

**RISK ASSESSMENT OF SOIL EXPOSURE TO ANTIBIOTIC RESIDUES IN MANURE  
IN LIVESTOCK REARING FARMS OF RONGAI SUB-COUNTY, NAKURU COUNTY,  
KENYA**

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**A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for  
the Master of Science Degree in Environmental and Occupational Health of Egerton  
University**

**EGERTON UNIVERSITY**

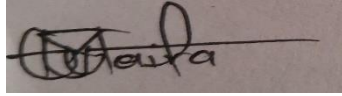
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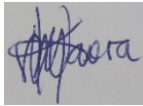
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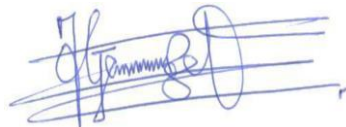
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## **DEDICATION**

This work is dedicated to my late grandfather Peter Nyakwaka, parents Meshack Obudho and Dr. Dorothy Nyakwaka, brother Jack Nyambok for their constant and persistent support and prayers throughout this journey. To them I say Thank you. God bless you.

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## ABSTRACT

Limited spaces within urban centers have forced farmers to practice intensive livestock farming within very small spaces, contributing to the prevalence of livestock diseases and increased veterinary drug use. In order to meet the growing demands for animal products, farmers have embraced intensive livestock production and thus heavy reliance on veterinary drugs for therapeutic, disease prevention and growth promotion purposes that introduce the risk of antibiotics in the environment from manure application. Antibiotic contaminated manure reaches agricultural ecosystems exerting antimicrobial resistance selection and toxicity pressure in soils and the environment. The main aim of this study was to assess the risk of soil exposure to antibiotic residues in livestock manure from livestock rearing farms in Rongai sub-county, Nakuru County. The study used a mixed method design consisting of cross-sectional survey, ecological survey and laboratory analysis. Questionnaires were used to obtain information on livestock reared, farm and farmer practices. Livestock manure samples were picked from compost heaps in farms while soil samples were collected from soil (0-10cm depth) in farms. Analysis for antibiotic drug residues in livestock manure and manure amended soils was done using HPLC-DAD. The common class of antibiotics used among poultry farmers were tetracyclines (35%), sulfonamide (40%), quinolones (20%) and others (5%). Tetracyclines (60%), sulfonamide (8%), penicillin's (20%) and macrolides (12%) were the most consumed class of antibiotics among cattle farmers. Concentration of tetracycline and sulphonamide antibiotics ranged from 1105-16240  $\mu\text{g}/\text{kg}$  and 375.5-4955  $\mu\text{g}/\text{kg}$  in poultry manure. In cattle manure, the concentrations ranged from 60-15231  $\mu\text{g}/\text{kg}$  for tetracycline and 8.9-3940  $\mu\text{g}/\text{kg}$  for Sulfonamide. In, manure-amended soils, antibiotic concentrations for tetracyclines ranged from 1880-29396  $\mu\text{g}/\text{kg}$  in poultry and 1532-14237  $\mu\text{g}/\text{kg}$  in cattle. Sulfonamide in soils had concentrations of 64.2-10556  $\mu\text{g}/\text{kg}$  in poultry and 11.74-7752  $\mu\text{g}/\text{kg}$  in cattle manure. Most of the risk quotient values calculated in this study presented low and medium risk. Sulfadiazine presented a higher ecological risk quotient value above the recommended value of  $\leq 1$ . Most of the manure and soils were contaminated with antibiotic residues indicating a significant threat to environmental and human health. Sensitization on proper antibiotic use and healthy manure management practice is recommended in order to reduce antibiotic residues released into the environment from livestock rearing farms. Ministry of Agriculture, livestock and fisheries and NEMA, should set and monitor maximum residues limits of antibiotics in manure to protect the environment.

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## **LIST OF ABBREVIATIONS AND ACRONYMS**

<b>AMR:</b>	Antimicrobial Resistance
<b>ARG:</b>	Antimicrobial resistant gene
<b>ARG:</b>	Antibiotic Resistant Genes.
<b>BRICS:</b>	Brazil, Russia, India, China and South Africa.
<b>CAFOs:</b>	Concentrated animal feeding operations
<b>CIDP:</b>	County Integrated Development Plan
<b>CGN:</b>	County Government of Nakuru
<b>EC:</b>	European commission
<b>FAO:</b>	Food and Agriculture Organization
<b>FDA:</b>	Food and Drug Administration
<b>GAP:</b>	Global Action Plan
<b>GDP:</b>	Gross Domestic Product
<b>HPLC/DAD:</b>	High Performance Liquid Chromatography/Diode-Array Detection
<b>Kd:</b>	Partitioning Coefficient.
<b>KNBS:</b>	Kenya National Bureau of Statistics.
<b>MEC:</b>	Measured Environment Concentration
<b>MoA:</b>	Ministry of Agriculture.
<b>MOALFI:</b>	Ministry of Agriculture Livestock Fisheries and Irrigation.
<b>MRL:</b>	Maximum Residue Limits
<b>NAP:</b>	National Action Plan
<b>NCIDP:</b>	Nakuru County Integrated Development Plan
<b>NEC:</b>	No effect concentration.

<b>NEMA:</b>	National Environment Management Authority
<b>pKa:</b>	Dissociation constant
<b>PPCPs:</b>	Pharmaceutical and Personal Care Products.
<b>PEC:</b>	Predicted Environmental Concentration
<b>PNEC:</b>	Predicted No effect concentration.
<b>TOC:</b>	Total Organic Carbon
<b>USEPA:</b>	United States Environment Protection Agency
<b>WHO:</b>	World Health Organization

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background Information of the study

Globally, the agricultural sector has been evolving rapidly spurred by the needs of an ever-growing population and globalization. However, growth within the sector has been linked to emerging challenges related to social, environmental and public health risks (Robinson *et al.*,2011). Livestock production contributes to nearly 40% of total agricultural output and 20% in developed and developing countries, respectively (FAO, 2018). Agriculture is rated as one of the most dominant sectors within the Kenyan economy, with a total of 6.4 million households practicing agriculture and 4.7million actively involved in livestock rearing (KNBS, 2019).

Livestock production plays an important role to the Kenyan economy contributing 42% of agricultural GDP, and 12% in the national GDP (FAOSTAT, 2005). The Kenyan Vision 2030 anchored in 3 main pillars has agriculture outlined under the economic pillar as innovative, commercially-oriented and modern farming (MoALFI, 2019). The need to meet growing demand for animal products has necessitated increased uptake of large-scale animal rearing and increased use of veterinary drugs (Ezenduka *et al.*,2014). Population growth has spurred the need for increased livestock production to meet the ever-increasing demand. This has contributed to the recent increase in the uptake of intensive animal rearing among many households as a source of income and to meet nutritional needs of families.

Antibiotics refers to a wide range of chemical substances produced naturally, semi-synthetically and synthetically, used in killing bacteria or bacterial growth inhibition (Kümmerer, 2009; Milić *et al.*,2013). They are manufactured mainly for prevention of disease outbreaks and treatment of animal diseases such as arthritis, brucellosis, gastrointestinal diseases, respiratory diseases and bacterial infectious diseases (Tollefson & Miller, 2000). Pharmaceuticals and personal care products (PPCPs) have been receiving considerable attention in recent years due to the high rates of consumption, detection in the environment and their reported eco-toxicological effects (Yang *et al.*,2017). The total annual use of antibiotics has increased from 100,000 to 200,000 tons globally (Gelband *et al.*,2015). A large number of veterinary antibiotics are produced and consumed annually with estimations of up to 420 tons in the United Kingdom, 14,000 tons in the United States (FDA, 2017) and 84240 tons in China (Zhang *et al.*,2015).

Antibiotic consumption is expected to increase in countries like Brazil, Russia, India, China and South Africa (BRICS) (Van Boeckel *et al.*,2015). This increase is attributed to high consumption levels of veterinary drugs within large scale animal rearing farms. China is regarded as the largest consumer of veterinary drugs in the world due to the high number of its concentrated animal feeding operations (CAFOs) (Manyi-Loh *et al.*,2018). A large number of antibiotics consumed are used in the treatment of animals making veterinary antibiotics the most consumed group of antibiotics (Gelband *et al.*,2015).

A study on the consumption of antibiotics among both human and veterinary drug stores in Kenya, showed veterinary antibiotics consumption was higher with sulfonamides, tetracyclines and penicillins among the most purchased antibiotic classes among dairy and poultry farmers (Muloi *et al.*,2019). According to Mitema *et al.* (2001), antimicrobial consumption patterns in Kenya from 1995-1999 showed tetracyclines accounted for 55% of all antimicrobials, 21% sulfonamide and 6% beta lactams used in food animals. Commonly used animal prophylactics included; beta lactams, sulfonamide and tetracycline's (Aboge *et al.*,2000).

Kenya has an estimated poultry and cattle population of 57 million and 22 million respectively (KNBS,2019). According to Van Boeckel *et al.* (2015), the average global scale of antibiotics consumption per annum is approximately 45mg/kg, 148mg/kg and 172mg/kg for cattle, chicken and swine, respectively. However, consumption patterns vary across regions, a phenomenon attributed to variations of farming types, agricultural advancement, availability of antibiotics, pests and diseases, and climatic conditions (Van Boeckel *et al.*,2015).

Tetracycline and sulfonamide groups generate a lot of interest since they are not easily absorbed in the gut of animals and end up excreted into the environment in their original physical and chemical structure (Conde-Cid *et al.*,2020). These antibiotics are widely used in treating and preventing infections in humans, plants and animals but can be introduced at very small doses in sub-therapeutic doses to promote growth and improve feeding efficiency of reared animals (Du & Liu, 2012).

Animal metabolic processes contribute to the levels of antibiotic doses released into the environment with up to 90% released as original compounds or metabolites via animal urine and feces (Rang *et al.*,2007). Poor absorption of antibiotics leaves livestock manure exposed to antibiotic residues that end up in the environment. The use of animal manure as organic fertilizer is a widely adopted agricultural practice used as an alternative to chemical fertilizers in arable soils that are of low fertility (Zhao *et al.*,2010). The resulting anthropogenic activities

have made livestock manure reservoirs of antibiotic residues and bacteria carrying antibiotic resistance genes (ARGs) that are resistant to many clinically important antibiotics (Lee *et al.*,2020).

Livestock feces and manure are important reservoirs for antibiotics and antibiotic resistant bacteria (Lima *et al.*,2020). Antibiotics in soils have a toxic effect on plants and soil microorganisms. Sulfadiazine which has been found to have toxic effects on crops such as wheat, Chinese cabbage and tomatoes. Chlorotetracyclines have also been found to have an effect on microbial communities in soils (An *et al.*,2009; Du & Liu, 2012; Reichel *et al.*,2015). They do not maintain their original state when exposed to the environment and end up degraded into their metabolites or adsorbed in soils.

Antibiotic metabolites can also be excreted with antimicrobial activity or can return to the initial active compound (Manzetti & Ghisi,2014). One example is the conjugation in the liver of sulfamethazine with sugars after its administration. After its excretion, bacteria are able to degrade the sugars and convert it back to its bioactive form (Kim *et al.*,2011). Active forms of veterinary antimicrobials are often found in manure and may disseminate in the environment after its application on soil (Sarmah *et al.*,2006). Antibiotic degradation and adsorption are mainly dependent on different physical-chemical properties of the residues, soil characteristics, temperature, rainfall and humidity (Kemper, 2008). They are also susceptible to microbial degradation in aerobic conditions in soils (Pan & Chu,2016). However, non-degraded antibiotics in manure and in soil may act as a selective pressure, contributing to the emergence and dissemination of antimicrobial resistance determinants.

Antibiotic residues have frequently been reported in the environment (Pikkemaat *et al.*, 2016). Their residues have also been found in animal manure in Turkey, Iran, Spain and Tanzania (Alavi *et al.*,2015; Conde-Cid *et al.*,2018; Karcı & Balcıoğlu, 2009; Mohameda *et al.*,2017) and manure amended soils (Pan & Chu,2016; Oliver *et al.*,2020). These compounds have a high affinity for soil organic matter (Figueroa *et al.*,2004). This leads to a rise in the prevalence of antibiotic residues in the agro-ecosystem, attributed to poor metabolization of drugs that end up in animal manure leading to public health concerns. The use of animal manure on agricultural soils not only adds to the amount of nutrients, but also introduces a considerable number of micro-organisms including bacteria and their genes that are resistant to antibiotics (Poulsen *et al.*,2013).

Livestock manure is a key ingredient in both organic and sustainable farming, applied as either compost or as raw manure leaving soils and water as a reservoir of antibiotic residues (Kumar *et al.*,2005). Emergence of antibiotic-resistant genes has been linked to extensive use of veterinary antibiotics in animal production used to control infections and as growth promoters in very small amounts (Pikkemaat *et al.*,2016).

Emerging livestock diseases have been linked to increased cases of disease resistance and climate change. Overuse of antibiotics in livestock production is increasingly posing a great risk to both agricultural and human health, linked to the spread of antibiotic resistant genes and residues in both soils, surface and groundwater resources. The application of antibiotic-laden manure introduces the risk of antibiotics in soils affecting the soil ecosystem. Uncontrolled use of antibiotics in livestock rearing has the potential of introducing antibiotics into the environment causing a risk to human health. It is against this background that this study was conceived to assess the risk of soil exposure to antibiotic residues in cattle and poultry manure in the study area.

According to Du and Liu (2012), the use of antibiotic contaminated water and manure for fertilization are sources of veterinary antibiotics released into the agro-ecosystem. Studies on different animal manure types have found significant levels of different antibiotics providing a critical entry point of antibiotics into soils amended by manure. Antibiotic residues have been found in different settings in the environment such as soils, water, and animal manure (Conde-Cid *et al.*,2018; Kummerer, 2009; Yang *et al.*,2016). Rongai-sub-County is among the leading sub-counties in agricultural production, with many households practicing both intensive and semi-intensive livestock rearing and crop production in Nakuru county.

However, there is limited data documenting consumption of veterinary antibiotics, and associated impacts likely to be generated by veterinary drugs in the environment. Most studies in Kenya have focused on the presence of antibiotic residues and antibiotic resistance genes in animal products. Furthermore, a number of recent studies in Kenya have increasingly focused on the presence of antibiotics residues in the environment and occurrence of antibiotic resistant genes. Antibiotic resistant genes have been found to affect human health, and have the potential of adversely affecting microorganism populations and diversity in the environment (K'oreje *et al.*,2018; Ngigi *et al.*,2019; Yang *et al.*,2016). This study sought to assess antibiotic use, farm practices, presence of antibiotic residues in livestock manure, manure amended soil and antibiotic risk levels in soils.

## **1.2 Statement of the Problem**

Veterinary antibiotics are important in livestock rearing. However, their presence in the environment have increasingly been of concern, antibiotic residues in the environment have been found to affect environmental and human health. Tetracyclines and Sulfonamide are the most consumed class of antibiotics among livestock farmers in Kenya. Furthermore, they are the most detected classes of antibiotics in animal manure and manure amended soils worldwide. In, Nakuru county both animal farming and agricultural activities are of economic and social importance. According to the 2019 census, Rongai sub-county has one of the largest number of poultry and cattle recorded within Nakuru County, with many livestock rearing farms practicing both intensive and semi-intensive farming. Application of antibiotic laden manure introduces the risk of antibiotics in soil affecting the soil ecosystem. Assessment and monitoring of veterinary antibiotics in the environment is important to safeguard environmental and human health. Although there are reports on the presence of antibiotic residues in Kenyan soils (Yang *et al.*,2016) little research has been done to study their levels in animal manure and manure amended soils. Therefore, there is a need to document the presence of antibiotics in animal manure and manure amended soils. The research findings were aimed at generating data that can inform development of proper and safe manure management practices, ensuring the protection of soils before their utilization in farming activities.

## **1.3 Broad Objective**

To generate knowledge on the risk of soil exposure to antibiotic residues in livestock manure from livestock rearing farms so as to safeguard human and environmental health.

### **1.3.1 Specific objectives**

- i. To assess the use of selected veterinary antibiotics in poultry and cattle rearing in Rongai sub-county, Nakuru County.
- ii. To assess knowledge and practices of livestock manure use in farms within Rongai sub-county in Nakuru County.
- iii. To assess the levels of tetracycline and sulphonamide residues in fresh poultry and cattle manure and manure amended soils within Rongai sub-county in Nakuru County.
- iv. To evaluate the contaminant risk levels of selected antibiotics from poultry and cattle manure in soils.

### **1.3.2 Research Questions**

- i. What are the patterns of use for selected veterinary antibiotics in poultry and cattle rearing in Rongai Sub- County of Nakuru County?
- ii. What are the levels of knowledge and practices of livestock manure use by farmers in various farms?
- iii. What are the levels of tetracycline and sulphonamide residues in poultry and cattle manure and manure amended soils?
- iv. What is the estimated exposure of antibiotic residues to soils through application of livestock manure?

### **1.4 Justification**

In Kenya, monitoring of antibiotics is mainly done on livestock products to ensure their safety for consumption. However, little has been done to assess the presence of antibiotic residues in livestock manure and soils, which may be attributed to inadequate resources and regulatory frameworks. Tetracyclines have been chosen for this study given that they are the most widely consumed class of antibiotics. Sulfonamide antibiotics unlike tetracyclines are not widely consumed but have a high mobility in soils having a higher potential risk to the environment.

Antibiotic residues in soils have the potential of affecting soil micro-organisms largely contributing to changes in bacterial genomes, their diversity and transfer of antibiotic-resistant genes significantly affecting soil microbial populations. Introduction of antibiotic residues and antibiotic-resistant genes from agricultural practices have the potential of degrading environmental resources ending up in water and soil contributing to environmental pollution and potential adverse effects on human health. Antibiotic in the environment have the potential of contributing to the development of antibiotic-resistant genes in soils and water. They have potential of being taken up by crops or end up leeching and contaminating ground and surface water in soils.

Antibiotic residues in soils are not directly mentioned in the sustainable development goals (SDGs). However, it is an important factor in sustainable agriculture target 2.4 on Goal number 2 that seeks to ensure sustainable food production systems and resilient agricultural practices that are able to increase production and productivity capacities. This helps to maintain ecosystems while also strengthening their capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters that have the potential of progressively improving the quality of both land and soils. It is important that antibiotic residues are assessed

within the environment to ensure water sources are safe and in line with sustainable development Goal No.6 on clean water and sanitation.

Assessing antibiotic residues is key in ensuring that there is limited environmental degradation attributed to livestock rearing, ensuring sustainable consumption and production in line with sustainable development Goal No.3 and 12 on good health and wellbeing. This is key to ensuring all agricultural practices are sustainable. Antibiotic residues are linked to the development of antibiotic-resistant genes that have the potential to increase disease incidence. Vision 2030 and the Bottom-up economic transformation agenda (BETA) under the social pillar, food security, nutrition and agriculture respectively contribute to the country's strategic agenda. Nakuru County has an operating county development integrated plan (CIDP) anchored on vision 2030. Environment and climate change features as one of the concerns resulting from human activities in the county (Nakuru County Integrated Development Plan,2023).

The study contributes significant knowledge in creating a balance in environmental, animal and human health under the One-health concept. Many of the livestock rearing farms within Rongai sub-county practice both intensive and semi-intensive farming with an approximate population of 308,212 for cattle and poultry reared within farms (KNBS,2019). Therefore, assessing the presence of antibiotic residues in livestock manure and manure amended soils is important for educating farmers on undertaking sustainable farm activities within Rongai sub-county, and providing information on the impacts of livestock production in the study area and beyond.

Data from this study provides important information on antibiotic pollution and the development of future monitoring strategies for antibiotics in livestock manure and soils. It will be used in developing guidelines for the safe management of livestock manure before application in soils. It will also be useful in policymaking with respect to livestock manure use for crop production and contribute to the growing knowledge of the one health concept.

### **1.5 Scope and limitations of the study**

The study covered livestock rearing farms within Rongai sub-county in Nakuru County. Livestock manure samples and manure amended soils were collected from the farm environment. Poultry and Cattle within Rongai sub-county have the highest population with many farmers taking up their production. Intensive and semi-intensive livestock rearing is associated with high levels of antibiotic use in treating and managing livestock in farms. Livestock waste from these farms have the potential of introducing antibiotics into the

environment, affecting human and environment health. The study assessed veterinary drug consumption patterns among livestock rearing farmers, manure uses in farms, levels of veterinary antibiotics in livestock manure and manure amended soils and ecological risks levels associated with antibiotics in soils.

The farms from each sampling unit were purposely selected depending on farm size and the type of livestock reared. The study covered farms that did not mix livestock waste during management. Manure and soil samples were analyzed to determine occurrence and levels of antibiotics. Manure and soil analysis included measuring of antibiotic levels. Sampling was conducted between September 2022 and February 2023.

The limitations of the study included;

- i. The study design entailed assessing the level of antibiotic residues in soils at one point in time and did not consider seasonality. This was overcome by collecting samples at a scheduled period to avoid any difference in the samples collected.
- ii. Some respondents were unwilling to participate in filling the questionnaires. This limitation was mitigated by use of the local contacts who created a rapport with the respondents hence making it easier for the researcher to conduct the research with relative ease.

## 1.6 Assumptions

During the study the following assumptions were made:

- i. Antibiotic residues in the soils are solely from livestock manure.
- ii. Soil properties did not affect the level of antibiotics in soil.

## 1.7 Definition and operationalization of terms

<b>Antibiotic</b>	Is a type of antimicrobial substance active against bacteria.
<b>Antibiotic Resistance</b>	The adaptive change in bacteria that allows them to grow in the presence of a drug or an antibiotic.
<b>Antibiotic Resistance Genes</b>	Genes that enable bacteria and fungi to survive exposure to antibiotics and antifungals.
<b>Composting:</b>	Organic waste management and disposal under oxygen rich conditions.
<b>Composite Sample</b>	Refers to a homogenous mixture of a sample.

<b>Consumption Rate</b>	Refers to the amount of manure and veterinary antibiotics used by farmers.
<b>Environmental exposure</b>	Refers to coming into contact with antibiotic residue through the application of livestock manure in soils.
<b>Livestock</b>	Cattle and poultry kept on farms in this study.
<b>Manure Amendment</b>	Any addition to soil that improves its physical conditions resulting to better abilities.
<b>Risk Assessment</b>	Identifying risks that are likely to be caused resulting from the presence of antibiotics in manure.
<b>Sulfonamide</b>	Antibiotics containing Sulfonamide (SO <sub>2</sub> NH <sub>2</sub> ) groups in their chemical structure
<b>Tetracyclines</b>	Group of broad-spectrum antibiotic compounds having a common basic structure.
<b>Veterinary antibiotics</b>	Wide-range of drugs used in the treatment of livestock.

