-
	4	800000	9200000	8200000	-
	5	7700000	8800000	7700000	500000
	6	8200000	8000000	6500000	6100000
	7	8300000	8100000	6900000	6600000
	8	8500000	8300000	6800000	4300000
Test group (CL 1)					
	1	1E+08	-	-	-
	2	9100000	6200000	-	-
	3	9800000	7200000	4200000	-
	4	8900000	6500000	-	-
	5	9900000	550000	-	-
	6	8800000	7000000	-	-
	7	7000000	-	-	-
	8	8800000	5400000	4800000	-

Appendix 7 continues

Control group					
	1	9200000	9400000	-	-
	2	9900000	9800000	-	-
	3	9100000	9000000	9200000	-
	4	9500000	9500000	9600000	-
	5	9000000	8600000	8900000	9100000
	6	8800000	8900000	9200000	9000000
	7	9900000	10000000	9600000	9600000
	8	9100000	9000000	9100000	8800000

Legend:

CL1:-code for salmonella isolate from human clinical source

KNG14:- code for salmonella isolate from water source

APPENDIX 8: Haemoglobin content (grams per decilitre) in *Salmonella* infected and control mice

		Days post inoculation			
KNG 14 test group	Animal no.	0	3	7	14
	1	16	15.33	-	-
	2	15.67	15	-	-
	3	15	15	14	
	4	15	14.33	14.33	
	5	15.33	14	14.67	13.67
	6	15.67	14.67	13.67	14
	7	15	14	13.33	13.33
	8	15.67	15.33	13.67	13.67
CL1 test group	1	16.33	-	-	-
	2	15.38	14.20		-
	3	15	13.67	12.67	-
	4	16	13.67	-	-
	5	15.67	13	-	-
	6	15.33	14.60	-	-
	7	15	-	-	-
	8	15.33	13.33	13	-

Appendix 8 continues

Control group	1	14.67	14.67	-	-
	2	15	14.67	-	-
	3	15.67	15.37	15.67	-
	4	15.33	15.67	15.67	-
	5	15	15	14.67	15
	6	15	14.67	14.67	14.67
	7	15.33	15.67	15.67	15.67
	8	15.67	15.33	15	15.01

Legend:

CL1:-Code for *Salmonella* isolate from human clinical source

KNG14:- Code for *Salmonella* isolate from water source

APPENDIX 9: Total leukocyte count of *Salmonella* infected and control mice

		Days post inoculation			
Test group (KNG 14)	Mice number	0	3	7	14
	1	4000	3900	-	-
	2	3800	3900	-	-
	3	3500	3600	4900	-
	4	3100	3100	3500	-
	5	3200	3400	3200	5100
	6	3400	3300	4800	6000
	7	2900	3000	5200	5000
	8	3000	2800	4800	5600
Test group (CL 1)					
	1	3500	-	-	-
	2	3200	3800	-	-
	3	3800	3800	6600	
	4	3000	4100	-	-
	5	3400	3500	-	-
	6	3800	4200	-	-
	7	3000	-	-	-
	8	2900	3600	6700	-

Appendix 9: continues

Control group					
	1	3500	3600	-	-
	2	3600	3700	-	-
	3	3800	3700	3400	-
	4	3100	3200	3500	-
	5	3500	3600	3700	3700
	6	3600	3700	3600	3600
	7	3700	3700	3700	3700
	8	3800	3900	3800	3800

Legend:

CL1:- code for *Salmonella* isolate from human clinical source

KNG14:- code for *Salmonella* isolate from water source

APPENDIX 10: Percent leukocyte differential count of *Salmonella* infected and control mice

		DAYS POST INOCULATION																			
		0					3					7					14				
Test group (KNG 14)	Animal no.	N	L	E	B	M	N	L	E	B	M	N	L	E	B	M	N	L	E	B	M
	1	17	80	2	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	18	80	1	0	1	24	73	1	0	1	-	-	-	-	-	-	-	-	-	-
	3	16	81	1	1	1	26	71	1	2	0	27	70	2	1	0	-	-	-	-	-
	4	20	78	0	0	2	20	78	0	0	2	30	70	0	0	0	-	-	-	-	-
	5	18	79	1	2	0	17	81	1	1	1	32	67	0	1	0	34	64	1	2	0
	6	21	77	1	1	0	29	71	0	0	0	29	69	1	0	1	36	62	1	1	0
	7	18	80	1	0	1	25	75	0	0	0	33	65	2	0	0	38	60	0	0	2
	8	15	84	1	0	0	27	70	1	1	0	35	65	0	0	0	36	62	1	1	0

