Effect of dairy farming practices on intake of antibiotic residues in milk consumed in Kiambu County, Kenya

BY

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Dedication

I dedicate this work to my dear wife, Carol Nyambura for her support and prayers during my study. For my son Jesse Kimani and my daughter Tamara Wanjiru for the time I spent without giving my attention during the study.

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Abbreviations and acronyms

ATMs	Automated machines
AVMA	American Veterinary Medical Association
CAC	Codex Alimentarius Commission
EU	European Union
FDA	Food and Drug Administration
GDP	Gross Domestic Product
HPLC	High-performance liquid chromatography
IARC	International Agency Research of Cancer
ILRI	International Livestock Research Institute
KCC	Kenya Creameries Co-Operation
KDB	Kenya Dairy Board
MRLs	Maximum Residue Limits
RDCoDE	Regional Dairy Centre of Excellence
SPSS	Statistical Package for Social Science
USAID	United States Agency for International Development
OIE	Organization of Animal Health
PCPs	Progressive Control Pathways

DEFINITION OF TERMS

- **Food safety-** practices and conditions that preserves the quality of food against contamination and foodborne illness.

- **Antibiotics-** are medication that slow down or destroy the growth of bacteria.

- **Antibiotic residue- are** metabolites found in trace amounts in any portion of the animal product after administration of the antibiotics.

- **Traceability** – ability to track any products through all stages of production.

- **Acceptable daily intakes (ADI)-** is a measure of a specific substance e.g. veterinary drug residue in food or drinking water that can be ingested on daily basis over a life time without any health risk.

- **Dairy practices-** ways through which dairy farming activities are coordinated to maximize production.

- **Maximum residue limits (MRLs)** – is a maximum concentration of veterinary drug residue mg/kg likely to occur in food as result of the use of vet drugs in animal treatment**.**

- **Quality control-** is a system through which standards are maintained against outlined specifications.

General abstract

The dairy sector in Kenya contributes to the daily diet of the population despite its being subsistence in nature. Quality control and assurance have been the most significant hurdle due to the loose of regulations enforcement. Therefore, the sector poses significant food safety risks to consumers. The fact that milk contributes significantly to the diet, especially for children, makes its safety paramount hence the need for quality checks at the farm level.

The current study evaluated dairy practices that compromise milk quality and safety along the dairy supply chain. The main risk factor being antibiotics residue in raw milk consumed in Kiambu County. A cross-sectional survey was conducted using a structured questionnaire to determine the suitability of milk equipment, storage of milk, health management and animal treatment at farms, the level of knowledge of risks associated with antibiotics residue in milk, and farm management. Raw milk samples were also collected and analyzed to determine the levels of antibiotic residues.

The results showed that small scale farmers keep 2 to 3 cows, which accounted for 98% of the respondents. The record management at the farm level was done by less than 40% of the respondents. Hygiene and poor storage highly contributed to milk rejection, as reported by 97% of the respondents. The buyers lacked testing gargets for determining contaminants such as antibiotics and preservatives. According to the present study, brokers who accounted for 14% of the respondents play a significant role in milk vending in the sub-urban centers in Thika town, Ruiru town, and Nairobi. The use of health records was not a priority among the farmers, coupled with irregular withdrawal periods ranging from 48 hours to 72 hours.