## CHAPTER TWO

### LITERATURE REVIEW

#### **2.1 Consumption of veterinary antibiotics**

Veterinary antibiotics are mainly consumed by farmers in animal rearing to maintain animal health and productivity. Introduction of various animal farming techniques have led to unprecedented high levels of consumption in veterinary antibiotics (O'Neill,2015). Antimicrobials are widely used in both humans and animals playing a critical role in the treatment of a wide range of diseases. They have different effects on targeted disease-causing pathogens and are mainly used for therapeutics and prophylactic purposes to address or prevent occurrence of diseases or as growth promoters in animal production.

Demand for antimicrobials over the years has been driven by increased uptake of livestock rearing to meet rising demand levels of animal proteins by an ever-growing human population. According to the state of the world's antibiotics, in 2015 two thirds of all antibiotics produced globally each year are used in animal husbandry (Gelband *et al.*, 2015). Consumption of antimicrobials in food animal production in 2010 was estimated at 63,151 tons and is projected to rise to 67% to 105,596 by 2030, with the rise attributed to an increase in animal rearing (Van Boeckel *et al.*,2015).

China is among key consumers of world antibiotics that is largely attributed to high levels of meat production, a total of 210,000 tons of antibiotics produced was used in animal farming in 2015 exceeding consumption levels in the United States (Collignon & Voss, 2015). Consumption of veterinary antimicrobials and antibiotics for animal rearing in countries such as Brazil, Russia, India, China and South Africa (BRICS) are expected to increase by 90% by the year 2030 compared to human antimicrobials that are projected to increase by 13% in the same timeline.

According to Van Boeckel *et al.* (2015), this trend is attributed to increased uptake of animal rearing. This scenario paints a picture likely to be experienced in the future among both developed and developing countries due to the introduction of various animal husbandry technologies to improve production and demand for quality animal proteins. There is a difference in amount of antibiotics administered in animals that is largely dependent on the size and type of an animal (Kumar *et al.*,2005; McEwen & Fedorka-Cray,2002). This is an important aspect since manures from various animals may contain varying levels of antibiotics.

Veterinary antibiotics are key inputs in animal production. However, there is limited data on their consumption levels. Consumption patterns of veterinary antibiotics in farms are not well documented, limited studies have been undertaken to look into their consumption (Lawal & Babalola, 2014). Consumption patterns of veterinary antibiotics differ from one country to another with developed countries leading in consumption partly attributed to high intensification of animal farming. Consumption of antimicrobials in developing countries is expected to increase as many households turn to animal rearing as a source of income to meet the demands of a growing population characterized by high consumption of animal proteins.

Statistics show that there has been an increase in the purchase of livestock drugs and medicines from 2013-2017 rising from 2,988,000 to 4,610,000 within a span of 4 years (KNBS,2018). Tetracycline and Sulfonamide antibiotics were noted as the most consumed classes of antibiotics among Kenyan farmers (Mitema *et al.*,2001). Antibiotics are important inputs in animal husbandry in the management of animal health mainly used by farmers in food animals such as cattle, sheep, poultry and fish.

## **2.2 Types of antibiotics**

According to the US Food and Drug Administration (2019), a total of 18 classes of antimicrobials are approved for use in rearing food-producing animals. These classes include the following: aminocoumarins, aminoglycosides, amphenicols, cephalosporins, diaminopyrimidines, fluoroquinolones, glycolipids, ionophores, lincosamides, macrolides, penicillin's, pleuromutilins, polymyxins, polypeptides, quinoxalines, streptogramins, sulfonamides, and tetracycline's administered primarily through animal feeds, water or through injections, orally, intramammary, oral and topical means. Antibiotics are of a wide class and have different physical and chemical properties that make them useful in treating various diseases.

### **2.2.1 Tetracyclines**

Tetracyclines were discovered in the 1940s and exhibited activity against a range of microorganisms including gram-positive and gram-negative bacteria, chlamydia, mycoplasmas, rickettsiae and protozoan parasites (Chopra & Roberts,2001). They are inexpensive and are used widely in human and animal prophylaxis, infectious therapy and sub-therapeutic levels as growth promoters (Chopra & Roberts,2001). They are relatively cheap and this contributes to its high levels of consumption among animal rearing farmers. Tetracycline's antibiotics are polyketides comprising of a naphthalene ring structure. They are

amphoteric compounds characterized by three pKa values of 3.3, 7.7 and 9.0, which allows that they can exist as cation, zwitterion and/or anion, depending on the pH of the medium.

Tetracyclines are relatively stable in acids, but not in bases, and form salts in both media (Halling-Sørensen *et al.*,2002). They form complexes chelate with divalent metal ions and b-dike tones and strongly bind to proteins and silanolic groups (Oka *et al.*,2000). Most TCs are sparingly water-soluble, while the solubility of the corresponding hydrochlorides is higher. Tetracyclines are excreted as active compounds through feces and urine after administration, which are spread in the environment as animal manure (Ricker *et al.*,2020; Xiong *et al.*,2018). However, unlike Sulfonamide they do not sorb strongly to the soil and have been widely detected in surface water, groundwater and soil pore water (Weghst-urich *et al.*,2014).

### **2.2.2 Sulfonamide**

In general, amphoteric sulfonamide (SAs) behave as weak acids and form salts in strongly acidic or basic solutions. Mostly, SAs, substituted at the amino-N, have greatly reduced antibacterial activity. Antibiotics from the class of aminoglycosides are basic, strongly polar polycationic compounds. Their molecular structure is characterized by two or more amino sugars that are glycosidically bound to aminocyclitol. They are water-soluble, mostly hydrophilic, and susceptible to photo-degradation.

### **2.3 Commonly used antibiotics**

Consumption of veterinary antibiotics by farmers in the management of animal health is important to maintain high productivity and yields in farms. Antibiotic doses in animal feeds vary by compound, animal, and region. Bolan *et al.* (2010) assembled a list of antimicrobial doses in poultry production that included maximal doses of 77 mg/kg amprolium (coccidiostat), 26 mg/kg chlortetracycline (tetracycline), 152 mg/kg ncarbazine (coccidiostat), 29 mg/kg oxytetracycline (tetracycline), and 25 mg/kg penicillin (beta-lactam). According to McEwen and Fedorka-Cray. (2002) growth promoters are typically administered at dose levels of between 2.5 – 125 mg/kg.

Consumption of various antibiotics classes is expected to increase with an increase in the number of farms taking up animal rearing, attributed to increased demand for animal products. Globally, China is considered the largest consumer and producer of antibiotics. In 2003, China produced 28,000 and 10,000 tons of penicillin and oxytetracycline (OTC) that represented 60% and 65% of the total global output (Yang *et al.*,2010). Tetracyclines seem to be the most consumed class of antibiotics, followed by sulfonamides and macrolides. Their use accounts

for approximately 90% of the total antibiotics used in the UK and 50% in Korea (Kim *et al.*,2011).

According to Mitema *et al.* (2001) commonly used antibiotic drugs consumed by animal rearing farmers in Kenya included tetracycline and Sulfonamide that accounted for more than 70% of the total consumption. Tetracyclines, Sulfonamide, trimethoprim, nitrofurans, aminoglycosides, b-lactams and quinolones were the most commonly used drugs in food-producing animal in Kenya (Darwish *et al.*,2013). The use of antimicrobials in animal rearing in Kenya has also been assessed in various regions. According to Muthuma *et al.* (2016) the most frequently used veterinary drugs in Nairobi were reported to be sulfonamides (59%), tetracycline (23%) and amprolium (9%) among peri -urban broiler farmers.

#### **2.4 Antibiotic residues in the environment**

Antibiotics residues in the environment have been linked to various anthropogenic activities. Veterinary antibiotics in the aquatic environment have the potential to enter the environment through leaching and run-off from animal manure (Vijver *et al.*,2016). Antibiotics that have a higher K<sub>d</sub> value are considered to be more mobile and have the highest probability to be found in both ground and surface waters (Taylor *et al.*,2011). Antibiotic residues in agricultural soils have been found at levels that are more than 10m deep (Aminov & Mackie ,2007).

Agricultural activities within farms and poor wastewater treatment techniques are major contributors to the rising levels of antibiotic residues in the environment. The dominant entry point for antibiotics into the environment is mainly through the application of animal manure and bio solids that contain a considerable level of antibiotics to soils (Kemper, 2008). Antibiotics can also be introduced into agricultural land through wastewater irrigation since they have been frequently detected in both treated and raw wastewaters (Gulkowska *et al.*,2008).

Various studies undertaken to assess the availability of antibiotics in different animal manures and manure amended soils have found significant levels of antibiotic residues in manure of various animals such as cattle, swine and poultry, animal manure amended soils and existing water bodies adjacent to the animal rearing farms. Antibiotics within agricultural systems are excreted through animal urine and feces that are introduced into the environment as animal manure slurries and have the potential to accumulate in soil or leech into underground and surface water bodies and end up being taken up by plants.

### **2.4.1 Antibiotic residues in soils**

The presence of antibiotic residues in soils has recently generated some concern. Due to increased use of antibiotics, various aspects of the environment have been left exposed to antibiotic pollution. This might be attributed to the use of polluted water in farming or the use of polluted animal manure as fertilizer in farming. The rising levels of antibiotic residues in manure amended soils has been linked to the use of animal manure containing antibiotics. According to Poulsen *et al.* (2013) the main source of veterinary antibiotic at the field level were manure fertilizers, runoff and aerial transport. It is of increasing concern due to increased uptake of animal rearing practices in various countries.

Antibiotics in soils have been found to affect soil microorganisms (Liu *et al.*,2009). This makes it important to undertake constant monitoring activities on the levels of antibiotics in soils. Antibiotic residues in soils have been reported in various countries. In China, studies on the presence of various antibiotics in soils found significant levels of antibiotic residues in various manure amended soils (Li *et al.*,2011). In Spain, significant levels of antibiotic residues were found in agricultural soils (Conde-Cid *et al.*,2018). In Turkey, significant levels of antibiotic residues were found in manure amended soils (Karci & Balcıoğlu, 2009).

A similar study done to assess the levels of tetracycline, sulfonamide and quinolone antibiotics in poultry and cattle manure in Tanzania noted significant levels of antibiotic residues in animal manure (Mohameda *et al.*,2017). In Kenya, the presence of tetracycline, sulphonamide, fluoroquinolones antibiotics residues in soils within suburban areas have been noted (Yang *et al.*, 2016). Veterinary antibiotics present in animal manure applied in soils have the potential to move through soils. Antibiotics in soils have been linked to poor seed germination, poor crop growth, and can be taken up by crops being of potential risk to soil ecosystems (Hu *et al.*,2010; Liu *et al.*, 2009). Repeated application of animal manure on agricultural soils has the potential of affecting existing soil microorganisms affecting soil quality.

## **2.5 Persistence of Tetracycline and Sulphonamide antibiotics in the environment**

The transfer of antibiotics through various media in the environment is affected by adsorption/desorption (Fernández-Calviño *et al.*,2015; Zhou *et al.*,2017) as well as degradation processes acting on antibiotics (Liu *et al.*,2017).

### **2.5.1 Tetracycline's in the Environment**

Tetracyclines are stable in acid media, but not in alkaline conditions, form salts in both conditions (Doi & Stoskopf, 2000; Halling-Sørensen, 2002). Their water solubility ranges

between 230 and 5200 (mg L<sup>-1</sup>) with three pKa values of 3.3, 7.7, and 9.0, having amphoteric characteristics (Thiele-Bruhn, 2003). They are susceptible to photo-degradation (Doi & Stoskopf, 2000) and form complexes with chelate divalent and trivalent metal ions such as Mg<sup>+2</sup>, Ca<sup>+2</sup>, Fe<sup>+2</sup>, Zn<sup>+2</sup>, and Al<sup>+2</sup>, Fe<sup>+3</sup>, Al<sup>+3</sup>, and  $\beta$ -diketones (Oka, 2000; Thiele-Bruhn, 2003). Chelating reduces bioavailability and reduces the antibacterial effect of tetracyclines. Complexation is therefore an important factor to determine the fate and effect of tetracyclines as divalent metals usually are present in high concentrations in the environment.

Tetracyclines are known to bind strongly to proteins and silanolic groups (Oka, 2000; Thiele-Bruhn, 2003). There is slow degradation of tetracycline in manure. A study on the degradation of tetracyclines showed that the process of degradation followed first-order kinetics (Bansal, 2012). The rate of degradation increased with increased moisture content, temperature, and amount of farm yield manure or nitrogen, and decreased with increasing antibiotic concentration. The detection level of tetracyclines in manure ranged from 84.9 to 96.8% with a maximum concentration of residual chlortetracycline recovery reaching 764.4 mg kg<sup>-1</sup> (Pan *et al.*, 2011)

Tetracycline have been reported to have different half-lives in soils as per different studies conducted. This is because for most, they are sparingly water soluble and have been found to have strong binding affinities for clay materials and soil organic matter (Thiele-Bruhn, 2003). Hence, microbial degradation of tetracyclines is primarily linked to manure type, soil and climatic conditions. Various studies assessing the presence of tetracyclines in soils have noted considerable levels under various conditions. A field study undertaken reported half-life of 18-79 days (Pan & Chu, 2016). In two different greenhouse-based studies undertaken oxytetracyclines half-lives were found to be at 23 and 33 days respectively in manure amended soils (Pan & Chu, 2016; Wang & Yates, 2008).

A field study in Germany in which soil was fertilized with manure found 4000  $\mu\text{g}/\text{kg}$  tetracycline and 100  $\mu\text{g}/\text{kg}$  chlortetracycline in the liquid manure, 86,200  $\mu\text{g}/\text{kg}$  at the top layer (0-10 cm depth) and 171,700  $\mu\text{g}/\text{kg}$  at the deeper layer of the soil (i.e. 20-30 cm depth) (Hamscher *et al.*, 2002). Tetracyclines could not be detected at soil depths between 30 and 90 cm, in soil or groundwater (Hamscher *et al.*, 2002). A study in Canada that used manure from feedlots found the concentration of chlortetracycline in manure was 401  $\mu\text{g kg}^{-1}$ , in soil 87  $\mu\text{g kg}^{-1}$  at 0-30 cm depth and 55  $\mu\text{g kg}^{-1}$  at 40 cm depth indicating a decrease in concentration with

increasing soil depth (Aust, 2008). In addition, Tetracyclines were detected in surface water in China at concentrations of up to 1322 ng/L (Zhang *et al.*,2023).

Tetracycline and chlortetracycline were found to be more persistent in soils than in manure (Bansal, 2012). Several studies showed that tetracyclines can adsorb strongly to clays (Allaire, 2006), soil (Bansal, 2012) and sediments (Rabølle & Spliid, 2000) indicating their persistence in different environments. According to Pikkemaat *et al.* (2016), Tetracyclines introduced into the environment in significant amounts build-up persistent residue concentrations in soil. This poses a considerable risk of antibiotic resistance development in the environment that is linked to their constant use and physiochemical properties. Thus, they have the potential to pose a considerable risk of antibiotic resistance development in the environment.

### **2.5.2 Sulfonamide in the Environment**

Sulfonamides in nature are polar compounds with a solubility ranging from 0.1 to 8 g L<sup>-1</sup> depending on the compound within the group. Sulfonamides function as weak acids in the physiological pH range (Thiele-Bruhn, 2003). They occur in neutral form between pH 2.5 – 6 and negatively charged in alkaline conditions. Sulfonamides are characterized by low chelating ability, low binding constants, and high-water solubility (Sukul *et al.*,2006). Sulfonamides have a low sorption coefficient and are considered to be the most mobile antibiotics (Boxall *et al.*,2002; Tolls, 2001).

Photo-degradation is considered an important degradation pathway (Batchu *et al.*,2014) and has been subjected to many studies. Ingerslev and Halling-Sørensen. (2000) concluded that, according to the applied formal screening test, sulfonamides should not be classified as readily biodegradable. Sulfonamide adapted bacterial cultures, however, were able to degrade sulfonamides very rapidly between 0.2 to 3 days (Ingerslev & Halling-Sørensen,2000). The accelerating effect of previous exposure was also shown in a study on manure (Islas-Espinoza *et al.*,2012). Drillia *et al.* (2005) suggested that little or no biodegradation occurs in manure and soils whenever there are other easily biodegradable sources of carbon and nitrogen present.

Sulfonamides have the potential to enter soils, animal wastewaters, sediments, groundwater, and surface waters (Kolpin *et al.*,2002; Lindsey *et al.*,2001; Thiele-Bruhn,2003; Zhang *et al.*,2011), and have been observed in environmental samples. A detectable concentration of sulfamethazine was found in agricultural soil in Germany seven months after application of manure (Christian *et al.*,2003). A concentration of up to 590 µg L<sup>-1</sup> of sulfachloropyridazine was detected in drainage waters in the Netherlands seven days after manure application on clay

loam soil (Boxall *et al.*,2002). Based on their physiochemical properties and relative stability, sulfonamides are likely to occur in the environment in a bioavailable form and may therefore pose a risk to antimicrobial resistance development.

## **2.6 Agricultural Manure Treatment**

Agricultural processes release a lot of waste that ends up being taken back to farms as inputs for soil fertilization. Sewage treatment, composting and anaerobic digestion are some of the techniques used to reduce the concentrations of antimicrobials, antibiotic resistant genes and microorganisms introduced in soils through manure fertilization (Collignon & McEwen,2019). The growing concept of circular economy has been taken up in agricultural production, with the aim of reusing, recycling and nutrient use efficiency in farming. This has led to increased demand for livestock waste as food waste from farms is used in the breeding of insect larvae, notably black soldier fly larvae (Van Zanten *et al.*,2015).

Poultry waste can also be used as feed in cattle rearing and fish farming (Adewumi *et al.*, 2011; Kariuki *et al.*, 2023; Usman *et al.*, 2019). Composting is an important process since farm waste from animals and plant refuse are composted to make animal manure. The use of animal manure for soil fertilization should be considered as the main agricultural contributor to environmental contamination and transmission of antibiotic residues, resistant bacteria and resistant genes (Pikkemaat *et al.*,2016).

Composting is a spontaneous and biological process of aerobic digestion that involves mineralization and humus formation of organic matter. This bio-oxidative process involves environmental microorganisms which have the ability to break down organic materials, resulting in the formation of a stable final product, known as compost (Bernal *et al.*,2009; Fan *et al.*,2020). Composting is a suitable option for manure management since it aids in reducing the volume of animal wastes and the elimination or reduction of pathogenic microorganisms and has significant economic, environmental and public health advantages. Composting procedure requires controlled conditions such as bulk density, porosity, particle size, nutrient content, carbon/nitrogen ratio, temperature, pH, moisture and oxygen supply that determine the optimal conditions of microbial development and organic matter degradation (Bernal *et al.*,2009).

In Kenya, animal manure is considered an important input in farming due to its ease of availability, low cost, and soil nutrient content that aid in improving the quality of agricultural soils. Declining levels of soil fertility in Kenya has led to intensified use of both inorganic and

organic fertilizers in farming. However, over the years there has been increased concern on the suitability and safety of animal manure in farming due to increased levels of mechanization in animal farming. This is a result of antibiotic laden animal waste once antibiotics are administered to animals.

Antibiotic residues in animal manure varies from one group to another and could be attributed to individual/group treatment, duration of treatment and moment of sampling related to the treatment period. The highest detected class of antibiotic residues detected in animal manure is the tetracycline group (Pikkemaat *et al.*,2016). Tetracycline levels in various studies have been found to reach levels of 100,000 µg/kg (Hu *et al.*, 2010; Karcı & Balcıoğlu,2009; Pan *et al.*, 2011; Widyasari-Mehta *et al.*,2016)) with reported extremes of up to 764,000 µg/kg for chlortetracycline in swine manure (Pan *et al.*,2011). Sulfonamide have also been identified in high levels in animal manure with levels of up to 20,000 µg/kg for sulfadimidine and 91,000 µg/kg in sulfadiazine (Martínez-Carballo *et al.*,2007).

Various studies show that animal manure fertilization has the potential of increasing abundance of drug-resistant bacteria and the presence of antibiotic-resistant genes in soils (Garder *et al.*,2014; Marti *et al.*,2014; Udikovic-Kolic *et al.*,2014). Manure management methods have the potential of reducing the number of veterinary antibiotics present in animal manure due to the degradation process. Antibiotics are degraded into their metabolites while some are significantly degraded in animal manure. However, other revert to their original structure once they are spread in the environment.

## **2.7 Antibiotic Resistant Genes in the Environment**

Antibiotics residues in the environment have been contributing to the menace of public health linked to antimicrobial resistance, which is largely attributed to the growing uptake of animal husbandry (Topi & Spahiu,2020). Animal manure contaminated with veterinary antibiotics, applied to the agro-ecosystems may affect the soil microbial community by creating an ideal selective pressure to the bacterial strains carrying antibiotic-resistant genes (Gullberg *et al.*,2011). Significant amounts of veterinary antibiotics end up remaining in soils after application of animal manure as fertilizer (Kuppusamy *et al.*,2018). A significant portion leaches into the groundwater and surface water bodies by infiltration and run-off from agricultural fields contributing to the spread of antibiotic resistant genes in the environment (Kivits *et al.*,2018).

Veterinary antibiotics administered to animals for selective advantages contributes to antibiotic resistant bacteria (ARBs) development in animal intestines that end up in animal manure and eventually in the environment (Looft *et al.*,2012). However, the presence of antibiotic resistant bacteria is not limited to only areas next to agricultural land amended using animal manure. Resistant bacteria have also been found in pristine environments because of the natural reservoirs of antibiotic resistance genes in the environment (Allen *et al.*,2010; Heuer *et al.*,2011). There is widespread agreement and support for the idea that agricultural antibiotic resistance should be reduced, with an emphasis on reducing transfer of resistance from practices such as land application of animal manures (Heuer *et al.*,2011; Marti *et al.*,2014). However, the details of attaining total reduction of antibiotic use cannot be easily attained to reduce resistance. It is important to first obtain baseline information on how basic agricultural practices are involved in resistance transfer.

## **2.8 Legal Framework**

Globally, antibiotic use is increasingly generating lots of concern because of their increased presence in the environment. It has been linked to increased occurrence of antibiotic resistance genes in the environment that have the potential to affect animal, human and environmental health. This cause of concern has led to the development of laws to guide on the sustainable use of antibiotic residues. However, there has been limited changes with regards to antibiotic consumption and use.