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Appendix 10 continues

Test group CL 1																					
	1	20	78	1	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	22	77	0	1	0	20	80	0	0	0	-	-	-	-	-	-	-	-	-	-
	3	18	80	1		1	23	76	1	0	0	-	-	-	-	-	-	-	-	-	-
	4	15	83	1	1	0	24	74	1	0	1	38	62	0	0	0	-	-	-	-	-
	5	23	75	0	2	0	23	74	1	2	0	-	-	-	-	-	-	-	-	-	-
	6	17	80	1	1	1	26	70	2	1	1	-	-	-	-	-	-	-	-	-	-
	7	19	78	2	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	8	22	74	2	2	0	21	77	1	0	0	36	60	2	1	1	-	-	-	-	-
Control group																					
	1	22	74	2	2	0	18	80	2	0	0	-	-	-	-	-	-	-	-	-	-
	2	17	80	1	1	0	16	83	1	1	1	-	-	-	-	-	-	-	-	-	
	3	19	78	2	1	0	20	78	1	2	0	17	80	2	1	0	-	-	-	-	-
	4	20	78	1	1	0	19	80	0	1	0	20	78	1	1	1	-	-	-	-	-
	5	24	76	0	0	0	24	76	0	0	0	17	80	1	1	0	17	80	1	1	0

Appendix 10 continues

	6	17	80	2	1	0	15	85	0	0	0	22	74	2	2	0	22	74	2	2	0
	7	20	78	2	0	0	17	80	1	0	1	19	78	2	1	0	19	80	0	1	0
	8	15	84	1	0	1	21	78	1	0	0	15	83	1	1	0	16	83	1	1	1

Legend:

CL1:-code for *Salmonella* isolate from human clinical source

KNG14:- code for *Salmonella* isolate from water source

APPENDIX 11: Total protein, albumin and globulin (grams per decilitre) of *Salmonella* infected and control mice

		DAY 0			DAY 3		
Test group KNG 14	Animal No.	Total protein	Albumin	Globulin	Total protein	Albumin	Globulin
	1	7.41	4.52	2.89	7.02	4.21	2.81
	2	7.22	4.31	2.91	6.96	4.02	2.94
	3	7.03	4.42	2.61	6.87	4.31	2.56
	4	7.44	4.51	2.93	7.79	3.94	3.25
	5	7.37	4.5	2.87	7.02	4.11	2.91
	6	7.48	4.21	3.27	6.83	4.01	2.82
	7	7.69	4.31	3.38	6.55	3.81	2.74
	8	7.84	4.55	3.29	7.06	3.99	3.07
Test group CL1							
	1	7.41	4.61	2.8	-	-	-
	2	7.67	4.41	3.21	7.02	3.12	4.39
	3	7.59	4.22	3.35	6.83	3	3.9
	4	7.66	4.55	3.11	6.74	3.41	3.83
	5	7.41	4.59	2.82	6.95	3.16	3.33
	6	7.32	4.71	2.61	6.45	2.06	3.79

Appendix 11 continues

	7	7.54	4.31	3.23	-	-	-
	8	7.45	4.46	2.99	6.59	2.09	4.5
Control							
	1	7.81	4.72	3.09	7.21		2.35
	2	7.62	4.66	2.96	7.61		3.1
	3	7.43	4.33	3.1	7.52		3
	4	7.34	4.56	2.78	7.62		3.05
	5	7.44	4.79	2.65	7.43		2.78
	6	7.05	4.61	2.44	7.33		2.9
	7	7.26	4.68	2.58	7.77		3.3
	8	7.47	4.66	2.81	7.51		2.85
		DAY 7			DAY 14		
Test group KNG14		Total protein	Albumin	Globulin	Total protein	Albumin	Globulin
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-
	3	6.11	3.12	2.99	-	-	-
	4	6.33	3.04	3.29	-	-	-
	5	6.12	3.14	2.98	5.75	2.66	3.09
	6	6.04	3.21	2.83	5.66	2.61	3.05
	7	6.51	3.02	3.49	4.87	1.52	3.33
	8	6.37	3.14	3.23	4.81	1.71	3.1
Test group CL1							

Appendix 11 continues

	1	-	-	-	-	-	
	2	-	-	-	-	-	-
	3	4.67	1.22	3.45	-	-	-
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
	6	-	-	-	-	-	-
	7	-	-	-	-	-	-
	8	4.22	1.16	3.306	-	-	-
Control group		-	-	-	-	-	-
	1	-	-	-	-	-	
	2	-	-	-	-	-	-
	3	7.54	4.31	3.23	-	-	-
	4	7.63	4.49	3.14	-	-	-
	5	7.26	4.76	2.5	7.15	4.65	2.5
	6	7.49	4.6	2.89	7.63	4.5	3.13
	7	7.68	4.69	2.9	7.24	4.6	2.62
	8	7.52	4.56	2.96	7.11	4.6	2.51

Legend:

CL1:-code for *Salmonella* isolate from human clinical source

KNG14:- code for *Salmonella* isolate from water source

APPENDIX 12: Bacterial viable count (colony forming units per gram) in the livers of *Salmonella* infected and control mice