Additionally, the present study found the presence of antibiotics in raw milk. Among the samples, 10 % tested beta-lactam positive on screening through the rapid test; namely: Gatei 11.7%, Gatundu 6%, and Kiganjo 12.1%. Consequently, eight derivatives were quantified and identified as follows: Ampicillin 0.007±0.0 µg/ml, Amoxicillin 0.02±0.022 µg/ml, and Penicillin G 0.016±0.017 µg/ml were above 0.004µg/ml recommended MRLs. However, Cloxacillin (0.008±0.004 µg/ml), Dicloxacillin (0.007±0.0 µg/ml), Nafcillin (0.010±0.004 µg/ml), Oxacillin (0.009±0.0.002 µg/ml), and Phenoxymethyl-penicillin (0.009±0.005 µg/ml) were below 0.030µg/ml recommended MRLs. The exposure assessment for the antibiotic residues, revealed that consumer under the study were safe, having ADI below the set standard by Codex. The levels of antibiotics residues quantified, including ampicillin, amoxicillin, and penicillin G were above the Codex standards maximum recommended levels, hence posing a food safety risk to the consumer. The study concluded that the prevalence of antibiotic residues in raw milk is 10%; this poses a high food safety risks to the milk consumers. Inappropriate dairy farming practices on food safety have heightened the situation. The creation of food safety awareness and improvement in dairy practices can mitigate the situation.

CHAPTER ONE: INTRODUCTION

1.1 Background information

The dairy sector in Kenya approximates 4.5 % of the Gross Domestic Product GDP (Kenya Dairy Master Plan 2010-2030). Agriculture accounts for 19 %, hence referred to as the backbone of Kenya's economy (National Livestock Policy, *2008*). According to the Kenya Dairy Board (*KDB*, 2016), milk production has exceeded 4.6 million tonnes a year; of this, 1.5 million are from small scale holders, producing more than 80 % of milk. Officially the total herd size is about 3.5 million heads of dairy cattle (Muriuk*i et al*., 2011). Today the actual herd is presumably bigger as reported by Kenya Dairy Board. *(KDB*, 2016) Rains patterns have changed due to global warming; seasons have changed; short and long rain seasons are at times delayed. These have brought acute shortage and oversupply hence fluctuating demand and supply, which affects the milk price. KDB has come on board with marketing tools to assist farmers though many benefits have not resulted.

In Africa, the Kenya dairy sector is one of the most developed. However, it expects to suffer a deficit of milk due to an increase in demand in Nairobi and other urban markets due to an ever-growing increase in the urban population. Dairy farming in Kenya is characterized by small scale farmers having 2-3 cows, which account for 70 % of dairy farming. According to Smallholder Dairy Research and Development Project (2010), 70 % of jobs in the dairy sector are in the informal sector. The informal sector is characterized by milk hawking, milk bars, and upcoming milk Automated machines (ATMs) (*KDB* 2013). The milk ATMs have taken a sizeable share of the market without considering the milk consumers' safety. Traceability of milk has been an issue due to the influx of unscrupulous traders. These have led to contamination of milk with additives such as water and preservatives. The milk sold in these places is unpasteurized, and in some instances, it is chilled, and at times it is not. This has led to milk preservation by the use of hydrogen peroxide; hence contamination and safety issues arise to the consumer of this product. (Mwangi *et al.*, 2000).The Kenya Dairy Board recently championed milk safety and control of milk hawking countrywide. They have championed the use of milk for a healthy nation, especially in school feeding programs.

The informal sector dominates the dairy industry. Leading in milk processing are Brookside Dairy, New KCC, Githunguri Dairy, Sameer Agriculture and Livestock Company (Daima brand), and other small scale processors. Their mode of operation is characterized by collecting, chilling, bulking, and transporting to facilities where the processing occurs. The processed products are distributed for sale in various urban centers.

Antibiotic residues in milk have been an issue of great concern; it has been a challenge not only in developed countries but also in developing countries. Developing countries lack well-coordinated safety and quality management systems.Quality assurance channels hence a potential for public health risk (Aboge *et al*., 2000). Due to modernization and development in the dairy sector, production in line with antimicrobial usage will be estimated to increase to 67 % between 2010 and 2030, hence more significant concern in terms of risk factor *(*Van Boeckel *et al*., 2015). Small farmers characterize the dairy industry in Kenya. Due to its amorphous nature, the dairy sector lacks proper coordination; hence controls are not checked. Studies have shown the presence of antibiotics along the market chain (Aboge *et al.,* 2000). The antibiotics can be found in contaminated feeds, which end up in milk. (Kangethe *et al.*, 2005). Aboge *et* al. (2000) found that antibiotic residue in milk was three times higher in rural areas than in urban areas. Kangethe *et al*. (2005) found that the level of antibiotic residues at the consumer level was higher than at the market level, 9.4 %, and 5.7 %, respectively.