Global collaborations on AMR have fueled concern on the use antibiotics and spread of antimicrobial resistance, largely initiated by organizations such as the World Health Organization, Food and Agriculture Organization and the World Organization on Animal Health. The 68th World Health Assembly endorsed the Global Action Plan (GAP) on AMR jointly developed by the WHO, OIE and FAO contributing to the development National Action Plans (NAP) from a One Health approach among member states (Global Antibiotic Resistance Partnership, 2011).

Kenya developed a policy covering resistance monitoring; antimicrobial use in public health and agriculture; and the disposal of antimicrobials in the environment contributing to combating AMR. Several laws have been developed to aid in regulating antibiotic use in various industries. There are various laws that regulate the use of drugs in food animal, including Pharmacy and Poisons Act, Drug and Chemical Substances Act (Cap 254), Meat Control Act (CAP 356), Pests Control Products Act (Cap 345) and Fertilizer and animal

foodstuff act (CAP 345) and their management during disposal under the Waste Management Regulation Act 2006. The Livestock (Poultry Industry) Regulations, 2023 outlines appropriate measures for the use, treatment and storage of poultry waste.

Veterinary medicine is mainly regulated by the ministry of health through the poison and pharmacy board. However, challenges have been noted with regards to inadequate monitoring and enforcement of protective legislation, drug adulteration, lack of post market testing and unprofessional repackaging of veterinary medicines. Studies on antibiotics on other parts of the world have found the levels of antibiotics to be falling above the recommended residual limits, including in Germany and Netherlands (Abjean *et al.*,2000; Kress *et al.*,2007). Other African countries have also been reported to have milk contaminated with antibiotic residues. Some of these countries include Ghana and Kenya (Addo *et al.*,2011; Orwa *et al.*,2017). A study conducted in Kenya to estimate AMR patterns in various bacteria isolated from chickens found a high prevalence of resistance to commonly used antibiotics, including ampicillin (76%), tetracycline (71%), sulfamethoxazole (70%), and co-trimoxazole (66%) (Deng, 2017).

Laws and policies controlling the use of antibiotics and their presence in the environment should be strengthened to increase monitoring and regulation. The European Union has set maximum residues limits (MRL) for antibiotics in food origin but there is none set to regulate their levels in livestock manure and manure amended soils. Regulations for veterinary drugs in foods have been set at maximum residual limits of 100 µg/kg for sulfonamides, while tetracyclines should not exceed 100 µg/kg (FAO & WHO,2021). However, despite there being various laws and regulations on antibiotic use and their levels in human food. Higher residues have been noted in food products with this product ending up in the environment through animal waste contributing to their high levels in the environment.

## **2.9 Driver, Pressure, State, Impact, Response framework**

The DPSIR framework is often described as a conceptual framework used for the description of environmental problems and resulting relationships that are linked to socio-economic aspect linked to existing policy (Environmental European Agency, 2006). According to its terminology DPSIR framework stand for D- Drivers, P-Pressures as a consequence, S-state of the environment, I- resulting impact on ecosystems, human health and society that are likely to Response that have the potential to influence Driving forces, State and Impact by initiating various mitigation, adaptation and restoration (Gabrielsen & Bosch, 2003; Smeets & Weterings, 1999).

Antibiotics play a key role in the management of infectious diseases in humans and animals. It is used in the management and treatment of livestock and aquacultures in many parts of the world. However, release of antibiotics in large amounts into water and soils has a high potential threat for all microorganisms and plants in the environment (Cycon *et al.*,2019). Growing demand for livestock products to meet the demands of an ever-growing population has led to increased uptake of animal rearing activities among various households. This has led to increased consumption of veterinary drugs in animal husbandry to increase quality of products produced and reduce disease occurrence of various animal rearing units.

Increased use of veterinary antibiotics has the potential to contribute to environmental pollution. Animal waste and the environment have borne the brunt of the ever-changing patterns of use of this drugs as they have been left exposed to veterinary drugs used in animal husbandry. Increased use of antibiotic drugs has left the environment exposed to antibiotic residues from animal waste. Poor waste management within animal rearing farms has the potential of affecting environmental and human health negatively. Hence, this study determined the presence of antibiotic residues in animal manure and the possibility of transfer to soils amended using livestock manure.

Antibiotics used in the treatment of livestock are not fully absorbed into their system as some are excreted through urine and feces. These excreta are utilized as soil amendments in farms to improve on soil fertility providing an avenue to which antibiotic residues are introduced to soils and the environment. This study believes that when livestock manure contaminated with antibiotic residues are applied in soils, they end up introducing antibiotic residues into the environment. Therefore, this research seeks to assess antibiotic use among livestock rearing farmers and create awareness on the availability of antibiotic residues in the environment linked to livestock rearing. Knowledge gained by famers during this study will be important in reducing amounts of antibiotics used and their presence in the environment.

## 2.10 Sub-section on research gaps.

**Table 2.1: Research Gaps for the study.**

<b>Author and Publication</b>	<b>Scope of study</b>	<b>Research gaps</b>
Residue levels and discharge loads of antibiotics in wastewater treatment plants (WWTPs), hospital lagoons, and rivers within Lake Victoria basin, Kenya (Kimosop <i>et al.</i> ,2016)	Contribution of hospital waste water on the presence of antibiotics in waste water treatment plants and hospital lagoons and rivers in Lake Victoria Basin.	Only focused on the presence of antibiotics in surface waters and wastewaters and hospitals as a source did not outline the contribution of activities such as agriculture.
Occurrence, fate and removal of pharmaceuticals, personal care products and pesticides in wastewater stabilization ponds and receiving rivers in the Nzoia Basin, Kenya (K'oreje <i>et al.</i> ,2018)	Contribution of effluent water on the presence of antibiotics, personal care products and pesticides in waste water treatment plants, and rivers in Nzoia Basin.	Only focused on the presence of antibiotics in surface waters and wastewaters and hospitals as a source did not outline the contribution of activities such as agriculture.
Occurrence of antibiotics residues in hospital wastewater, wastewater treatment plant, and in surface water in Nairobi County, Kenya (Ngigi <i>et al.</i> ,2019)	Contribution of effluent water on the presence of antibiotics, personal care products and pesticides in waste water treatment plants, In surface waters in Nairobi County.	Only focused on the presence of antibiotics in surface waters and wastewaters and hospitals as a source did not outline the contribution of activities such as agriculture.
Occurrence of antibiotics and risk of antibiotic resistance evolution in selected Kenyan wastewaters, surface waters and sediments (Kairigo <i>et al.</i> ,2020)	Focused on presence of antibiotics and antibiotic resistance products and pesticides in waste water treatment plants and in surface waters in Nairobi County.	Only focused on the presence of antibiotics in surface waters and wastewaters and not the sources of the antibiotics.

Occurrence, composition and risk assessment of antibiotics in soils from Kenya, Africa (Yang <i>et al.</i> ,2016)	Focused on presence of antibiotics in soils and the risk assessment on the presence of antibiotics in various soils in Kenya.	Only focused on the presence of antibiotics in soils and not the sources of antibiotics in the environment.
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Over the years there has been developing interest on assessing the levels of antibiotics in the environment and their effects on human health and the environment. Many studies assessed environmental exposure levels focusing on water bodies and wastewater treatment sites in Kenya. There are fewer studies that have focused on assessing risks associated to direct toxicity of antibiotics in soils (Gao *et al.*,2008). According to Li *et al.* (2012) lack of environmental legislation covering release of antibiotics into the environment has the potential to cause more undesired effects into the environment. The current study is unique in that it provides an assessment of antibiotic exposure to soils fertilized by animal manure and information that can be used in developing environmental legislation related to antibiotics. It was conducted within animal rearing farms that are growing rapidly with little amount of information on the risk associated with contaminated animal manure use.

In the previous years there has been increasing literature mainly focused on the presence of antibiotics compounds within surface waters and the role played by sewerage water treatment plants in reducing the quantities of this substances into the environment (Kairigo *et al.*,2020; Kimosop *et al.*,2016; K'oreje *et al.*,2018; Ngigi *et al.*,2019). However, there is a need to assess the impact of agricultural activities in contributing to antibiotic pollution in the environment, with rising concern of this veterinary drugs within the study area. Lack of data on the contribution of antibiotic residues by animal manure have made it difficult to develop measures enhancing environmental and public health. The study used HPLC-DAD for antibiotic residues assessment that provides a more effective and efficient technique for environmental assessment.

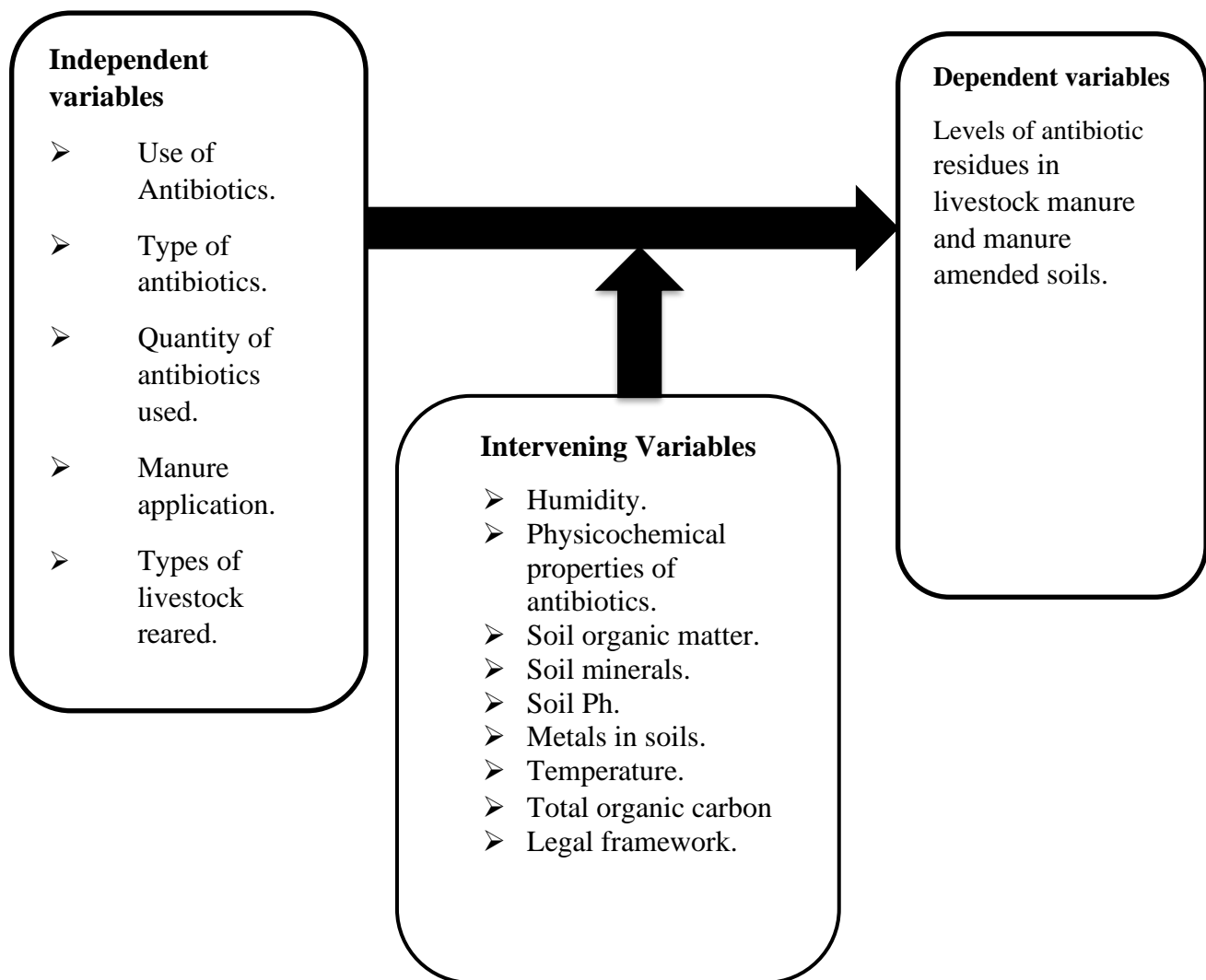
In this study, specific livestock manure and the levels antibiotics in manure and soils were analyzed. It identified manures likely to provide a higher level of antibiotic pollution to the environment. Increased number of households taking up livestock rearing as a source of income has led to rising consumption levels of veterinary drugs among farmers. The findings of this

study will make a significant contribution to the knowledge, and evidence of antibiotic exposure to the environment resulting from these activities.

### **2.11 Conceptual framework**

The conceptual framework illustrates the relationship of variables that determine the level of antibiotic residues in both livestock manure and in manure amended soils. Antibiotic residues in livestock manure and the environment have been of concern due to the emergence of resistant genes. The independent variables include the type of antibiotics, frequency and quantity of use of veterinary antibiotics in rearing animals, manure application on soils, types of livestock reared. These factors have an influence on the levels of antibiotics found in animal manure and in manure amended soils. Intervening variables for the study include; humidity, physicochemical properties of antibiotics, soil organic matter, soil minerals, soil pH, metals in soils, temperature, total organic carbon and existing government policy have the potential to affect the levels of antibiotic residues in both manure and soils.

Veterinary drugs use and livestock metabolic processes affect the number of veterinary drugs released through urine or manure by livestock. However, their levels in soils are largely attributed to amounts and types of livestock manure used for soil amendment, and physicochemical properties in soils. These factors determine the amount of veterinary antibiotics found in soils.



**Figure 2.1: Conceptual framework outlining various interactions among study variables.**

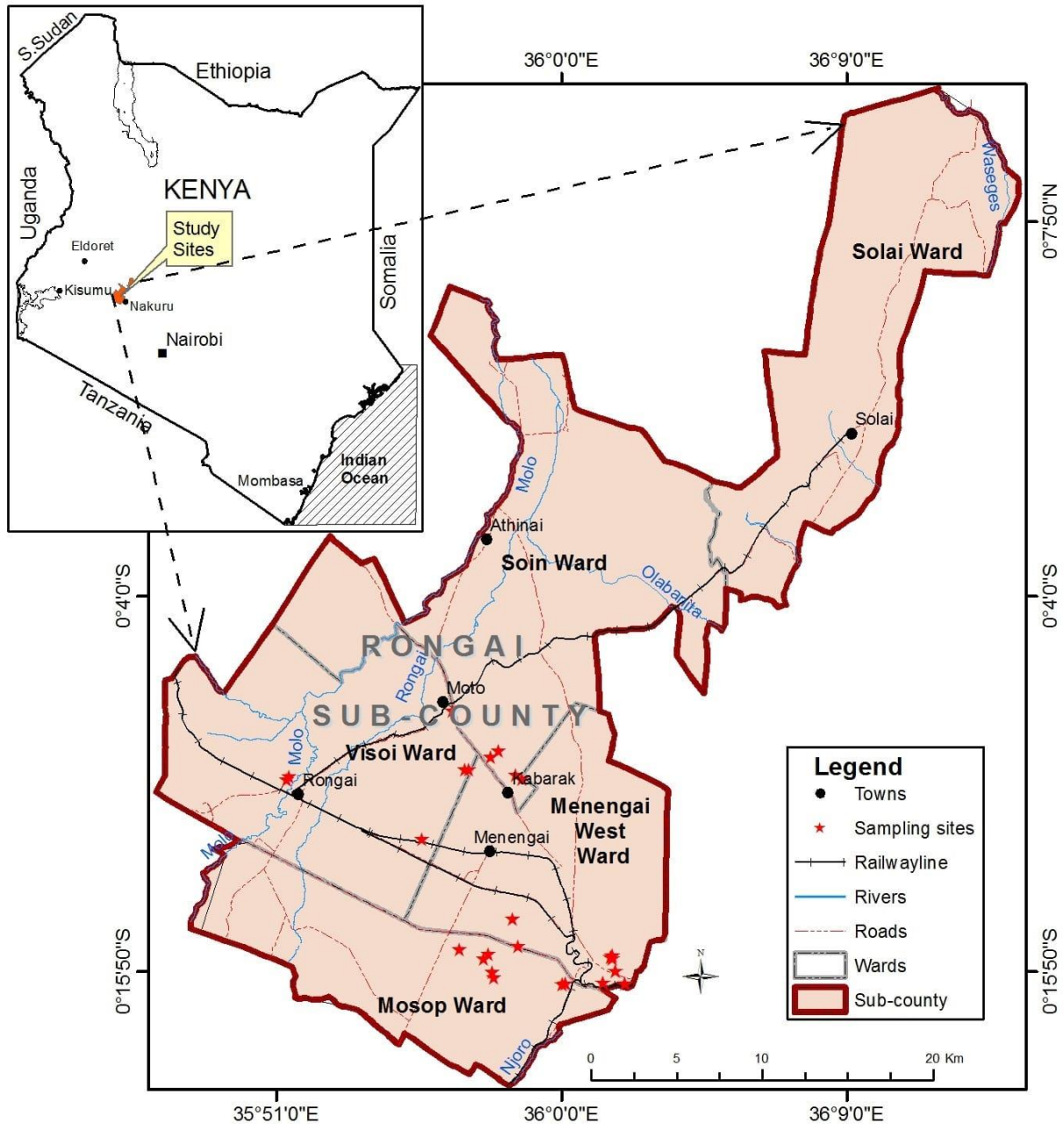
## CHAPTER THREE

### METHODOLOGY

#### 3.1 Description of the Study area

Rongai Sub-County is found within Nakuru County, Kenya. It lies within a Latitude of 0° 10' 23.99" N and a Longitude of 35° 51' 49.75" E. It has total of 5 administrative units namely Menengai west, Rongai, Visoi, Mosop and Solai, covering an area of 1049.1 square Kilometer. It has a total population of 199,004, with 31% of the households actively involved in cattle rearing, poultry rearing and farming (KNBS, 2019). It falls within an altitude range of between 1650 and 1850 m above sea level (a.s.l) with a temperature range of between 17 and 29°C annually. There are three agro-ecological zones namely Lower Highland 3 (LH3), Upper Midland 2 (UM2) and Upper Midland 3 (UM3). The annual rainfall is between 600-1000 mm (Jaetzold *et al.*,2010).

The population is projected to increase attributed to the growing demand for agricultural products mainly food crops and other resources such as water and land. Nakuru County economy is mainly reliant on the vast number of households practicing agriculture with an increase in the number of agricultural activities within the urban area. There are also commerce and industrial sectors that are both agriculturally based and non-agricultural industries in manufacturing, service, tourism and informal trade (KNBS, 2018).



**Figure 3.1: Map of Study area.**

**(Source: Regional Centre for Mapping Resources for Development)**

### 3.2 Research Design

The study used a mixed methods-design that included a cross-sectional survey, ecological survey and laboratory analysis. This entailed use of both qualitative and quantitative methods. The qualitative aspects explored the types of antibiotics used by farmers and practice with regard to manure use that largely relied on common knowledge of the respondents as well as literature sources on various factors associated with antibiotic use among farmers. The

quantitative factors assessed levels of antibiotics in livestock manure and soils sampled in various farms at a single point in time. A semi-structured questionnaire was used to obtain information on veterinary antibiotics and manure use within farms. Finally, samples were collected from different manure heaps, and in manure amended farms for scientific laboratory analysis.

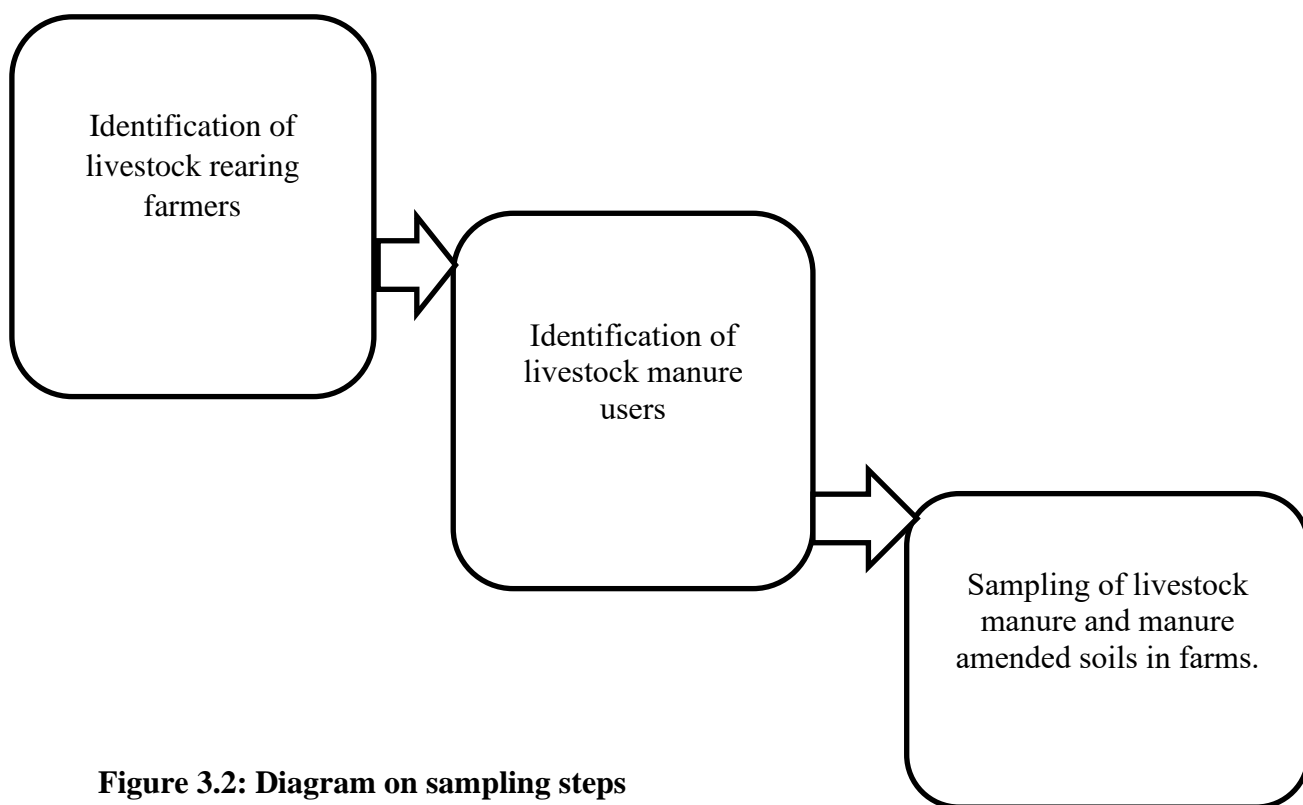
### **3.3 Sampling Design**

The study design used a mixture of methods to select suitable sampling units for the study. Rongai sub-county was divided into strata based on existing administrative units. Purposive sampling was used within each stratum to select livestock rearing farms to be included in the study based on the livestock population size and farming practices within farm, to enable collection of sufficient data for this study and adequate representativeness of the study site. Semi-structured questionnaires were used for the social survey to obtain information on the types of antibiotics used and livestock manure use in farms (APPENDIX I). Laboratory analysis was used to assess concentrations of veterinary antibiotics from collected samples of livestock manure and manure amended soils (Mamani *et al.*,2009; USEPA, 2023). Veterinary doctors involved in treating livestock and livestock production officers were consulted on commonly used antibiotics and livestock reared in the area.