Test group KNG 14	Animal no.	day2*	day3	day 5*	7	14
	1	-	NG	-	$-1.8x10^2$	-
	2	-	NG	-	-	-
	3	-	-	-	$1.4x10^3$	-
	4	-	-	-	NG	-
	5	-	-	-	-	NG
	6	-	-	-	-	NG
	7	-	-	-	-	$4.8x10^3$
	8	-	-	-	-	NG
Test group CL1						
	1	$1.6x10^3$		-		
	2	-	$4.0x10^4$	-		
	3	-	-	$6.4x10^6$	$3.2x10^3$	-
	4	-	-	-	-	-
	5	-	-	$3.8x10^2$	-	-
	6	-	$4.4x10^3$		-	-
	7	$3.6x10^4$	-	-	-	-
	8	-	-	-	$2.2x10^5$	-
Control group						
	1	-	NG	-	-	-
	2	-	NG	-	-	-
	3	-	-	-	NG	-
	4	-	-	-	NG	-
	5	-	-	-	-	NG
	6	-	-	-	-	NG
	7	-	-	-	-	NG
	8	-	-	-	-	NG

Legend:

- Sacrifice not done/ already done earlier

*- death due to salmonellosis

CL1:-code for *Salmonella* isolate from human clinical source

KNG14:- code for *Salmonella* isolate from water source

APPENDIX 13: Antibiotic sensitivities of the *Salmonella* isolates

	Salmonella isolates						
ANTIBIOTIC	CL1	CL2	CL3	BO890	BO973	KNG14	E.COLI ATCC 25922
Ampicillin/Cloxacillin	7R	16PS	6R	21S	20S	15PS	8
Cefuroxime	6R	22S	23S	23S	24S	19S	20
Co-Trimoxazole/Trimethoprime	7R	29S	6R	32S	29S	29S	29
Erythromycin	7R	9R	6R	12S	12S	14S	6
Ceftriazone	9R	35S	32S	34S	33S	27S	32
Chloramphenicol	6R	36S	10R	33S	33S	28S	25
Ciprofloxacin	35S	38S	40S	43S	44S	40S	40
Nalidixic acid	26S	27S	24S	24S	26S	27S	26
Penicillin	6R	6R	6R	10R	12R	6R	10

Legend:

R:-resistant

PS:- partial sensitivity

S:-rensitive

CL1:-code for *Salmonella* isolate from human clinical source

CL2:- code for *Salmonella* isolate from human clinical source

CL3:- code for *Salmonella* isolate from human clinical source

BO890:- code for *Salmonella* isolate from kale

BO973:- code for *Salmonella* isolate from kale

KNG14:- code for *Salmonella* isolate from water source

E.COLI:- *Escherichia coli*

ATCC 25922:- American type culture collection

Appendix 14: Inhibition zone diameter size interpretive standards for selected antimicrobial disks appropriate for *Salmonella*

	Susceptible (millimeter)	Intermediate (millimeter)	Resistant (millimeter)	*E.coli* ATCC 25922 (millimeter)
Antimicrobial				
Ampicillin/Cloxacillin[10]	≥17	14-16	≤13	16-22
Cefuroxime[30]	≥18	14-17	≤13	
Co-Trimoxazole/Trimethoprime[25]	≥16	11-15	≤10	23-29
Erythromicin[15]	≥12	10-11	≤9	6-10
Ceftriazone[30]	≥25	16-24	≤15	32-42
Chloramphenicol[50]	≥18	13-17	≤12	21-27
Ciprofloxacin[30]	≥21	16-20	≤15	30-40
Nalidixic acid[30]	≥19	14-18	≤13	22-28
Penicillin[10]	≥16	13-16	≤12	10-18

Source: NCCLS (2002)

NB: The superscript figures denote the antibiotic concentration of the respective sensititivity disks

Appendix 15: Names of the manufacturers of the media and reagents used

MEDIA/REAGENT	MANUFACTURER
MEDIA	
MacConkey Agar	Oxoid LTD., Bangingstoke, Hants., England
Trypton Soya broth U.S.P. (CM 0129)	Oxoid LTD., Bangingstoke, Hants., England
Urease Agar Base (CM 53)	Oxoid LTD., Bangingstoke, Hants., England
Triple Sugar Iron Agar (CM 0277)	Oxoid LTD.,Bangingstoke, Hants., England
Buffered Pepton water (CM 109)	Oxoid LTD., Bangingstoke, Hants., England
Muller Kauffman Tetrathionate Broth Base (9221)	Oxoid LTD.,Bangingstoke, Hants., England
Rappaport- Vasiliadis Soy peptone Broth (9220)	Oxoid LTD.,Bangingstoke, Hants., England
MacConkey purple broth	Oxoid LTD.,Bangingstoke, Hants., England
Brilliant Green Bile Agar	Oxoid LTD.,Bangingstoke, Hants., England

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Appendix 15 continues

Violet Red Bile Agar	Oxoid LTD.,Bangingstoke, Hants., England
REAGENTS	**COMPANY**
API 20100	Biomeriux LTD., USA
Serological reagents Diagnostica's *Salmonella* sero-quick ID kit	Statens Serum Institut (SSI) , USA