Purposive sampling was used in selecting farms rearing cattle and poultry within the study area. Selected farms were a representation of cattle and poultry rearing farming activities undertaken in the area. Sampling of manure amended field soils was done after farmers identified the type of manure they used to amend their soils. This information was used in calculating a suitable sample size of manure amended farms to be sampled in the study. The number of samples to be collected in the study was identified after farms were set in to clusters within the strata's developed in the study area.

### **3.4 Sampling frame**

The sampling frame for this study were livestock rearing farms, livestock rearing farmers, livestock manure and manure amended soils in farms within Rongai sub-county.



**Figure 3.2: Diagram on sampling steps**

### **3.5 Sample size determination and Sampling.**

Two sets of sample size were worked in this research:

1. A sample size of farmers involved in livestock rearing within Rongai sub-county, Nakuru County.
2. A sample size for livestock rearing farms within Rongai sub-county, Nakuru County.

#### **3.5.1 Sampling procedure for livestock rearing Farmers**

The formula applicable for sample size calculation for this study is  $(n=z^2pq/d^2)$  (Bartlett *et al.*, 2001). The formula is used since the proportion of the population consuming the selected veterinary antibiotics within the study area is not known.

Where;

N =sample size,

$Z^2$  = desired confidence level,

$d^2$  = desired level of precision,

p = estimated proportion of the population that consume the selected veterinary antibiotics,

q = estimated proportion of the population that does not consume the selected veterinary

antibiotics,

Z = 95%,

p and q = 0.5

$d^2 = 0.05$ .

Therefore, by substituting the formula with the given figures the sample size for this study was;  
 $n = 1.96^2 \times 0.5 \times 0.5 / (0.05)^2 = 230$ .

The total households involved in the study area were 5,894. The calculated proportionate sample size using the population size gave a sample size of 230 for the study. The respondents were distributed proportionately based on the strata in the study area.

### **3.5.2 Livestock Manure sampling**

Sampling of manure was done once livestock rearing farms had been identified. The information generated was used to calculate the sample size to be used for the study. The formula  $n_f = n / (1 + n/N)$  was used to calculate the sample size (Araoye, 2004). Where  $n_f$  was the desired sample size when the sources are less than 10,000.

$n / (1 + n/N)$

$n_f = n / (1 + n/N)$

$n_f$  = desired sample size when the population is <10,000.

The sample size was then divided proportionately according to the number of farms involved in livestock rearing within Rongai sub-county. The number of samples to be selected for the samples were allocated proportionately using the proportionate sampling formula. The number of samples to be chosen from each cluster were proportionately allocated using the proportionate sampling formula  $n_w = (N_w/N) * n$  (Barlett *et al.*, 2001). Specific farms to be included in the study were selected through simple random sampling by lottery. A total of 28 farms were sampled with 56 samples collected representing both animal manure and manure amended soil samples from farms. The number of farms sampled in this study provided sufficient representation considering the central limit theorem.

### **3.5.3 Sampling of Manure and manure amended soils**

Manure and soil sampling was done once livestock rearing farms were identified during the interviews. Sampling was done in a single visit to the farms. A total of 28 farms were sampled in this study representing 15 and 13 cattle and poultry rearing farms respectively. The farms

were then distributed proportionately within the study area. Manure samples were collected from animal waste heaps within the farms. Sampling procedure used was similar to Martínez-Carballo *et al.* (2007), Soil sampling was done once livestock manure had been sampled. Soil samples were collected using a shovel and augur at selected points within farms amended by livestock manure at depths of between 0-10 cm. Composite samples of both manure and soil weighing 500 grams were collected from selected farms, the samples were then stored in a cool box with sufficient ice and transported to the lab for analysis.

### **3.6 Validity and Reliability**

The pilot study was conducted in Njoro sub-county that had similar climatic conditions and farmers practicing livestock rearing. Questionnaires were pre-tested for reliability using an approximate of 25 livestock rearing households. The questionnaires were also shared with experts in this field to ensure validity. Data from the questionnaires were then subjected to a reliability test using the Cronbach's alpha to identify the most reliable instrument to be used for the study. The score attained by the questionnaires was 0.76. The test revealed the questionnaires were suitable to be administered among livestock rearing farms.

The high-performance liquid chromatography used for antibiotic residue analysis used for the study was pretested to ensure it worked properly. Antibiotic standards of antibiotics to be assessed were analyzed using high performance liquid chromatography to ensure that the machine could detect them. This was done to enhance validity and reliability of the machine to analyze antibiotic residues in the samples.

Calibration graphs were first determined by the preparation of different concentration of standard solutions. From a stock solution of 1 g/ml of each standard the following concentrations were prepared; 50 g/ml, 100 g/ml, 200 g/ml, 500 g/ml using mobile phase A. The calibration graphs produced by the standards were then used to determine the concentration of antibiotic residues in the samples. Regression equation for the calibration curves were,  $y=0.0037x-0.0196$ ,  $y=9.0761x-7.8752$ ,  $y=137.84x-3.6953$ ,  $y=179.94x-0.588$  for tetracycline, oxytetracycline, sulfadiazine and sulfamethoxazole respectively the  $R^2$  value was 0.999.

### **3.7 Equipment Calibration and Method Validation**

#### **3.7.1 Calibration**

Calibration graphs were developed by preparing different concentrations of standard solutions. From a stock solution of 1 g/ml of each standard the following concentrations were prepared; 50 g/ml, 100 g/ml, 200 g/ml and 500g/ml using mobile phase A. The calibration curves were

used to provide information on recovery, retention factor and the standard deviation. The Limits of detection were also provided, but these were equipment and procedure specific provided by the manufacturer of the HPLC-DAD.

### **3.7.2 Method Validation**

The method was validated by the use of blank samples concentration of 200 g/ml of all the Sulfonamide (sulfadiazine (SDZ), sulfamethoxazole (SMX) and tetracyclines (Tetracycline hydrochloride (TC) and oxytetracycline (OTC). The spiked samples were passed through sample preparation as other soil and manure samples.

### **3.7.3 Chemicals and Materials**

The standards used were sulfonamide (sulfadiazine (SDZ), sulfamethoxazole (SMX) and tetracyclines (tetracycline hydrochloride (TC) and oxytetracycline (OTC) all purchased from (Sigma-Aldrich).

### **3.7.4 Reagents**

The reagents included; Acetonitrile (J.T Baker 9017), Methanol (J.T Baker 8402), Disodium hydrogen phosphate (J.T Baker0326), Citric acid (Acros 124912500), Sodium-EDTA (J.T Baker 1073), Calcium Chloride (Merck 1.2378.0500), Sodium Cetate (J.T Baker 0258), Ammonium acetate (J.T Baker 0011). Mobile Phase A was prepared by mixing Na acetate (0.075 M) and Calcium Chloride (0.035 M) to Sodium EDTA (0.025 M) and the pH adjusted to 7.0. Mobile Phase B was prepared by mixing 75% methanol to 25% Acetonitrile.

### **3.7.5 Equipment**

The HPLC Agilent 1260 series used four main steps that included; Sample preparation drying and grinding, sample purification by vortexing and centrifugation and citric acid, McIlvaine-EDTA Buffer and Methanol, sample extraction and filtration, quantification by HPLC with isocratic mode on C18 column and DAD-detection. The equipment used in the study was Shimadzu HPLC Agilent-1260 series. Equipped with a Diode Array Detector (DAD), Reverse Column Oven-CTO-10ASVP, X-TerraR MS C18 (4.6  $\mu$ m, 2.1  $\times$  100 mm column Waters made in Ireland), an XTerra Guard column C18 (3.5  $\mu$ m, 2.1  $\times$  10 mm) solvent delivery module, LC 20AT, degassing unit DGU-20A3, an auto sampler SIL-20AHT and system controller CBM-20A connected to a HP integrator with LC Solution Version 3.5 Shimadzu Corp-Japan.

### **3.7.6 Sample Preparation and Analysis**

Laboratory analysis was conducted to obtain data on the concentration of antibiotic residues in livestock manure and manure amended soils. To achieve this, a composite manure sample was

prepared from multiple subsamples taken with clean shovels and augur, placed in a plastic bucket and mixed thoroughly. A subsample of 500 g from the mixed composite samples was placed in a plastic ziplock bag. Soil samples representing various points from the farm were collected randomly in selected sites and mixed to create a composite sample for laboratory analysis. The sample was collected, packed, labelled according to the site, wrapped and stored in a cool box with sufficient ice for transportation to the laboratory. Samples were stored at -20° C in the laboratory. The sample were then extracted for analysis and analyzed for antibiotics using a HPLC-DAD. This was carried out at Egerton University Animal Science Laboratory.

Sample extraction of target antibiotics was carried out employing the QuEChERS extraction procedure (Guo *et al.*,2016). Composite samples were oven dried, grinded and one gram of the samples extracted successively using a solution mixture (5 ml of methanol, 1 ml of Na<sub>2</sub>- EDTA and 2 ml of citrate buffer at pH 5.0). The mixture was collected and vortexed for 5 mins, then centrifuged at 4000rpm for 10 mins. The clear supernatant was poured out to a new 25ml centrifuge with the sample remaining on the tube wall. To the old centrifuge containing the supernatant, 5 ml of methanol, 1 ml of Na<sub>2</sub>- EDTA and 2 ml of citrate buffer at pH 5.0 mixed by vortexing for 5 mins and centrifuged at 4000rpm for 10 mins. The resulting supernatant was mixed with the old supernatant initially collected from the sample. The supernatants were filtered through 0.2 µm syringe filter to HPLC vials and placed inside the HPLC and results generated after the run time was completed.



**Plate 3.1:Animal manure samples in the lab.**



**Plate 3.2: Extracted samples ready for analysis.**

### **3.7.7 Quantification by HPLC**

Tetracycline and sulfonamide were detected at a wavelength of 270-277 nm. The column temperature was set at 30<sup>0</sup>c, sample run time was 15mins and flow rate set at 0.5ml/min with an isocratic elution. The injection volume was set at 50  $\mu$ L. Retention time for tetracycline, oxytetracycline, sulfadiazine and sulfamethoxazole antibiotics in this study were 4mins, 1.5mins, 2.4 mins and 3.0 mins respectively. Mobile phase B (Methanol 75% +Acetonitrile 25%). The sample was the filtered through a 0.2  $\mu$ L syringe filter to HPLC vials and loaded into a HPLC and results generated after the runtime was completed.

### **3.8 Environmental Risk assessment**

Environmental risk assessment refers to the acute toxic risk that are likely to occur within the environment resulting from exposure to a particular chemical. Risk Assessment of antibiotics in soils was calculated using the risk quotient formula used in characterization of potential contaminants within the environment (Gonzalez-Pleiter *et al.*,2013; Xu *et al.*,2013). Risk quotient (RQ) values were applied to assess the ecological risk of antibiotics in soils, which were calculated as the ratio of the measured environmental concentrations (MEC; or predicted environmental concentrations, PEC) to the predicted no-effect concentrations (PNEC) for the specific pollutants. (European commission, 2003). The risk values were calculated based on the ratio between the predicted environmental concentration (PEC) of the compounds, and the highest predicted no-effect concentration (PNEC) of these compounds. In general,  $RQ < 0.1$  indicates low risk;  $0.1 \leq RQ < 1$  means medium risk, and  $RQ \geq 1$  symbolizes high risk (European Commission, 2003; Verlicchi *et al.*,2012).

### **3.9 Safety and ethical considerations**

Before data collection, approval was sought from the County Government of Nakuru and the National Council for Science and Technology (NACOSTI) in Kenya, both who issued researchers with permit to conduct the study (Permit number NACOSTI/P/22/20346; APPENDIX VIII). The researcher thereafter gained informed consent from respondents to participate in the study. Disinfectants were available for body and sanitization of appliances. Strict compliance to COVID-19 protocols were undertaken during questionnaire administration in households.

### **3.10 Data analysis**

Data collected on questionnaires and observation checklists were analyzed using statistical package for social sciences. Descriptive statistics was used to organize data collected using questionnaire, arrange the most commonly used classes of antibiotics, determine variations in antibiotic residues concentrations among different types of manure and manure amended soils sampled. Independent T-test was used to determine if there was a significant difference in antibiotic concentration in cattle and poultry manure, and also a significant difference in antibiotic concentration in livestock manure and manure amended soils. The risk quotient values of antibiotics were calculated to determine if the selected antibiotics had a significant risk on the environment. The P-value used for the statistical test was  $P < 0.05$ . Summary of data analysis is given in Table 3.1.

**Table 3.1: Summary of data analysis.**

<b>Research Question</b>	<b>Variables</b>	<b>Statistical tool</b>
What are the consumption patterns of veterinary antibiotics in poultry and cattle rearing within Rongai, Nakuru County.	<p><b>Independent</b></p> <p>Type of antibiotic used.</p> <p>Estimated usage.</p> <p>Frequency of consumption.</p> <p><b>Dependent</b></p> <p>Level of tetracycline's and Sulfonamide.</p>	Descriptive statistics
What are the levels of awareness, and practices of animal manure use in farms in within Rongai sub-county in Nakuru County.	<p><b>Independent</b></p> <p>Manure use within farms.</p> <p>Composting</p> <p>Biogas use</p> <p>Feeds</p> <p>Level of awareness on antibiotics presence in animal manure.</p> <p><b>Dependent</b></p> <p>Level of tetracycline's and Sulfonamide.</p>	<p>Descriptive statistics.</p> <p>Likert scale for level of awareness.</p>
What is the level of tetracycline and sulphonamide residues in cattle and poultry manure and in manure amended soils?	<p><b>Independent</b></p> <p>Tetracycline</p> <p>Sulfonamide</p> <p><b>Dependent</b></p> <p>Level of tetracycline and sulphonamide in animal manure and manure amended soils</p>	<p>Descriptive statistics.</p> <p>Independent T-test.</p>
What is the estimated exposure of antibiotic residues to soils through application of animal manure?	<p>Concentration of antibiotics in soils.</p> <p>Frequency of manure application in farms.</p> <p>Amount of manure applied in farms</p>	Risk Quotient.

## CHAPTER FOUR

### RESULTS AND DISCUSSION

#### 4.1 Representation of farmer's respondents per ward

Rongai sub-county has a total of 5 wards. This table is a representation of the respondents per ward. A total of 230 questionnaires were administered with a response rate of 71%. The data in Table 4.1 represents the number of households assessed.

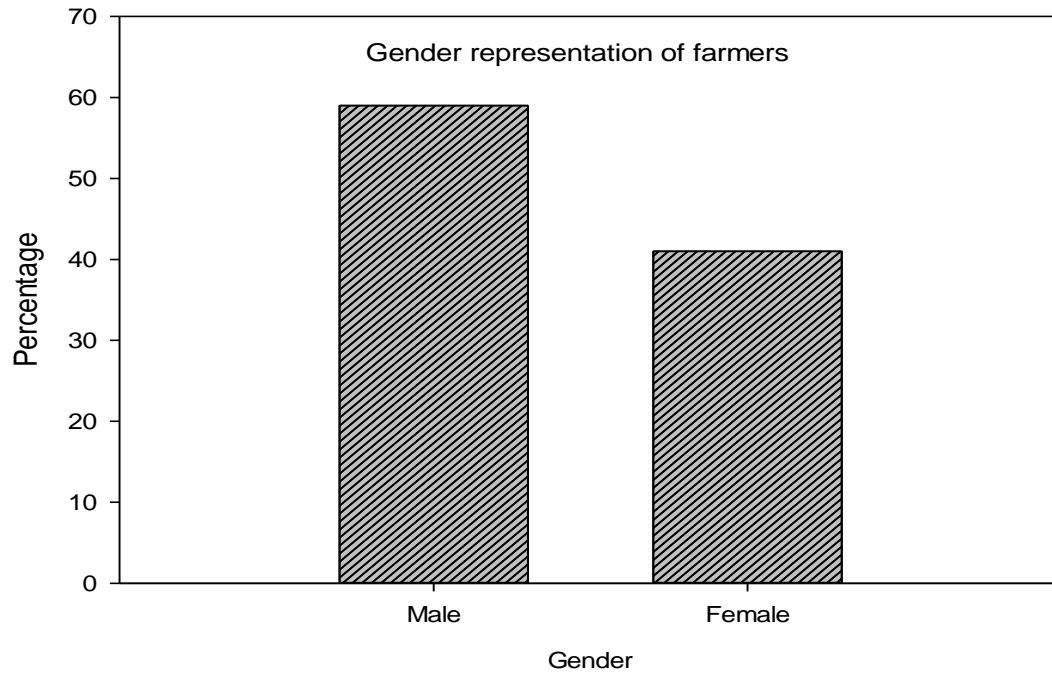
**Table 4.1: Representation of farmer respondents per ward in Rongai sub-county**

<b>Wards</b>	<b>No of Farms</b>	<b>Valid Percentage</b>
Visoi	24	14
Mosop	58	34
Menengai	64	38
Soin	10	6
Solai	14	8
<b>Total</b>	<b>170</b>	<b>100%</b>

#### 4.2 Socio Demographic data of respondents in the study

##### 4.2.1 Gender representation of farmers

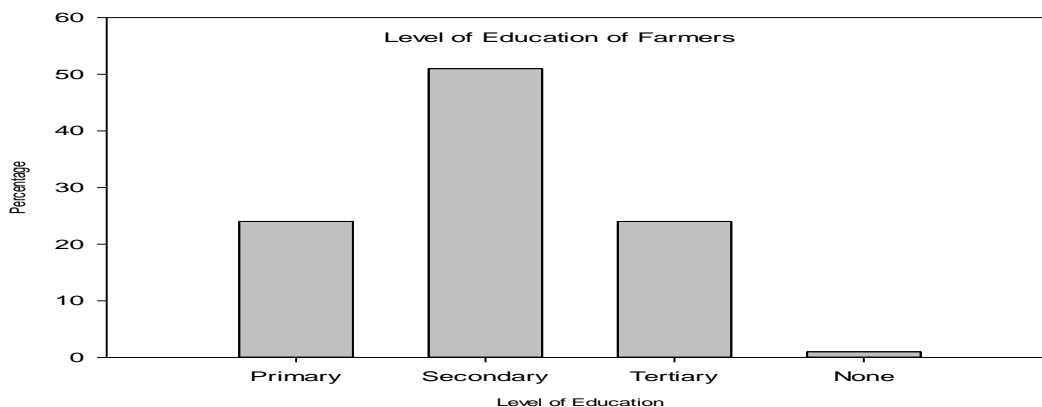
A total of 170 interviews were conducted across Rongai sub-county. The households were randomly selected from five administrative units that formed strata in this study. Out of the 170 households in the study 59% had male respondents, while 41% were female respondents in the study as shown in Figure 4.1. Most respondents in this study were male and this is because most livestock farms had males tending after their livestock. The results were similar to studies assessing livestock keeping practices in Nairobi, Baringo and Ghana (Alarcon *et al.*,2017; Mutua *et al.*,2017; Phares *et al.*,2020).



**Figure 4.1: Gender representation of farmers in Rongai Sub- County.**

#### 4.2.2 Level of Education

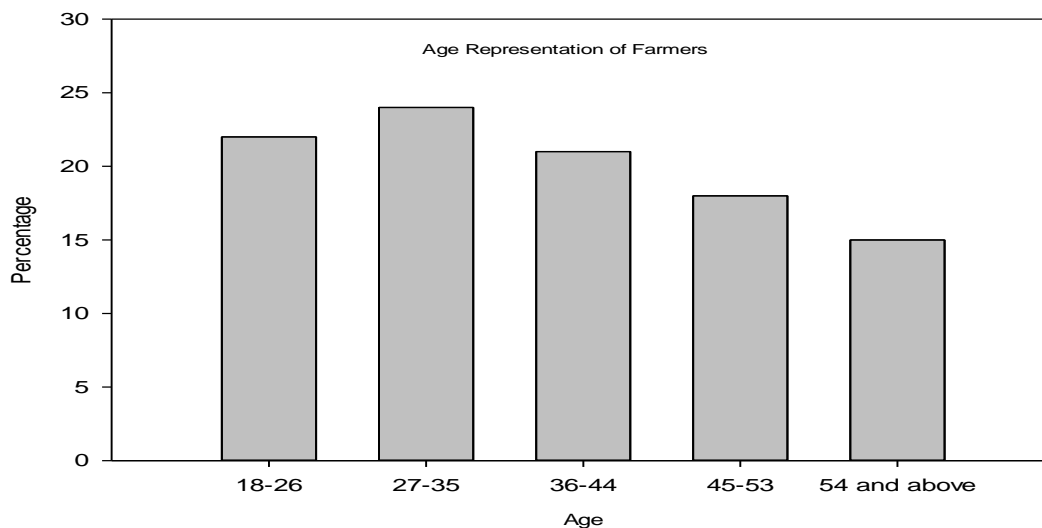
Results from the study showed there was a high rate of literacy recorded among farmers as majority of the respondents had attended secondary and tertiary education recorded at 51% and 24% respectively as shown in Figure 4.2. This was important as they could easily understand livestock health, make sound judgement with regards to their treatment and ensure livestock waste at the farm is managed properly. Most respondents in the study had attended formal schooling, the high literacy levels 99 % in this study are higher compared to the high literacy levels 81.5% and 87% in Kenya and Globally (Buchholz, 2022; UNESCO,2018).



**Figure 4.2: Level of Education among respondents in Rongai Sub- County.**

### 4.2.3 Age of respondents

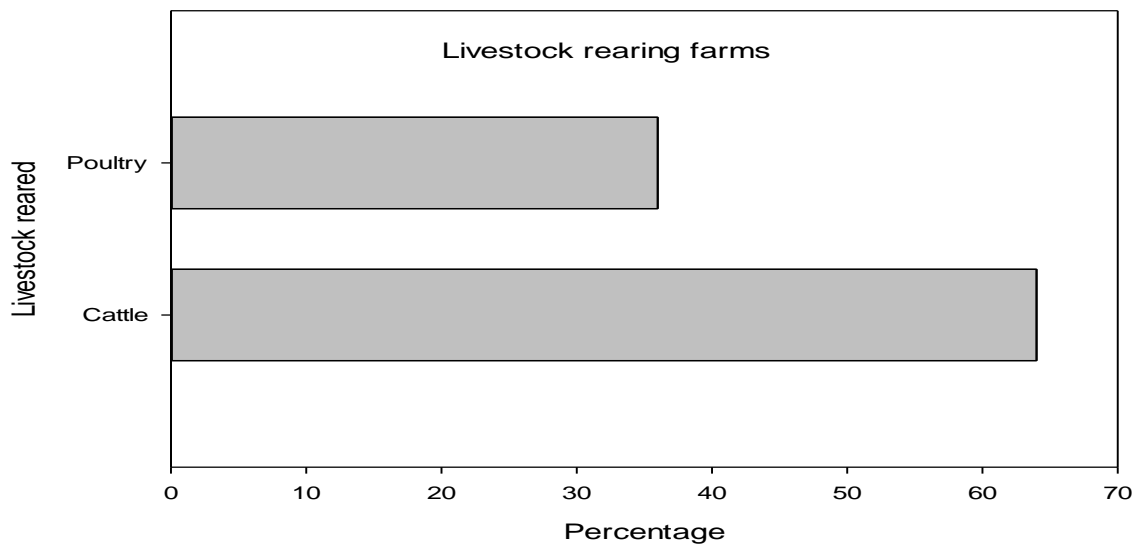
Out of the 170 households, age representation between 36 years and above were the highest number of respondents while those within the age bracket of 54 years and above were the lowest responders in the study. This was evident as a larger number of those involved in responsibly tending for livestock were mostly above 35 years. Results in this study were similar to studies in Kenya and Ghana that found most respondents above the age of 35 (Mutua *et al.*, 2017; Phares *et al.*,2020). Most of the respondents in the study responsible for tending to livestock were older and may have had better experience in drug administration and disease management.



**Figure 4.3: Age of respondents in Rongai Sub- County.**

### 4.3 Livestock rearing farmers within Rongai sub-county

Out of the 170 households evaluated during the study, 64% were actively involved in cattle rearing, while 36% were involved in poultry rearing. Results from the study showed a majority of the farms in the study utilized cattle manure for soil amendment compared to poultry manure. The study findings were similar to a study in Baringo that found cattle rearing as the most preferred compared to poultry rearing (Mutua *et al.*,2017).

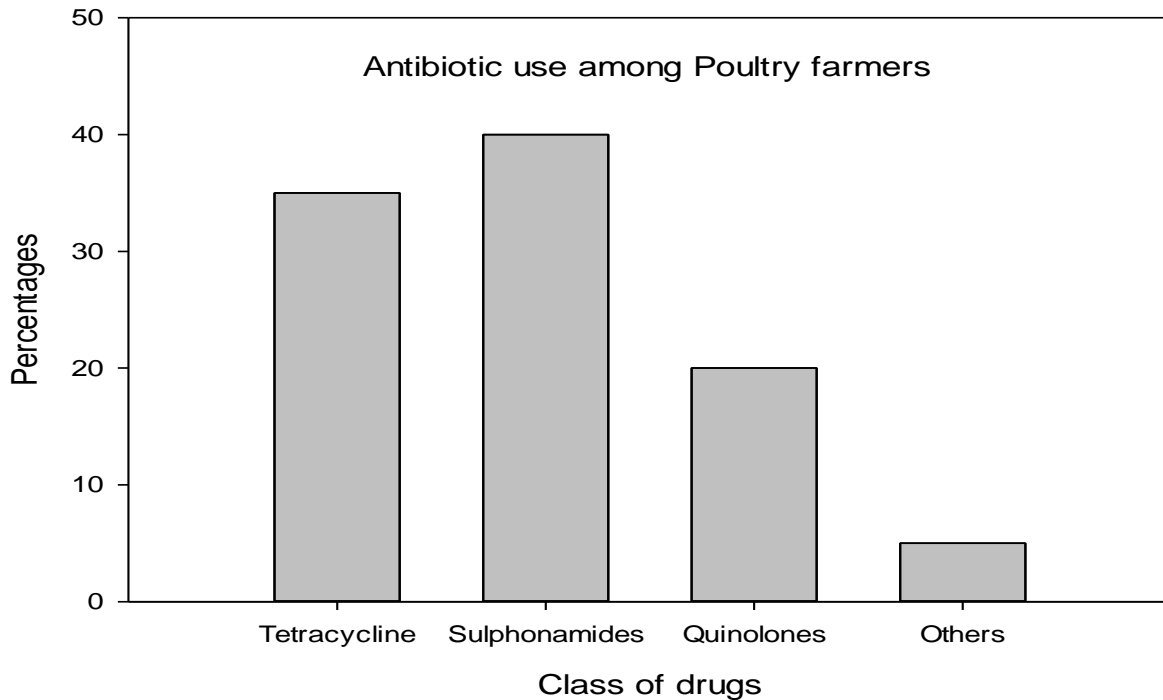


**Figure 4.4: Livestock rearing farmers in Rongai Sub- County**

#### **4.4 Antibiotic use among poultry farmers**

The study assessed antibiotic use by poultry rearing farmers. A total of 62 poultry rearing farms were assessed in the study. The mostly used class of antibiotics within the farms were tetracyclines 35%, sulfonamide 40%, quinolones 20% and 5% that included the use of other classes of drugs. These drugs were mostly used for therapeutics and prophylaxis. However, antibiotics were largely used together in combination in some instances. There were instances where farmers also used herbal medicine such as (Aloe Vera) to manage diseases.

Results were similar to a study assessing antibiotic use among poultry farmers in Kenya, sulfonamide and tetracyclines were found to be the most consumed class of antibiotic drugs among poultry farmers (Mumbua, 2013; Nsofor *et al.*,2013). A similar study assessing the use of antibiotics in poultry rearing in Kiambu County found tetracycline and sulphonamide as the most consumed class of drugs (Kariuki *et al.*,2023). Tetracycline and sulfonamide are the most preferred class of antibiotics due to their wide spectrum in the treatment of livestock diseases. Increased reliance on antibiotic use in the treatment of livestock has the potential of increasing levels of antibiotic residues excreted through animal waste. This has the potential of introducing antibiotic residues into the environment contributing to the development of antibiotic-resistant genes that end up affecting environmental and human health.



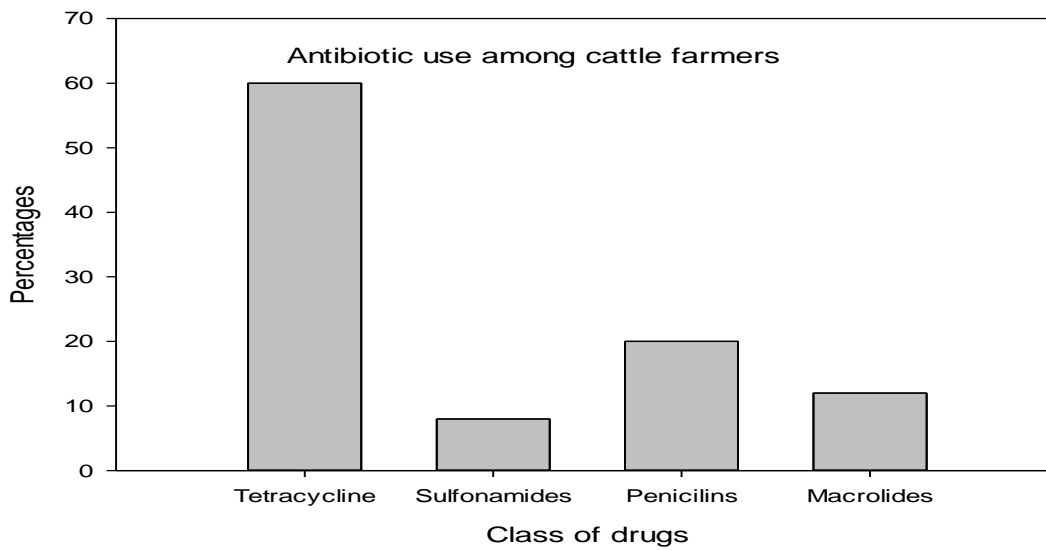
**Figure 4.5: Antibiotic use among poultry farmers in Rongai Sub- County**

#### **4.5 Antibiotic use among cattle farmers**

The study assessed antibiotics used among cattle rearing farmers, a total of 108 cattle rearing farms were assessed in the study. The commonly used class of antibiotics within cattle rearing farms were tetracyclines 60%, sulfonamide 8%, penicilins 20%, and macrolides 12%. A similar study in Pennsylvania reported that tetracycline and penicilins were among the most consumed class of antibiotics (Sawant *et al.*,2005). Results were similar to studies done in Nigeria and Peru that recorded tetracycline (oxytetracycline), followed by beta-lactam/aminoglycoside (penicillin with or without streptomycin) and trimethoprim-sulfonamide as among the most consumed class of antimicrobials (Alhaji *et al.*,2019; Redding *et al.*,2014).

Similar studies assessing antimicrobial use in dairy farming found tetracycline, sulfonamide, penicillins, beta-lactamase, aminoglycosides and macrolides as the most consumed class of drugs in Tanzania (Azabo *et al.*,2022; Katakweba *et al.*, 2012). Results were similar to other studies assessing antibiotic use that found tetracycline and penicillin as the most widely used antibiotics (Cameron & McAllister, 2016; Kisoo *et al.*,2023).

Uncontrolled use of veterinary antibiotics has the potential of increasing their presence in the environment. Antibiotics in the environment can have negative effects, they have the potential to increase development and spread of resistant bacteria affecting both environmental and human health negatively.

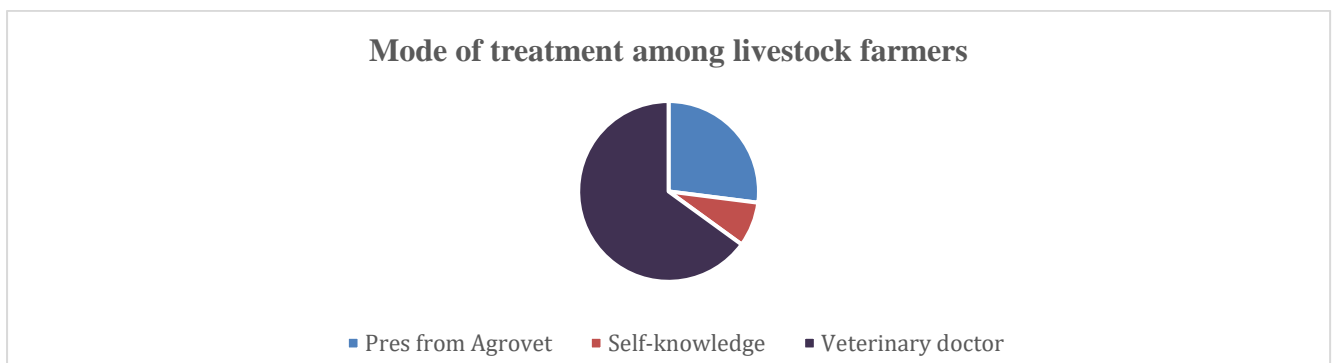


**Figure 4.6: Antibiotic use among cattle farmers in Rongai Sub- County**

#### 4.6 Awareness and Practices among livestock rearing farmers

##### 4.6.1 Treatment of livestock preference by farmers

Out of the 170 farmers interviewed on the preference to treat their livestock, 65% consulted a veterinary doctor for treatment of their livestock (Figure 4.7). These results were similar to a study assessing practices among antimicrobial users in western Kenya that found veterinary doctors and drug prescribed from agrovets as the most preferred mode of treating livestock (Kemp *et al.*,2021). 65% of the farms heavily relied on veterinary doctors for the treatment of their livestock within farms. This has a positive implication on livestock health and disease prevention as livestock are tended to by professionals. Proper administration of veterinary drugs reduces the level of veterinary antibiotics misused during treatment.

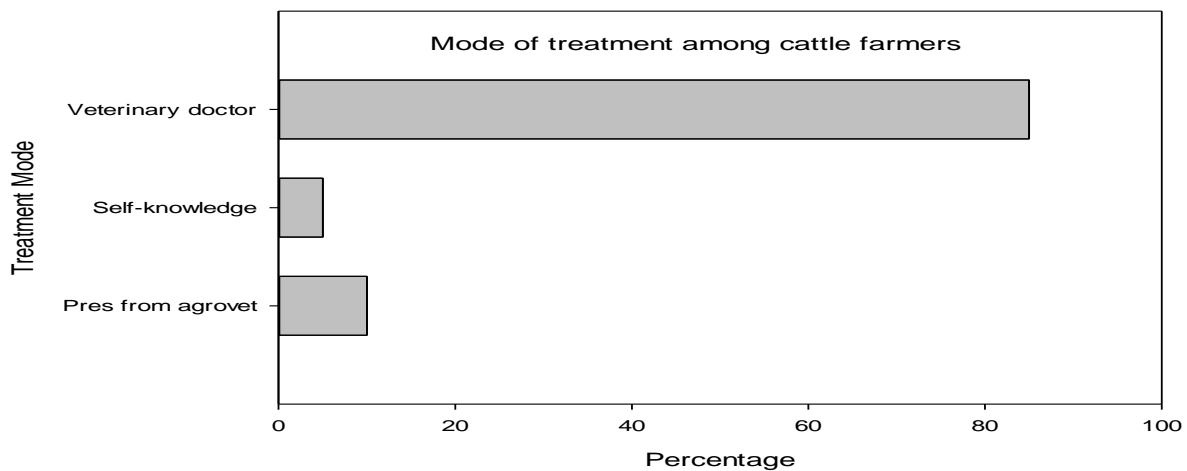


**Figure 4.7: Treatment of livestock preference by farmers in Rongai Sub- County**

##### 4.6.2 Treatment preference among cattle farmers

A representation of 10% of cattle farmers preferred getting prescriptions from agrovet stores, 5% treated their livestock through self-knowledge, while 85% consulted a veterinary doctor to

treat their livestock (Figure 4.8). Results in this study showed that farmers were more likely to consult a veterinary doctor in the treatment of their cattle. These results were similar to a study in Tanzania that found veterinary doctors as the most preferred in the treatment of cattle in farms (Katakweba *et al.*,2012). This was attributed to high value of cattle hence increased care in their management (Caudell *et al.*,2017; Nthambi *et al.*,2023). High reliance on veterinary doctors in the treatment of cattle in this study reduces the chances of self-diagnosing and administration of wrong doses of antibiotics by farmers. This reduces the possibility of antibiotic misuse within farms as recommended doses will be administered to cattle.



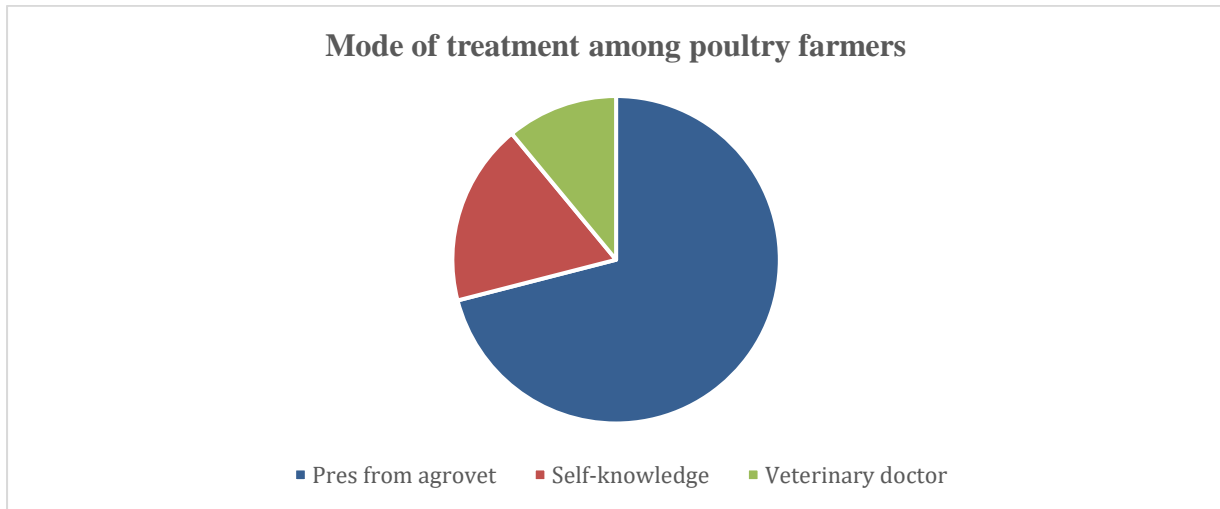
**Figure 4.8: Treatment preference among cattle farmers in Rongai Sub- County**

#### **4.6.3 Treatment preference among poultry farmers**

Of the 68 poultry rearing farmers interviewed during the study, 71% of them preferred getting prescriptions from agrovet stores, 18% treated their livestock through self-knowledge while 11% consulted a veterinary doctor for treatment. The results of this study were similar to studies in Ghana and Kenya assessing practices on antibiotic use by poultry farmers, that found prescription from agro-veterinary shops and self-prescription as the most preferred mode of treatment (Boamah *et al.*,2016; Kariuki *et al.*,2023).

Prescriptions from agrovet stores were the most preferred mode of treatment among poultry farmers. This meant that farmers were at risk of getting livestock disease misdiagnosed leaving farmers at the risk of purchasing a variety of drugs to treat livestock diseases at farm level. Treatment of poultry without prescriptions has the potential to lead to the administration of high doses of veterinary antibiotics. That are eventually excreted into the environment contributing to antibiotic pollution. However, farms practicing intensive poultry farming relied mostly on veterinary doctors. Farmer preference on the mode of treatment in poultry rearing is largely

attributed to the level of education, rearing system and income levels of the farmer (Nthambi *et al.*, 2023).



**Figure 4.9: Treatment preference among poultry farmers in Rongai Sub- County**

#### 4.6.4 Use of livestock waste in farms

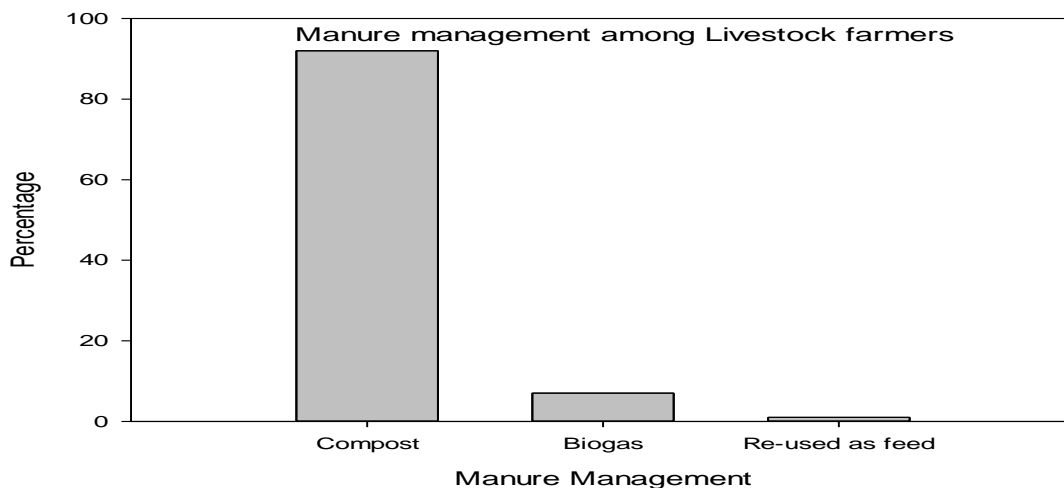
Out of the 170 household farmers interviewed on their utilization of livestock waste 92% used it as compost, 7% used livestock waste for biogas and 1% used livestock waste generated as feed that was mainly from poultry droppings (Figure 4.10). The main use of livestock waste within farms in this study was compost that was used in the farms, in farms with biogas unit's livestock waste was used to produce biogas and the slurry from the biogas systems later used in farms as manure. Poultry litter from some of the farms was dried and sieved to be used as cattle feed. However, a number of farms also used poultry litter for biogas and compost.

Results were similar to a study that found composting and conversion of waste into bioenergy as the main forms of manure management within farms (Zhu & Hiltunen,2016). The use of animal manure as compost is linked to increased soil quality and productivity and offers a chance to reduce on animal waste generated from farms (Goldan *et al.*,2023). The use of poultry dropping as animal feed was noted in a similar study in Kiambu County, this practice presents the risk of introducing sub-therapeutic doses of antibiotics to livestock consuming poultry waste as feeds (Kariuki *et al.*, 2023).

Animal manure application in farms as fertilizer has been practiced for centuries, it is considered a cheap alternative compared to manufactured fertilizer. It is a more natural effective way in enriching soils containing a wide range of nutrients that include nitrogen, phosphorus and potassium. It also provides a more sustainable way for waste management at

the farm level. Animal waste from the farm can be mixed with organic waste generated from the farms. This ensures that nutrients are not lost and are re-utilized within the farm in improving yield, enhancing sustainable waste management at the farm level. Waste from animal dropping can be used for energy production in generating biogas, slurry generated from biogas units can be used in the farm as manure.

The re-use of animal waste at the farm level is important in ensuring sustainability and efficiency of farm activities. Cleaner and circular agricultural production have increasingly been adopted in livestock and poultry rearing (Li *et al.*,2016). Recent changes in livestock rearing practices to incorporate cleaner production methodologies have been necessitated as a measure of mitigating against negative environmental effects. It also offers an alternative to energy production by creating sustainable energy sources and resource use in farms (Choi *et al.*,2017; Xu *et al.*,2017). Effective manure management at the farms reduces the likely negative impacts of livestock rearing activities at the farm level.



**Figure 4.10: Use of livestock waste in farms in Rongai Sub- County**

#### **4.6.5 Manure preparation in farms**

The study assessed the number of months used by farmers to prepare livestock manure before they are spread on farms. Out of the 170 households assessed, 62% of the farmers prepared manure for 0-3 months (Figure 4.11). The number of times taken to prepare the manure was mainly influenced by the planting season and livestock reared within farms. Animal waste was applied directly in some of the farms as a form of managing waste.

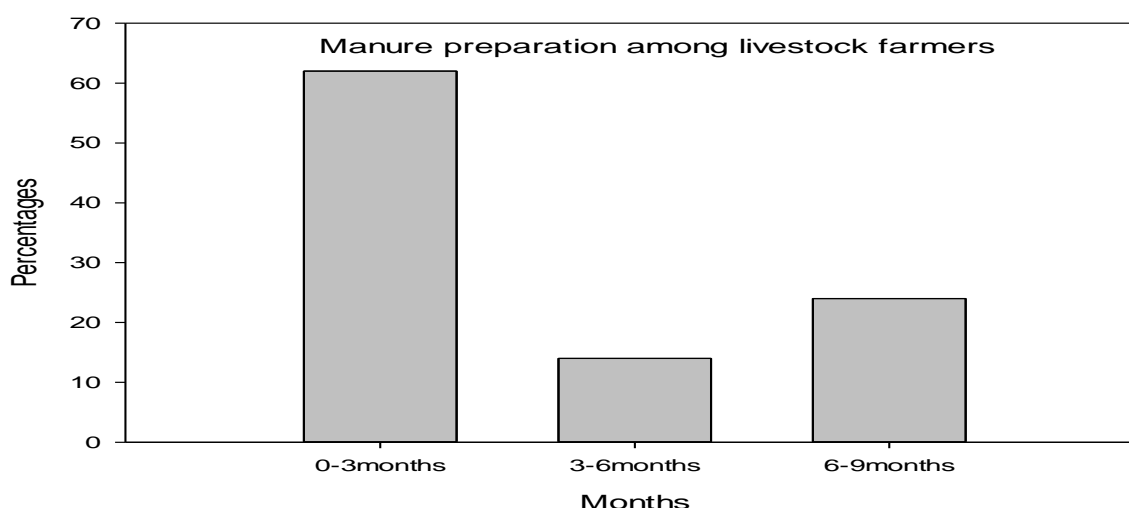
However, the results differed with a study in Nyando assessing manure management among smallholder livestock systems that found most farmers prepared manure for more than 6

months before application on farms (Casu, 2018). This implied that most of the manure applied in farms in this study had not been well prepared and could have high levels of antibiotic residues, thus presenting an opportunity for the introduction of antibiotic residues and antibiotic-resistant bacteria in the soil that end up in the environment.

Antibiotics have been found to have degradation rates ranging from 0-3466 days in soils from various studies (Cycon *et al.*,2019). Degradation rates of antibiotic residues in soils have been found to range from 14-69 days for tetracyclines concentrations ranging from 0.05-0.2 mg/kg(Pan & Chu,2016), 21- 63 days for oxytetracycline concentrations ranging from 0.3-50 mg/kg (Blackwell *et al.*,2007; Cycon *et al.*, 2019), 2-59 days for sulfamethoxazole concentrations ranging from 1-300 mg/kg (Liu *et al.*,2009; Lin & Gan,2011) and 4.8-30 days sulfadiazine concentrations ranging from 0.71- 4 mg/kg (Sittig *et al.*,2014; Zhang *et al.*,2017) in different studies. Degradation rates of antibiotics in this study fell in between 4-69 days, with a majority of the farms in the study preparing their manure for between 0-3months before application in soils. Soils in this study could be exposed to antibiotic residues resulting from frequent application of manure loaded with antibiotics that has the potential of resulting to antibiotic accumulation in soils.

Composting and anaerobic digestion were the most preferred methods for manure processing in this study. Despite their suitability, they have the potential of contributing to antibiotic residues, antibiotic resistance bacteria and antibiotic-resistant genes in the environment (Hou *et al.*,2017). They are not fully reliable in eliminating antibiotic residues. Degradation rates of antibiotics in manure are mainly reliant on their chemical structure, type of manure and manure characteristic, their degradation rates however differ in different studies.

Abiotic and biotic factors have the potential of affecting the degradation in different groups of antibiotics in soils (Cheng *et al.*,2019; Kasumba *et al.*,2020; Lee *et al.*,2020; Liu *et al.*,2018). Poor preparation of compost in farms has the potential of introducing antibiotic residues into soils during manure application. Antibiotic residues in livestock manure used in farms in this study had high levels. Continuous application of antibiotic contaminated manure on farms is likely to affect the rate of antibiotic degradation eventually increasing their presence in soils.



**Figure 4.11: Manure preparation in farms in Rongai Sub- County**

#### 4.6.6 Knowledge on presence of antibiotics in manure and soils

Farmers were assessed on their level of awareness on the presence of antibiotic residues in livestock manure and manure amended soils. A total of 170 farmers were assessed, on the questionnaire statement on antibiotic presence in livestock manure and soils. 75% and 55% of the farmers in this study disagreed with it respectively, indicating low levels of knowledge among livestock rearing farmers (Table 4.2).

**Table 4.2: Farmer knowledge on the presence of antibiotics in manure and soils**

	A	SA	U	D	SD
<b>Antibiotics in manure</b>	29(17%)	3(2%)	6(4%)	128(75%)	4(2%)
<b>Antibiotics in soils</b>	63(37%)	3(2%)	8(5%)	94(55%)	2(5%)

A-Agree, SA-Strongly agree, U-Undecided, D-Disagree SD-Strongly disagree

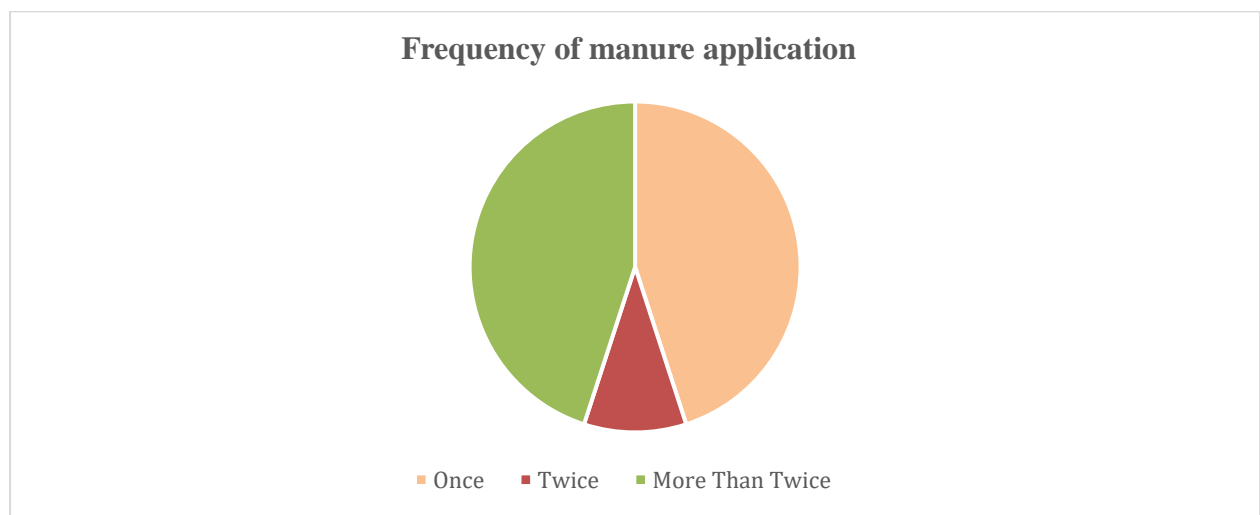
Most farmers involved in this study disagreed on the existence of antibiotics in both livestock manure and manure-amended soils. Farmers' practices in the use of veterinary antibiotics in treating their livestock is likely to influence antibiotic presence in livestock manure and manure amended soils. Low level of awareness among farmers on the presence on antibiotic residues in soils and livestock manure is likely to contribute to increased introduction of antibiotic residues in the environment. This is likely to be attributed to the slow adoption of safe use of antibiotics or poor manure management at the farm level. The results were similar to a study in Ghana that found farmers had low levels of awareness on antibiotics in soils and manure (Phares *et al.*,2020). Low levels of awareness on antibiotic presence in livestock manure and

soils has the potential to contribute to antibiotic resistance development that ends up affecting both environmental and human health negatively.

#### 4.6.7 Frequency of manure application

Application of livestock waste in farms provides an alternative to waste management and also benefits both soils and crops. In this study, 92% farmers used livestock waste as compost within their farms. Farmers gave information on the frequency of manure application on their farms. The results indicated that 89% of the farms assessed in the study applied manure in their farms either once or more than twice in a year (Figure 4.12). This was mainly dependent on the planting season throughout the year as the manure was used in preparing soils before planting. However, application on some of the farms was done more frequently by the farmers on their farm this practice differed from one farm to another.

Increased application frequencies of manure with antibiotic residues within farms is likely to cause an increase in the levels of antibiotics in soils. That has the potential of affecting soil microbial community and increased occurrence of antibiotic-resistant genes in the environment. Results in this study were similar to a study assessing organic manure use in Nepal that found majority of the farmers applied manure more than twice in their farms in a year (Maskey & Mihara,2019)



**Figure 4.12: Frequency of manure application.**

#### 4.7 Antibiotic residues in livestock manure and Soils

The study assessed the presence of four antibiotics in manure and soils representing two classes of antibiotics in various farms in the study. Farm samples of livestock manure and manure amended soils were collected and transported to the lab for analysis. Analysis for antibiotic

residues was done for soil samples and manure samples which included top soil samples for soil and manure samples from various manure heaps. The concentration of the antibiotic residues in the various farms is presented and discussed here below.

#### 4.7.1 Oxytetracycline in manure and soils

A total of 56 samples were collected representing both livestock manure and manure amended soils. Soils amended by cattle manure recorded the highest level of oxytetracycline residues ranging from 1653-14237  $\mu\text{g}/\text{kg}$ , while soils amended using poultry manure had oxytetracycline residues ranging from 1880-9240  $\mu\text{g}/\text{kg}$  (Table 4.3).

**Table 4.3: Concentration of antibiotics in manure and soils in the study**

Group of Antibiotic	Compound	Sample	Range $\mu\text{g}/\text{kg}$	(mean $\pm$ SE) $\mu\text{g}/\text{kg}$	Detection rate (%)
Tetracycline	Oxytetracycline	Manure	1105-16240(P)	3370.53 $\pm$ 1401.01(P)	54
			60-3299(C)	638.96 $\pm$ 273.35(C)	
			Soils	1880-9240(P)	
	1653-14237(C)	1405.4 $\pm$ 951.03(C)			
	Tetracycline	Manure	1247-15178(P)	1171.62 $\pm$ 1171.62(P)	25
			1316-15231(C)	2426.38 $\pm$ 1136.14(C)	
Soils			4241-29396(P)	2964.46 $\pm$ 2253.04(P)	
1532-13069(C)	2412.71 $\pm$ 1035.26(C)				

Sulphonamide	Sulfadiazine	Manure	375.5- 3971(P) 8.9- 748.7(C)	961.75±427.39(P) 90.91±52.23(C)	46
		Soils	103.4- 1847(P) 11.74- 389(C)	321.56±188.89(P) 43.92±27.66(C)	39
	Sulfamethoxazole	Manure	1212- 4955(P) 40.4- 3940 (C)	1605.8± 568.06(P) 665.82±336.28 (C)	50
		Soils	64.2 - 10556 (P) 34.5- 7752 (C)	2193.81±992.11 (P) 1345.56±685.97 (C)	50

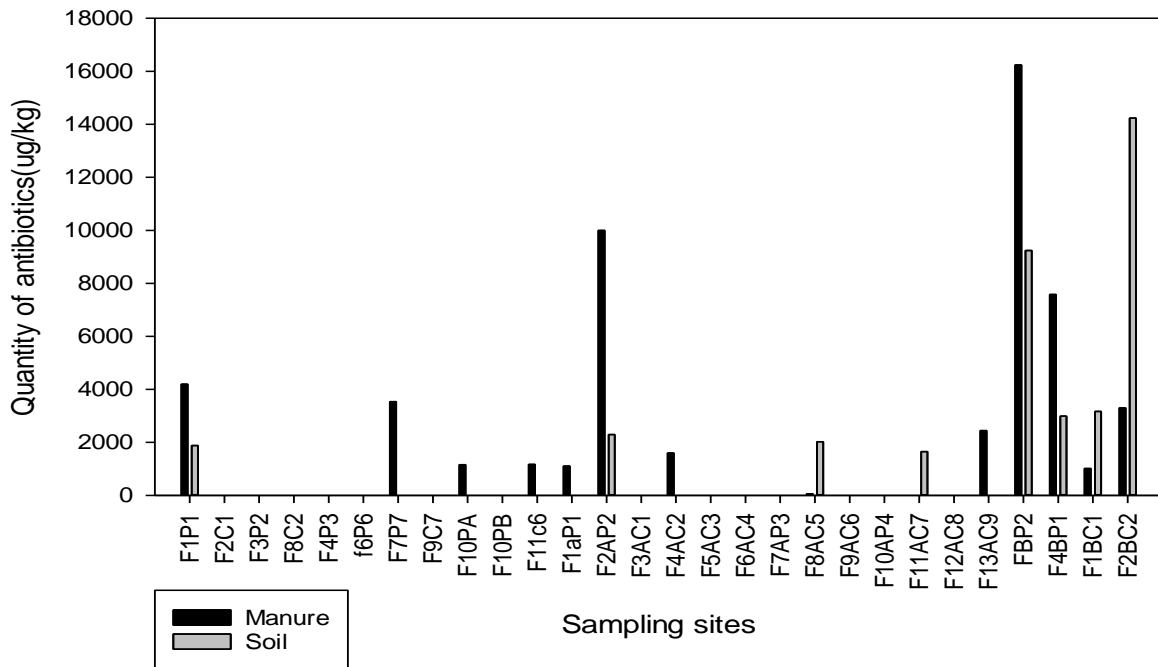
n=56, P=Poultry, C=Cattle, SE-Standard Error

High levels of oxytetracycline residues in this study can be attributed to its broad use in the treatment of diseases and high doses administered to livestock during self-prescribing that eventually end up in manure and the environment once they are excreted by livestock. Previous studies assessing the levels of oxytetracycline in manure found lower levels of oxytetracycline in manure. There was no significant difference in the concentration of oxytetracycline antibiotics in cattle and poultry manure samples analyzed, ( $T=2.04$ ;  $P=0.05$ ). In China, a study assessing the level of oxytetracycline in soils found residue levels of  $80\mu\text{g}/\text{kg}$  in vegetable farmland, a similar study in Guangdong China, assessing oxytetracycline residues found levels of up to  $9.6\mu\text{g}/\text{kg}$  in greenhouse vegetable production bases.

According to Zhao *et al.* (2010), oxytetracycline in livestock manure were found to reach levels of up to  $1550\mu\text{g}/\text{kg}$  from manure compost in farms. Similar studies assessing the presence of oxytetracycline in poultry manure in China, Iran and Egypt recorded tetracyclines levels of up

to 4,570 µg/kg, 13,770 µg/kg and 1.33 µg/kg (Alavi *et al.*,2015; Li *et al.*,2013; Mahmoud & Abdel-Mohsein,2019). Figure 4.13 outlines concentration levels of oxytetracycline residues in manure and soils recorded in the study.

#### Oxytetracycline residues in livestock manure and manure amended soils



**Figure 4.13: Oxytetracycline residues in farms within Rongai sub-county**

In an experimental study assessing the levels of tetracycline in poultry droppings treated for 14 days with 80,000 µg/kg, Oxytetracycline was highest in birds at 22,741 µg/kg and those recorded from poultry litter were less than <12.2µg/kg (Pokrant *et al.*,2021). Ji *et al.* (2012) recorded levels ranging from 1.87–4.24 µg/kg dry matter in farmland soils that were located near cattle and chicken farms in Shanghai, China. In Kenya, a study assessing the levels of oxytetracycline residues in soils found levels of up to 29.38µg/kg in soils (Yang *et al.*,2016). The concentrations were lower compared to findings in similar studies that recorded tetracycline levels of 47-13770 µg/kg in cattle manure in Malaysia and 78,516 µg/kg in poultry in Iran (Alavi *et al.*,2015; Ho *et al.*,2014). A similar study assessing oxytetracycline residues in soils recorded levels of up to 416,000 µg/kg in chicken manure (Zhang *et al.*,2015).

Oxytetracycline residues in soils contribute to the occurrence of antibiotic residues in rivers to levels of up to 652 µg/kg (Hu *et al.*, 2010). A study assessing the levels of Oxytetracycline residues in surface water in China recorded concentrations of 1530 l/kg and 2610 l/kg in Hailing Bay and Bosten Lake (Chen *et al.*,2015; Lei *et al.*,2014). Oxytetracycline residues that

end up in soils cause toxicity among soil bacterial communities. However, it is dependent on the soil properties such that an increase in soil pH and silt content resulted in a higher toxicity of (Santas-Miguel *et al.*,2020). Antibiotic residues have also been found to contribute to the occurrence of antibiotic resistant genes in water. Tetracycline resistant genes tet A and tet B2 were found in Pearl River water with detection rates of 40% and 43% respectively (Tao *et al.*,2010).

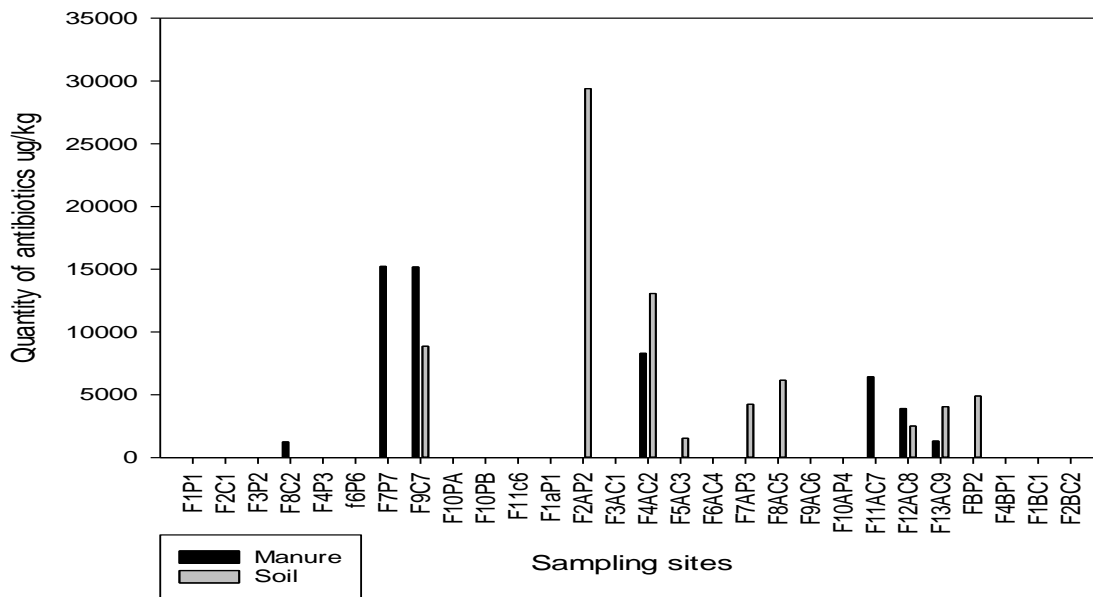
#### **4.7.2 Tetracycline in manure and soils**

Tetracycline residues in this study were found to be relatively lower in livestock manure compared to the levels in manure amended soils (Table 4.3). This was likely attributed to the slow degradation of tetracycline antibiotics in soils. Uncontrolled use of antibiotics in the treatment of livestock through self-prescribing among farmers is likely to contribute to the high levels of antibiotics recorded in animal manure. Tetracycline residues recorded the highest levels among various classes of antibiotics assessed in this study.

Poultry manure analyzed had higher levels of tetracycline ranging from 1316-15231 µg/kg compared to cattle manure 1247-15178 µg/kg (Table 4.3). However, there was no significant difference in tetracycline concentration in cattle and poultry manure ( $T=0.93$ ;  $P=0.36$ ). Further, soils amended by poultry manure recorded the highest level of tetracycline residues at 4241-29396 µg/kg, compared to soils amended using cattle manure ranging from 1532-13069 µg/kg. Table 4.3 presents various concentrations of tetracycline antibiotics in manure and soils recorded in the study. The study recorded higher levels of tetracycline residues in both livestock manure and soils compared to similar studies.

Over-reliance by poultry farmers on getting prescriptions from agrovets and self-prescribing for treatment could be linked to high levels of tetracycline residues in manure and soils. Self-prescribing of antibiotics is likely to contribute to administration of high doses of antibiotics in treating livestock. Figure 4.14 outlines the concentration levels of tetracycline residues in manure and soil samples in the study.

### Tetracycline residues in livestock manure and manure amended soils



**Figure 4.14: Tetracycline residues in farms within Rongai sub-county**

A similar study done in Tanzania assessing antibiotics levels in livestock manure recorded lower tetracycline levels of 1574  $\mu\text{g}/\text{kg}$  in poultry manure and 329.23  $\mu\text{g}/\text{kg}$  in cattle manure (Mohameda *et al.*,2017). A study in China found tetracycline residues levels of 80 $\mu\text{g}/\text{kg}$  of in soils in vegetable farmland (Li *et al.*,2011). Ji *et al.* (2012) recorded levels of up to 1870–4240  $\mu\text{g}/\text{kg}$  dry matter in farmland soils that were located next to cattle and chicken farms in Shanghai.

In Kenya, a study assessing the levels of antibiotics in soils found tetracycline levels of 16.02  $\mu\text{g}/\text{kg}$  in soils (Yang *et al.*,2016). Some of the soils showed higher levels of tetracycline residues than in livestock manure that might be attributed to previous use of antibiotic contaminated livestock manure or accumulation of antibiotics, while some had tetracycline residues that were not present in livestock manure. Tetracyclines are considered to be very persistent antibiotics that is likely to contribute to their high levels in the environment from frequent manure application in soils (Masse *et al.*,2014). Some of the samples analyzed for tetracycline residues in the study did not record the presence of antibiotic residues. However, this does not rule out the presence of other classes of antibiotics in both manure and soil samples.

Tetracyclines also have the potential of getting to water from soils through leaching and surface runoff from application of manure in soils. This contributes to the presence of antibiotic

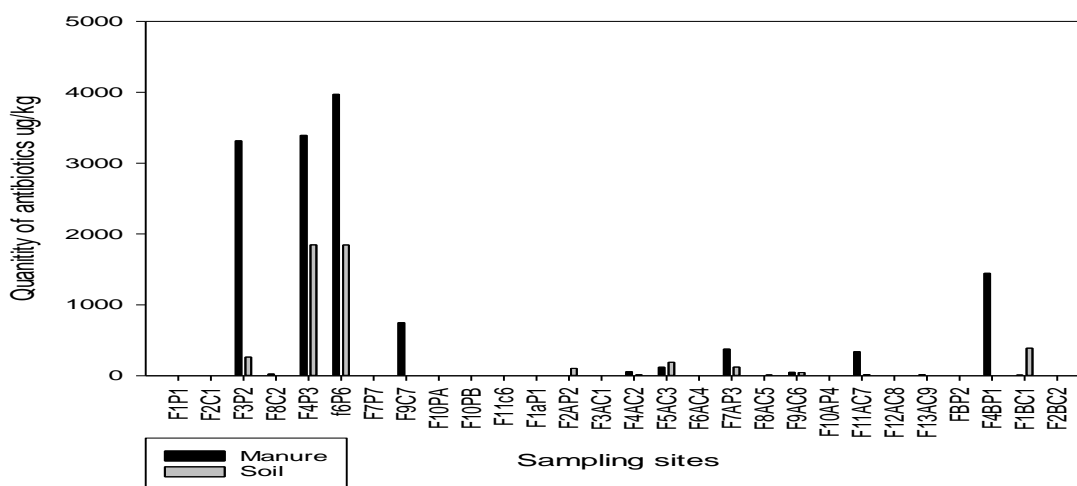
residues in water and occurrence of antibiotic-resistant genes in water that have the potential of causing animal and human diseases (Yang *et al.*,2018). A study assessing the levels of tetracycline residues in surface water in China recorded concentrations of 1608 l/kg and 13766 l/kg in Hailing Bay and Bosten Lake respectively (Chen *et al.*,2015; Lei *et al.*,2014).

Over-reliance on tetracycline residues in livestock rearing is increasingly becoming a cause for alarm in aquatic pollution, due to its relatively low metabolism in human and animal organisms and its stability in the environment (Xu *et al.*,2021). Tetracycline residues end up in soils leading to toxicity among soil bacterial communities (Santas-Miguel *et al.*,2020). A study assessing the responses of plankton in water to tetracycline found that increased levels have the potential to reduce species abundance and richness affecting both clarity and level of dissolved oxygen in water (Wilson *et al.*,2004). Figure 4.14 outlines the concentration levels of tetracycline residues in manure and soils samples in the study.

#### 4.7.3 Sulfadiazine in manure and soils

Poultry manure analyzed for sulfadiazine residues in this study had higher levels of antibiotics residues ranging from 375.5-3972 µg/kg compared to cattle manure 8.9-748.7 µg/kg. However, there was no significant difference in the concentration of sulfadiazine antibiotic in cattle manure and poultry manure (T=2.17; P=0.13). Soils amended by poultry manure in this study recorded a higher level of sulfadiazine residues at 103.4 -1847 µg/kg, compared to soils amended by cattle manure that had antibiotic residues ranging from 11.74-389 µg/kg. Sulfadiazine levels in this study were lower in both manure and soils compared to other antibiotics assessed in this study. Figure 4.15 outlines the concentration of sulfadiazine residues in manure and soil samples in the study.

Sulfadiazine residues in livestock manure and manure amended soils



#### **Figure 4.15: Sulfadiazine residues in farms within Rongai sub-county**

Sulfadiazine levels in livestock manure were relatively higher compared to those in manure amended soils largely attributed to a high degradation rate in soils (Table 4.3). Sulfadiazine has previously been identified at levels of 51,000 µg/kg in poultry feces (Martinez-Carbello *et al.*,2007). Ho *et al.* (2014) found concentrations ranging from 4500 to 18700 µg/kg in animal manure samples in Tianjing, China. A study in Netherlands assessed the presence of antibiotics in calve manure and found levels of 1-65µg/kg of sulfadiazine in manure samples (Jensen *et al.*,2019). In China, a similar study assessing the sulfadiazine levels in soils found antibiotic residue levels of 760 µg/kg, a similar study in four provinces in China, assessing the levels of sulfadiazine residue found levels of up to 0.7 µg/kg in manure fertilized farms(An *et al.*,2015; Wei *et al.*,2019).

In Kenya, a study assessing the levels of sulfadiazine in soils found levels of up to 3.85 µg/kg in soils (Yang *et al.*,2016). A study assessing the levels of sulfadiazine residues in surface water in China recorded concentrations of 1214 l/kg and 141 l/kg in Hailing Bay and Bosten Lake (Chen *et al.*,2015; Lei *et al.*,2014). Studies assessing the effect of antibiotics on soils have linked it to negative effects on soil microbiology. According to Hammesfahr *et al.* (2011), increased levels of sulfadiazine in soils has the potential of reducing soil microbial biomass. High levels of sulfadiazine antibiotics in soils in this study can result in reduced microbial biomass in soils. Exposure to high concentration of sulfadiazine in soils for a long time has the potential of affecting microorganisms due to increased toxicity. Results obtained in this study presented higher levels of antibiotic residues. This might be linked to the high level of antibiotic use by farmers in treating livestock within farms or increased accumulation of sulfadiazine in soils within the study area.

Most of the farmers in the study area prepared their manure for 0-3months before application onto farms. Sulfadiazine has been reported to have degradation rates of between 4.8-30 days for concentrations ranging from 710-4000 µg/kg (Sittig *et al.*,2014; Zhang *et al.*,2017). High levels of antibiotics in soils could be attributed to slow degradation rates or continuous application of manure with high levels of antibiotics in farms. Sulfadiazine levels recorded from livestock manure in this study show that manure can be considered as a key pollutant in the introduction of antibiotics residues in the environment that end up in soil, surface and groundwater.

#### 4.7.4 Sulfamethoxazole in manure and soils

Samples collected from farms were tested for sulfamethoxazole levels in both manure and manure amended soils. The study noted high levels of sulfamethoxazole residues in poultry manure compared to cattle manure (Table 4.3). Poultry manure analyzed in this study had higher levels of antibiotics residues ranging from 1212-4955  $\mu\text{g}/\text{kg}$  compared to cattle manure 40.4 -3940  $\mu\text{g}/\text{kg}$ . However, there was no significant difference in sulfamethoxazole concentration in cattle and poultry manure ( $T=1.46$ ;  $P=0.15$ ). Soils amended by poultry manure recorded a higher level of sulfamethoxazole residues at 64.2-10556  $\mu\text{g}/\text{kg}$  compared to soils amended using cattle manure ranging from 34.5 -7752  $\mu\text{g}/\text{kg}$ .

This meant that poultry manure presented a greater risk of introducing sulfamethoxazole antibiotics into the environment. The study recorded higher levels of sulfamethoxazole residues in both animal manure and soils compared to similar studies. In China, a study assessing the level of sulfamethoxazole in soils found levels of 0.06  $\mu\text{g}/\text{kg}$  in vegetable farmland (Li *et al.*,2011). A similar study in Southern China, assessing sulfamethoxazole residue found levels of up to 54.5  $\mu\text{g}/\text{kg}$  in farmland (Li *et al.*,2011). Figure 4.16 outlines the concentration of sulfamethoxazole residues in manure and soil samples in the study.

Sulfamethoxazole residues in livestock manure and manure amended soils

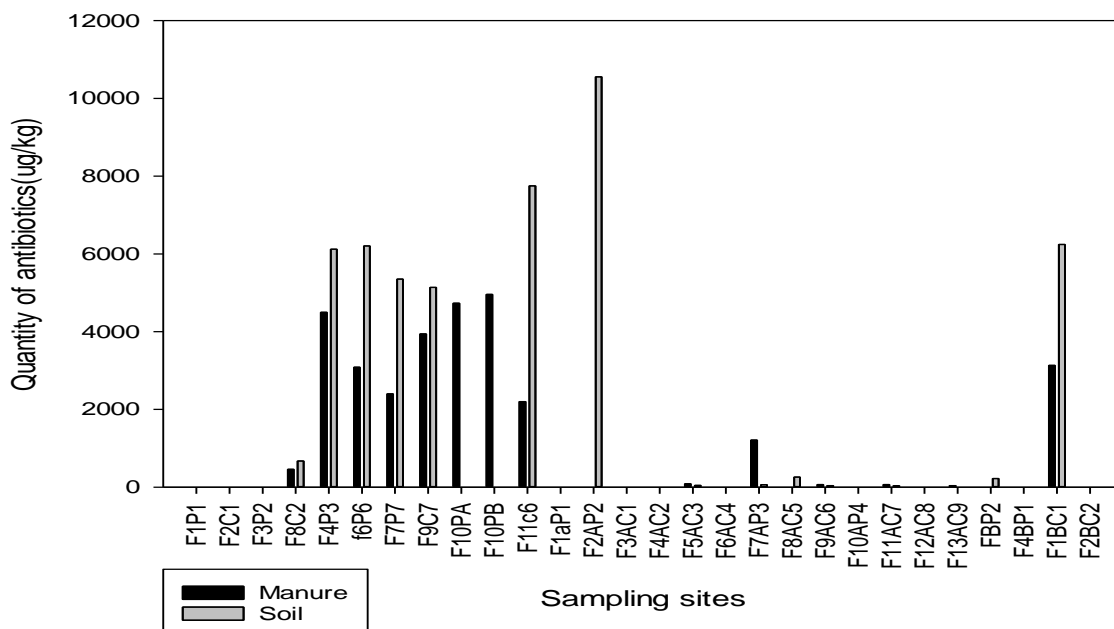


Figure 4.16: Sulfamethoxazole residues in farms within Rongai sub-county

Similar studies assessing sulfamethoxazole levels in livestock manure and soils recorded different levels. A study assessing sulfonamides in organic fertilizers in Poland found

sulfamethoxazole levels of up to 2000 $\mu\text{g}/\text{kg}$  in poultry manure (Osinski *et al.*,2022). Ji *et al.* (2012) recorded levels of up to 1290-2450  $\mu\text{g}/\text{kg}$  in farmland soils that were located near cattle and chicken farms in Shanghai. Hu *et al.* (2010) noted levels of up to 0.9  $\mu\text{g}/\text{kg}$  of sulfamethoxazole residues in farmland soils from organic vegetable bases in Northern China. A similar study assessing sulfamethoxazole recorded levels of 58.1 $\mu\text{g}/\text{kg}$  in manure amended soils in Northern China (Hou *et al.*,2015).

Li *et al.* (2011), recorded sulfamethoxazole levels of 54.5 $\mu\text{g}/\text{kg}$  in farmland soil in Pearl River delta area, southern China. According to García-Galán *et al.* (2008), sulfamethoxazole residues in soils were recorded in levels of up to 2.6  $\mu\text{g}/\text{kg}$  in Spain. A study assessing the levels of sulfamethoxazole found levels of up to 12.7  $\mu\text{g}/\text{kg}$  in poultry manure within poultry rearing farms in Ibadan, Nigeria (Ajibola *et al.*,2022). A similar study in Tanzania assessing antibiotics in animal manure found sulfonamide levels of 415.87  $\mu\text{g}/\text{kg}$  and 22  $\mu\text{g}/\text{kg}$  in cattle and poultry manure respectively (Mohammeda *et al.*,2017). In Kenya, a study assessing sulfamethoxazole in soils found levels of up to 14.47  $\mu\text{g}/\text{kg}$  (Yang *et al.*,2016). Results from soils analyzed recorded lower levels of sulfadiazine than in livestock manure.

High levels of sulfamethoxazole antibiotics in soils can be linked to increased retention in manure amended soils, this is attributed to the slow rate of degradation of sulfamethoxazole residues. The retention of sulfonamide compounds in soils is largely dependent on the physiochemical properties of soils as well as speciation and solubility of sulfonamide antibiotics (Liu *et al.*,2018). Soil properties have the potential of affecting the presence of antibiotics in soils by influencing the rates of degradation.

A study assessing the levels of sulfamethoxazole residues in surface water in China recorded concentrations of 22 l/kg in Wangyang River. Sulfamethoxazole has also been recorded in surface water in Kenya at concentrations of up to 6.8  $\mu\text{g L}^{-1}$  and 1000  $\mu\text{g L}^{-1}$  (K'oreje *et al.*,2018; Ngigi *et al.*,2019). Antibiotic residues have been found to contribute to the occurrence of antibiotic-resistant genes in water. Sulfonamide resistant genes Sul 1 and Sul 2 were found in Tianjin Haihe River water with detection rates of 100% (Luo *et al.*,2010).

#### **4.7.5 Concentration of antibiotics in manure from biogas slurry**

Livestock waste in farms can be used for energy production and as soil amendments in farms contributing to sustainability and agriculture circular economy at the farm level. Anaerobic digestion is one of the main processes to treat waste in livestock rearing farms. Livestock

manure used in biogas systems before being introduced into the farm as manure, recorded antibiotic residues ranging from 64.4 to 6439 µg/kg for various antibiotic. These levels were lower compared to the levels recorded from most compost manure samples in this study. (Table 4.4) presents antibiotic levels identified in livestock manure from biogas slurry.

**Table 4.4: Concentration of antibiotic residues in livestock waste from biogas slurry**

Group of Antibiotic	Compound	Quantity(µg/kg)
Tetracycline	Oxytetracycline	nd
	Tetracycline	6439
Sulfonamide	Sulfadiazine	339-375
	Sulfamethoxazole	64.4-1212

nd- not detected

Livestock wastes are a major source of pollution, they contain significant levels of organic compounds, nitrogen, phosphorus, and pathogens (Zhang *et al.*,2022).The uptake of manure treatment processes such as anaerobic digestion has increasingly been on the rise due to their ability to reduce organic matter pollution and pathogens and biogas production (Zubair *et al.*, 2023). This is an important aspect in managing waste and encouraging sustainability at the farm level. Antibiotic residues in biogas slurry were lower compared to manure from compost, implying that biogas units were more effective in degrading antibiotic residues in livestock manure in this study. Low levels of antibiotics recorded in biogas slurry can be attributed to digester conditions at the time of preparation or levels of antibiotics in livestock waste. Oxytetracycline residues were not detected in biogas slurry, these results were similar to a study assessing degradation of antibiotics in biogas units (Akyol *et al.*,2016).

Results in this study were similar to a study assessing the levels of sulfamethoxazole antibiotics in chicken manure that found anaerobic digestion units efficient in the elimination of sulfonamide residues in animal manure (Feng *et al.*,2023). In a similar study, oxytetracycline removal efficiency was found to be at 60% after 64 days yielding a half-life of 56 days in anaerobic digestion (Arikan *et al.*,2006; Arikan *et al.*,2008). Anaerobic digestion is considered effective in the removal of antibiotics although its potential to eliminate or remove antibiotics is linked to the concentration, class of antibiotics, time, temperature and reactor type (Masse *et al.*,2014).

Anaerobic digestion and composting are both efficient in the removal of antibiotics and antibiotic-resistant genes, however there are still important knowledge gaps on their operating efficiency and contribution to the development of antimicrobial resistance in the environment. High levels of antibiotics in animal manure have the potential to affect anaerobic digestion by suppressing or killing microbial communities in digesters, therefore, reducing their productivity (Koniuszewska *et al.*,2021;Wang *et al.*,2022).There is a need to assess effectiveness of biogas units in veterinary antibiotic removal in animal manure as their efficiency is mainly linked to a variety of conditions that vary within digesters.

#### 4.7.6 Concentration of antibiotics in poultry manure fed to cattle manure

Poultry manure has traditionally been used predominantly as fertilizer but over the years it has increasingly been used as an alternative cost saving feed supplement for ruminants and fish. The presence of urea in poultry manure improves the rumen environment making feed more efficiently utilized and better nourishment for cattle (Waziri & Kaltungo,2017). It is primarily obtained from laying hens as well as broilers and is mainly used as a source of supplemental protein (Muduli *et al.*,2019). Increased use of antibiotics in poultry rearing has made poultry manure unsafe due to the presence of antibiotic residues. In this study a number of poultry farms assessed used poultry waste as feeds in rearing their cattle. Poultry waste was collected, dried sieved into fine particles and used as feeds within farms. Poultry waste prepared as livestock feed in this study recorded antibiotic levels ranging from 3316-4955 µg/kg of antibiotic residues.

Samples analyzed did not show presence of oxytetracycline and tetracycline antibiotics residues, that could be attributed to complete degradation of antibiotics during feed preparation or very low levels of antibiotics. However, levels of sulfadiazine and sulfamethoxazole residues were recorded in the study. Table (4.5) presents antibiotic levels identified in poultry manure used as feeds in this study.

**Table 4.5: Concentration of antibiotic residues in poultry waste re-used as feeds.**

Group of Antibiotic	Compound	Quantity(µg/kg)
Tetracycline	Oxytetracycline	nd
	Tetracycline	nd
Sulfonamide	Sulfadiazine	3316
	Sulfamethoxazole	4955

nd- not detected

Studies have found a significant number of feed manufacturers add antibiotics in livestock feed to prevent diseases and improve yields (Erofeeva *et al.*,2021; Landers *et al.*,2012). The use of antibiotics in livestock rearing is linked to weight gain, by increasing nutrients within their diet feeding periods and feeds are reduced (Landers *et al.*,2012). However, prolonged antibiotic exposure to livestock contributes to the development of antibiotic resistance in livestock and the presence of antibiotic residues in livestock products.

Samples from poultry droppings reused as feeds in the study recorded levels of sulfadiazine and sulfamethoxazole antibiotics. This meant that cattle consuming poultry droppings as feeds in this study could be taking in antibiotics, that are absorbed by livestock and excreted through animal waste that end up farms as animal manure. Exposure to high antibiotic doses in livestock feeds is likely to contribute to the development and spread of resistant strains that have the potential of affecting both human and animal health (Ma *et al.*,2021). Resistant bacteria in livestock have the potential of directly or indirectly reaching humans through water, food and manure. Poultry waste used in farms have been found to have antibiotic residues. However, there are limited studies assessing the levels of antibiotic residues in poultry droppings re-used as feeds in farms.

#### **4.7.7 Comparisons of antibiotics residues against maximum residual limits**

According to Zhi *et al.* (2019), antibiotics in soils have the potential to pose potential risks to soil ecosystem health. Manure amended soils assessed in this study recorded antibiotic residues above the recommended levels of 100 µg/kg in soils (European Commission,2010). Antibiotics have the potential to alter soil microbial activity, increase percentage of resistant bacteria and abundance of antibiotic resistant genes and community composition (Xie *et al.*,2017). Globally, there is a lack of strict regulations linked to monitoring antibiotic concentration in soils, underground waters and wastewaters. Lack of specific regulations is largely attributed to a lack of consensus on safe environmental concentration of antibiotic residues in terms of development of resistance and (European Environmental Agency,2010;European Commission, 2011).

**Table 4.6: Antibiotic residues (Mean±SE) in livestock manure and manure amended soils**

<b>Antibiotic</b>	<b>Manure (mean±SE) µg/kg</b>	<b>Manure amended soils (mean±SE) µg/kg</b>
Tetracycline	1843.81±810.13	2668.88±1161.03
Oxytetracycline	1977.82±726.07	1338.80±600.37
Sulfadiazine	495.23±213.16	172.82±91.02
Sulfamethoxazole	1102.23±325.79	1739.39±583.48

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**SE-Standard Error**

According to data obtained in the study within the tetracycline group of antibiotics, there was no significant difference in the concentration of tetracycline antibiotics in livestock manure and manure amended soils ( $T=0.58;P=0.56$ ). In, Oxytetracycline residues, there was no significant difference in the concentration of antibiotics in livestock manure and manure amended soils ( $T=0.68;P=0.50$ ). Within the sulfonamide group of antibiotics, there was no significant difference in the concentration of sulfadiazine antibiotics in livestock manure and manure amended soils ( $T=1.39;P=0.17$ ). There was no significant difference in sulfamethoxazole residues in livestock manure and manure amended soils ( $T=0.95;P=0.34$ ).

This could be attributed to the slow degradation rates of antibiotic residues in livestock manure and the possibility of antibiotic accumulation in manure amended soils within farms. Slow degradation rates in livestock manure in the study is linked to the use of poor manure management methods and continuous heaping of manure piles that does not guarantee sufficient degradation of antibiotic residues. Accumulation of manure residues in soils in the study can be a result of slow degradation of antibiotics and continuous application of antibiotic contaminated livestock manure that has the potential of introducing antibiotic residues in soils.

Regulations have been developed to monitor antibiotic residues in livestock food products. However, regulations monitoring the presence of antibiotics in the environment are limited. There are no regulations on the levels of antibiotics in manure and soils in Kenya. The maximum amount of veterinary drug residues in livestock food products by EU regulation EG 37/2010 is set at 100-600 µg/kg (European Medicines Agency,2016;Hellmann & Radelof,2000).The EU directive CVMP/VICH/592/98-FINAL from 2000 (Committee for Medicinal Products for Veterinary Use 2016) proposed the predicted environmental

concentration (PEC) of veterinary medicinal products to be less than 100 µg /kg in soil, and less than 1 µg/L in groundwater (European Commission, 2010). 50% of the samples analyzed in the study had antibiotic residue levels that were higher than the recommended concentrations in soils.

These results were similar to other studies that found levels of antibiotic residues to be higher than the recommended limits (Conde-Cid *et al.*,2018; Hu *et al.*,2010; Sun *et al.*,2017). There are no residue limits on the levels of antibiotic residues in organic manure, hence once applied on farms, they have the potential to cause pollution in soils, ground water and river water (Karcı & Balcıoğlu,2009). Livestock manure is considered a cheaper alternative to fertilizer and a mode of waste management within farms. It has the potential to contribute to the presence of antibiotic residues and development of antibiotic resistance genes in soils if not well maintained. Composting and anaerobic digestion can reduce the levels of antibiotic residues in animal manure, hence the need to adopt these practices in minimizing the levels of antibiotics introduced into farms.

#### **4.8 Risk Assessment of antibiotic residues in soils**

Application of livestock manure containing antibiotic residues has the potential to cause ecological risks. High levels of antibiotic residues were recorded in manure amended soils sampled in this study. Assessment of ecological risks of antibiotics in soils in this study was done by the use of Risk Quotient values. Predicted environmental concentration in soils (VICH,2000).

$$\text{Equation 1} \quad PECs = CA \times M / (P \times 10 \times D)$$

Where CA is the concentration of the tested antibiotic (µg/kg<sup>-1</sup>), M is the amount of animal manure applied annually with the fresh weight set to 100 (t ha<sup>-1</sup>) according to Zhang *et al.* (2015); P is an agricultural soil density of 1300 kg/m<sup>3</sup> based on a previous study (Nguyen *et al.*,2018); D is the 0.2 m soil depth to which antibiotics may penetrate after manure application; and, 10 is a conversion coefficient. The water contents of manure were 44.54% for cow manure, 27.77% for chicken manure (Gao *et al.*,2019). The predicted no effect concentration was calculated using the ratio where TOX (µg/kg) is the toxicity of antibiotics to soil microorganisms that can be substituted with the lowest median effective ED<sub>50</sub>. ED<sub>50</sub> values for veterinary antibiotics in soils was sourced from literature. The assessment factor AF set as 1000 for acute and 100 for chronic toxicity (Li *et al.*,2013). The value of 1000 was used as the ED<sub>50</sub> values in this study were derived from acute toxicity tests.

$$\text{Equation 2} \quad PNEC = \frac{TOX}{AF}$$

Risk quotient was calculated through the predicted environmental concentration (PEC) divided by the predicted no effect concentration (PNEC).

$$\text{Equation 3} \quad RQ = \frac{PEC}{PNEC}$$

PNEC values for tetracycline, oxytetracycline, sulfadiazine and sulfamethoxazole in the study were derived from available literature. There are limited studies on the direct toxicity of antibiotics on soils that has made it had to estimate PNEC values in soils. The PNEC values for antibiotics in this study were 2735, 5000, 0.92 and 300 µg/kg-1 borrowed from various similar studies (Ghirardini *et al.*,2020; Kumar *et al.*,2005; Liu *et al.*,2009; Yang *et al.*,2016). Risk quotient values were applied to assess potential ecological risks linked to the agricultural application of livestock manure to soils. Table 4.7 presents risk quotient values for cattle manure calculated based on the above equations. The occurrence of veterinary antibiotics recorded in the study (µg/kg), Potential no effect concentration values (PNEC) and risk quotient values calculated for both poultry and cattle (RQ).

**Table 4.7: Risk quotient for cattle manure**

<b>Veterinary Antibiotic</b>	<b>Occurrence (%) Cattle(n=15)</b>	<b>Concentration(µg/kg) Cattle (n=15)</b>	<b>PEC</b>	<b>PNEC</b>	<b>Risk Quotient</b>	<b>Remarks on Risk quotient values</b>
TC	40	2412.67	91.66	2735	0.034	low
OTC	27	1405.4	53.40	5000	0.011	low
SDZ	40	43.92	1.67	0.92	1.815	High
SMX	53	1345.56	51.13	300	0.17	Medium

PEC- Predicted environmental concentration: PNEC- Predicted no effect concentration.

TC,OTC,SDZ, SMX represent Tetracycline, Oxytetracycline, Sulfadiazine, Sulfamethoxazole.

Risk quotient > 1-high; 0.1 < RQ < 1-medium; <0.1-low.

The risk quotient value obtained for sulfadiazine was greater than 1.0 presenting a higher ecological risk in soils, Sulfamethoxazole in soils presented medium risk. However, risk quotient values obtained for tetracycline and oxytetracycline antibiotic were low. This indicated a higher ecological risk for sulfadiazine antibiotic in soils amended using cattle manure. Antibiotics with a high ecological risk have the potential of contributing to the development of antimicrobial resistance genes in soils that is likely attributed to constant application of manure with antibiotic residues (Heuer *et al.*,2008). Table 4.8 presents risk quotient values for poultry manure.

**Table 4.8: Risk quotient for poultry manure**

<b>Veterinary Antibiotic</b>	<b>Occurrence (%) Poultry (n=13)</b>	<b>Concentration(<math>\mu\text{g}/\text{kg}</math>) Poultry (n=13)</b>	<b>PEC</b>	<b>PNEC</b>	<b>Risk Quotient</b>	<b>Remarks on Risk quotient values</b>
TC	23	2964.46	112.63	2735	0.041	low
OTC	31	1261.96	47.92	5000	0.009	low
SDZ	46	321.56	12.22	0.92	13.283	High
SMX	46	2193.81	83.33	300	0.277	Medium

PEC- Predicted environmental concentration: PNEC- Predicted no effect concentration.

TC,OTC,SDZ, SMX represent Tetracycline, Oxytetracycline, Sulfadiazine, Sulfamethoxazole.

RQ Risk quotient  $> 1$ -high;  $0.1 < \text{RQ} < 1$ -medium;  $<0.1$ -low.

The risk quotient value obtained for sulfadiazine in soils amended using poultry manure was greater than 1.0 presenting a higher ecological risk. Sulfamethoxazole antibiotic in soils presented medium risk, the risk quotient presented by tetracycline and oxytetracycline antibiotics was low. This indicated a high and medium ecological risk for sulfadiazine and sulfamethoxazole antibiotics respectively in soils amended using poultry manure in this study. Analysis of risk levels presented by antibiotics in farm soil, RQ values were classified into three levels of risk that include;  $< 0.1$ , low risk;  $0.1-1$ , medium; and  $>1$  high risk (European Commission,2003; Verlicchi *et al.*,2012). Most of the antibiotics assessed presented low and medium ecological risk in soils, attributed to low predicted environmental concentrations (PEC)values recorded in this study Majority of PEC values of antibiotics in soil in this study were lower than the antibiotic trigger value of  $100 \mu\text{g}/\text{kg}$  in soils, that has been reported to have effects in ecotoxicity studies conducted on earthworms, microbes and plants (VICH,2000).

The highest value recorded in the risk quotients was above the recommended ( $>1$ ). Application of cattle and poultry manure containing antibiotics may pose some ecological risk. The predicted environmental concentration of tetracycline antibiotic was above the trigger value of  $100 \mu\text{g}/\text{kg}$ , reported to have effects in ecotoxicity studies conducted on earthworms, microbes and plants (VICH,2000). Higher risk quotient values obtained for sulfadiazine antibiotic in this study were linked to high levels of predicted environmental concentration and low predicted no effect concentration. It can also be attributed to continuous application of animal manure

with high sulfadiazine levels or slow degradation in soils. Predicted environmental concentration of antibiotics in the environment can reduce, resulting from biodegradation, photolysis and hydrolysis of the antibiotic in the environment (European Medicines Agency,2016; Koschorreck *et al.*,2002; VICH, 2006).

Application of animal manure in soils can contribute to increased antibiotic levels in the environment, highlighting the risk associated with the use of antibiotic contaminated manure in soils. However, risk quotient values of most antibiotics assessed in manure amended soils in this study presented low and medium risk, indicating a low ecological risk largely attributed to the low PEC values in the study. Increased accumulation of antibiotics into the soil environment has the potential to cause risks on the soil ecosystem (Li *et al.*,2023).

Antibiotics in soils used for food production have the potential of ending up in the human body through the food chain causing potential risk to public health. (Zhao *et al.*,2019). Their presence in soils have the ability of affecting soil microbial composition, alter carbon and nitrogen cycling, modifying earthworm population and reducing crop yields (Grenni *et al.*,2018; Wepking *et al.*,2019). High risk level of sulfadiazine antibiotic recorded in soils has the potential to contribute to antibiotic resistance development in the environment when manure is used for fertilization in agricultural fields.

Veterinary antibiotics have been detected regularly in livestock manure and reached concentrations in soil well above 100 µg/kg (Menz *et al.*,2019). Similar studies found sulfadiazine antibiotic presented a high ecological risk recorded in soils (Li *et al.*,2013; Zhang *et al.*,2015). Sulfadiazine antibiotic presented a higher risk quotient value compared to other antibiotics in the study. Majority of the farms assessed in the study applied manure more than twice in a year, continuous application of antibiotic contaminated manure has the potential of increasing risk presented by antibiotics in soils. Tetracycline and oxytetracycline antibiotics in this study presented lower risk quotients, while sulfadiazine and sulfamethoxazole antibiotics had high and medium risk quotients respectively.

Results were similar to a study in Kenya that analyzed risk presented by antibiotics in soils (Yang *et al.*,2016). Sulfadiazine antibiotic residue in soils had a risk quotient >1, similar to a study in China assessing ecological risk of veterinary antibiotics in vegetable farming (Zhang *et al.*,2014). This meant they posed a high ecological risk to soil ecosystem health. Veterinary

antibiotics can adversely affect micro-organisms by causing mortality or inhibiting their growth (Grenni *et al.*,2018).

Antibiotics residues in soils therefore have the potential of altering the structure and abundance of the soil microbial community (Cycon *et al.*,2019). This is linked to their relative nature mainly those with a broad-spectrum. They have a selective effect on several microorganism and the potential to cause alteration in the relative abundance of microbial species affecting different species interaction in soils (Kuppusamy *et al.*,2018). The presence of antibiotics in the environment has the potential to contribute to emergence and spread of antibiotic resistance bacteria in the environment.

## CHAPTER FIVE

### SUMMARY OF FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

#### 5.1 Summary of findings

Commonly used class of antibiotics among poultry farmers were tetracyclines 35%, sulfonamide 40%, quinolones 20% and others 5%. Among cattle rearing farmers, the commonly used veterinary drugs were: tetracyclines 60%, sulfonamide 8%, penicillin's 20%, and macrolides 12%.

Sixty-five percent of livestock rearing farmers trusted veterinary doctors to treat their livestock while 27% and 8% preferred getting prescriptions from agrovets and treating their livestock on their own, respectively. Most of the households assessed 62% took 0-3months, 24% took 3-6 months and 24% took 6-9 months to prepare manure before they got applied into the farms. Majority of the farms applied livestock manure more than twice on their farms. A majority of the farms 92% utilized livestock waste as manure on their farms, while 7% used poultry and cattle waste in biogas before utilizing them as farm manure. 1% of farms re-used poultry waste as feeds in cattle rearing.

Most of the manure and manure amended soils in the study recorded both tetracycline and sulphonamide antibiotic residues. Some of the soil samples in the study had antibiotic residues despite being amended by livestock manure with no antibiotic residues.

Risk analysis in this the study area showed sulfadiazine antibiotics had a higher risk quotient in soils.

#### 5.2 Conclusions

Results from this study confirm that manure from livestock contributed significantly to the introduction of antibiotic residues into the environment. In conclusion,

- i. Most commonly consumed class of drugs in this study among both cattle and poultry farmers was tetracyclines, sulfonamides, quinolones and penicillin.
- ii. Most (65%) farmers in the study relied on veterinary doctors to treat their livestock; Majority (75%) and (55%) of the farmers had low levels of awareness on the presence of antibiotics in livestock manure and soils respectively. Livestock waste from farms in

the study were mainly used as manure (92%), slurry for biogas (7%) and re-used as feeds (1%).

- iii. Antibiotic residues recorded levels ranging from 60-16,240 $\mu\text{g}/\text{kg}$  for oxytetracycline, 1316-15,231 $\mu\text{g}/\text{kg}$  for tetracycline, 8.9-3,971 $\mu\text{g}/\text{kg}$  for sulfadiazine and 40.4-4955 $\mu\text{g}/\text{kg}$  for sulfamethoxazole in manure, while manure amended soils recorded levels ranging from 1653-14,237 $\mu\text{g}/\text{kg}$  for oxytetracycline, 1532-29,396 $\mu\text{g}/\text{kg}$  for tetracycline, 11.74-1,847 $\mu\text{g}/\text{kg}$  for sulfadiazine and 34.5-10,556 $\mu\text{g}/\text{kg}$  for sulfamethoxazole. Tetracycline class of antibiotics had higher residues compared to sulfonamides due to their persistence in the environment. Biogas units were efficient in reducing antibiotic levels in manure slurry with levels ranging from 64- 6439  $\mu\text{g}/\text{kg}$ . Biogas and composting were efficient in reducing antibiotic residues in manure. However, these values were above the recommended value of 100 $\mu\text{g}/\text{kg}$  in soils.
- iv. Risk quotient values for most antibiotics in soils in this study were within the recommended limit of  $\leq 1$ , except for sulfadiazine antibiotic. Risk quotient values obtained for tetracycline, oxytetracycline, sulfadiazine and sulfamethoxazole in cattle manure amended soils were 0.034, 0.011, 1.815 and 0.17 respectively. Risk quotient values in poultry manure amended soils for tetracycline, oxytetracycline, sulfadiazine and sulfamethoxazole were 0.041, 0.009, 13.283 and 0.277 respectively.

### 5.3 Recommendations

Based on the findings from the study, the researcher recommends that;

- i. Encourage proper and prudent veterinary antibiotic use in livestock rearing.
- ii. There is need for awareness campaigns on proper antibiotics use and manure management among farmers that will lead to improved veterinary antibiotic use within farms and reduced risk to human and soil ecosystem.
- iii. Composting and anaerobic system can be encouraged in the preparation of animal manure since they have the potential to reduce or eliminate antibiotic residues. Since, there are no existing documented standards on antibiotic residues in the environment, concerned authorities such as Ministry of Agriculture, livestock and fisheries and NEMA, should formulate and enforce the standards.
- iv. Animal manure on farms should undergo proper preparation before being utilized as soil amendments in order to enhance breakdown of the antibiotics in the manure and prevent their exposure to soils.

#### **5.4 Recommendation for further research**

- i. Effects of soil properties on the presence of antibiotic residues in soils.
- ii. Assess efficiency of anaerobic digestion and composting in the removal of antibiotic residues in livestock manure.
- iii. Assess antibiotic residues in poultry droppings reused as cattle feeds.

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## APPENDICES

### Appendix I: Questionnaire for animal rearing farmers

My name is Maira Joseph. I am a student at Egerton University taking a Master's Degree in Environmental and Occupational Health. I am carrying out research entitled **Determination of selected veterinary antibiotics in animal manure and manure amended soils**. This questionnaire is for the purpose of collecting data and information to aid in the research that I am conducting. I am kindly requesting for your time in filling the responses to the best of your knowledge. Responses for this research is only meant for academic purpose and will be treated as confidential.

Signature.....

### BASIC INFORMATION

Farm

ID.....

Area.....

....

### Section A: Background Information

Q 1. Respondent's gender:  Male  Female

Q 2. Respondent's age:

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Q 3. Level of education:  None  Primary  Secondary

Tertiary

### Section B: Farm Data

Q 1. Type of animal reared

[1] Cattle.

[2] Poultry.

Q 2. Number of animal reared

[1] 0-10.

[2] 10-20.

[3] 20-above.

**Q 3. Veterinary drugs used**

<b>Animal Reared</b>	<b>Drug used</b>	<b>Mode of administration</b>	<b>Purpose</b>	<b>Illness</b>	<b>Quantity</b>

**Q 4. Who treats your animals when they get sick?**

[1] Veterinary doctor [2] Prescriptions from agrovet. [3] Self-knowledge

**Q 5. Type of animal manure used?**

Cattle Manure ( ) Poultry Manure ( ) Both ( )

**Q 6. How do you utilize animal waste generated from the farm?**

Compost ( )

Re-used as feed ( )

Biogas ( )

Other methods.....

**Q 7. How long do you take to prepare your manure?**

[1] 0-3 months [2] 3-6 months [3] 6-9 months

**Q 8. How long have used animal manure on your farm?**

[1]1-10 years

[2]10-20 years

[3] 20-30 years

**Q 9.** How frequent do you use animal manure on your farm in a year?

[1] Once

[2] Twice

[3] More than twice

**Q 10.** Are you aware medicine used in animal can be found in manure?

Yes ( ) No ( )

If yes, what measures have you put in place to ensure animal manure that is used does not have drug residues.....

**Q 11.** Animal manure use

<b>Type of Manure used</b>	<b>Animal manure use amount and frequency</b>	
	Frequency of use per year/month	
	Amount used in (kg)	
	Amount of Land applied to	
<b>Type of Manure Used</b>	<b>Animal manure use amount and frequency</b>	
	Frequency of use per year/month	
	Amount used <ul style="list-style-type: none"> <li>● Number of wheelbarrows</li> <li>● Number of Bags</li> </ul>	
	Amount of Land applied to	

**Q 12. Level of Awareness**






Select the most appropriate response choice for the following statements/questions (1. Strongly disagree-SD, 2.Disagree-D, 3.Undecided-4.UN, Agree-A, 5.Strongly agree-SA)

	<b>SD</b>	<b>D</b>	<b>UD</b>	<b>A</b>	<b>SA</b>
<b>Antibiotics are present in animal manure</b>					
<b>Antibiotics are present in soils</b>					

## Appendix II; Farm Distribution

Farm	Elevation	E	N
F1P1	1836.83	828587.767	9982514.965
F2C1	1830.83	828366.081	9982526.842
F3P2	1842.83	830321.836	9983612.052
F8C2	1767.53	827598.099	9985964.012
F4P3	1846.73	829833.386	9983247.788
f6P6	1887.61	831665.771	9981980.463
F7P7	1888.08	831342.073	9982234.857
F9C7	1992.27	169003.262	9971694.083
F10PA	1988.67	168919.709	9971620.812
F10PB	1988.67	169014.847	9971514.409
F11c6	1928.77	169756.378	9970055.541
F1aP1	1941.87	169168.037	9970788.724
F2AP2	1922.87	168440.425	9970122.904
F3AC1	1861.83	817921.318	9981936.339
F4AC2	1871.83	818017.681	9982120.956
F5AC3	1987.87	166263.297	9970051.936
F6AC4	2002.87	166086.929	9970037.791
F7AP3	2104.93	830057.796	9970451.006
F8AC5	2087.93	829716.102	9971794.941
F9AC6	2093.93	829436.496	9971538.518
F10AP4	2109.93	829955.922	9970765.011
F11AC7	2053.83	831150.021	9973848.691
F12AC8	2093.93	831476.025	9972234.341
F13AC9	2090.56	828030.093	9972054.818
FBP2	2190.36	825829.964	9978480.567
F4BP1	1836.83	828587.767	9982514.965
F1BC1	1830.83	828366.081	9982526.842
F2BC2	1842.83	830321.836	9983612.052

**Appendix III; Research permit**

 <b>REPUBLIC OF KENYA</b>	 <b>NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY &amp; INNOVATION</b>
Ref No: <b>704838</b>	Date of Issue: <b>20/September/2022</b>
<b>RESEARCH LICENSE</b>	
	
<b>This is to Certify that Mr.. Maira Joseph Walter of Egerton University, has been licensed to conduct research in Nakuru on the topic: RISK ASSESSMENT OF SOIL EXPOSURE TO ANTIBIOTIC RESIDUES IN ANIMAL MANURE IN LIVESTOCK REARING FARMS OF RONGAI SUB-COUNTY, NAKURU COUNTY for the period ending : 20/September/2023.</b>	
License No: <b>NACOSTI/P/22/20346</b>	
<b>704838</b>	
Applicant Identification Number	Director General <b>NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY &amp; INNOVATION</b>
Verification QR Code:	
	
<b>NOTE: This is a computer-generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.</b>	

**Appendix IV; Research authorization for Rongai Sub-County**



**OFFICE OF THE PRESIDENT  
Ministry of Interior and Coordination of  
National Government**

Email: [ccnakurucounty@yahoo.com](mailto:ccnakurucounty@yahoo.com)  
[ccnakurucounty@gmail.com](mailto:ccnakurucounty@gmail.com)

COUNTY COMMISSIONER  
NAKURU COUNTY  
P.O. BOX 81  
NAKURU

When replying please quote:

Ref. No. CC. SR.EDU 12/1/2/VOL.VI1/15


29<sup>th</sup> September, 2022

Deputy County Commissioner  
RONGAI SUB-COUNTY

**RE: - RESEARCH AUTHORIZATION – MR. MAIRA JOSEPH WALTER**

This is to confirm that the above named from Egerton University, has been authorized to carry out research on: **RISK ASSESSMENT OF SOIL EXPOSURE TO ANTIBIOTIC RESIDUES IN ANIMAL MANURE IN LIVESTOCK REARING FARMS OF RONGAI SUB-COUNTY**, in Nakuru County for a period ending **20<sup>th</sup> September, 2023**.

Please accord him all the necessary support to facilitate the success of his research.

  
**COUNTY COMMISSIONER  
NAKURU COUNTY  
P.O. Box 81  
NAKURU**  
**MWANGI NYAGA  
FOR: COUNTY COMMISSIONER  
NAKURU COUNTY**

**Appendix v; Pictures from the farms.**

**SITE VISITS**

**A)**



**B)**



**A)** Picture taken with farm workers in Ngata-Opposite Boenix high school taken on 3/10/2022

**B)** Picture taken with poultry rearing farmer in Mangu taken on 17/05/2022.

**LIVESTOCK REARING UNITS**

**C)**



**D)**



**C)** Picture taken of livestock rearing unit in Verovian-Ngata taken on 23/06/2022

**D)** Picture taken of poultry rearing cages in Roret-Ngata taken on 5/10/2022

## BIOGAS UNITS AND SLURRY MANAGEMENT

**E**



**F**



**G**



**H**



**E)** Picture of biogas inlet in a farm in Ngata-Oasis area taken on 8/2/2023

**F)** Picture of manure management in farm within Ngata-Oasis area taken on 8/2/2023

**G)** Picture of biogas tanks in a farm in Roret- Ngata taken on 5/10/2022

**H)** Picture of biogas slurry managed in a farm in Roret- Ngata taken on 5/10/2022

**I)**



**J)**



**I)** Picture of site visit with supervisors on a farm in Ngata taken on 5/10/2022

**J)** Picture of manure slurry managed on a farm in Rongai taken on 5/10/2022

### **COMPOST HEAPS IN FARMS**

**K)**



**L)**



**M)**



**K)** Picture of livestock waste heaped in a farm in Verovian taken on 03/10/2022

**L)** Picture of livestock waste heaped in a farm in Soilo taken on 26/09/2022

**M)** Picture of animal waste heaped in a farm in Kabarak area taken on 13/05/2022

# APPENDIX VI; Assessment of veterinary antibiotic use and occurrence of veterinary antibiotics in livestock manure from farms in Rongai Sub-County, Kenya



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## Assessment of Veterinary Antibiotic use and Occurrence of Veterinary Antibiotics in Livestock Manure from Farms in Rongai Sub-County, Kenya

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### Abstract

Veterinary antibiotics are commonly used in livestock rearing to prevent diseases and stimulate growth. The release of antibiotics into the environment has become a significant environmental and public health concern. This research evaluated antibiotic use, livestock treatment, manure utilization, livestock waste treatment methods and antibiotic residues in livestock manure. Questionnaires were administered to 170 farmers rearing both cattle and poultry. Subsequently, 28 livestock manure samples from 15 cattle and 13 poultry rearing farms were collected from various farms to assess concentrations of tetracyclines (Tetracycline, Oxytetracycline) and sulfonamides (Sulfadiazine, Sulfamethoxazole) residues. Residues analysis was done using High Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD). Veterinarians were the most preferred in treating both cattle and poultry in farms. Tetracyclines and sulfonamides were the most consumed class of antibiotics among both poultry and cattle rearing farmers. Compost manure and Biogas were the most preferred use of animal waste within farms. Antibiotic presence in samples was detected in 80% and 93% of cattle and poultry manure respectively. Maximum antibiotic concentrations of 16.24 and 15.18 (mg/kg) were recorded in poultry and cattle manure, respectively. There was a statistically significant difference in antibiotic concentrations in poultry and cattle manure ( $P < 0.05$ ). The results of this research are important in monitoring rising concerns about veterinary antibiotics on environmental and public health.



### Article History

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Antibiotics;  
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Tetracyclines.