In Kenya, sulfonamides, beta-lactam, aminoglycosides, and tetracycline are mostly used to treat livestock (Aboge *et al.,* 2000). Drug residue in Kenya has increased since market liberalization. In 1978 penicillin was found to be 1% of a milk sample; in the year 2000, the residue was found to be 16 % (Kangethe *et al.*, 2005). In the year 2004, Shitandi studied farm practices related to veterinary drug usage. Only 22 % of small scale farmers documented drug usage. According to Orwa *et al.* (2017), tetracycline was the most used antibiotic drug (55 % of farmers) followed by sulfonamides (21 %) and beta-lactam (6 %).

1.2 Statement of the problem

Small-scale farmers who account for 70-80 % of milk production in Kenya lack equipment and tools such as strip cups for mastitis testing at milking sites. Due to harsh economic conditions, farmers only allow a one-day withdrawal period on lactating animals, posing a higher risk to the consumers by exposing them to antibiotic residues. Farmers have little or no knowledge of associated health risks of the residues of the administered drugs. Therefore risk factor becomes a matter of importance to food safety regulatory authority (Mitema et *al*., 2001; Hou *et al*., 2014).

Additionally, population growth and reduction in land size, have led to zero-grazing practices. Intensive dairy farming has led to poor hygiene controls at the farm level, consequently the emergence of diseases at the farm. The animals are fed from commercial feeds and grass. Due to economic constraints, most small-scale farmers end up feeding animals with grains and vegetable wastes, which compromise the health status of the dairy herd. Antibiotic and other microbial agents gain access to milk through the therapeutic and prophylactic treatment of animals, feed additives, or be added directly to the milk (Cabello, 2006, Jank *et al.*, 2014). Loose dairy practices pose risks to the consumer in terms of the health hazard, which may result in bacterial resistance to medical treatment and allergic reactions due to drug residues or their metabolites. It also results in alteration and eventual destruction of gastrointestinal microflora leading to the growth of opportunistic microbes. There has been a concern of inhibition of culture hampering fermentation processes in the manufacturing industry, hence antibiotic residue in milk being a technological disadvantage to processors (Muriuki *et al*., 2011).

The dairy farming management for small scale farmers becomes more complicated due to the lack of record-keeping and follow-up in health management operations. At times, the farmers, due to the high cost of veterinary doctors, resort to self-treatment of their animals based on past treatments. Therefore they don't have proper administration of drugs and withdrawal periods are not followed to the letter (Omore *et al.*, 2005

1.3: Justification of the study

It is essential to identify the critical control points during milk production, which introduces antibiotics. This will ensure the safety and quality of milk along the supply chain. Every household in Kenya consumes milk from a farm animal, milk vendors, milk bars, or packed milk from shops and supermarkets. Milk contributes a substantial portion of the diet to Kenya's population; hence its' hygiene and safety are of more significant concern. The safety and hygiene aspects become paramount across the food chain.

Consumption of contaminated products over a long time will harm the large population across the boundaries; hence intervention is warranted through a comprehensive survey of milk safety and hygiene. The primary milk producer being a small scale holder lacks proper coordination; therefore, management becomes a challenge, and safety assurance becomes a concern, which warrants an evaluation of milk product safety. The effective practices should not just be developed but also translated into working methods suitable for practical use. The intervention on quality and safety will save the population against possible immunological disorders. If critical control points are well-identified, this can save the government a lot of revenue in disease control and associated losses.

1.4 Study aim

This study aims at assessing dairy practices that compromise the safety of milk produced and consumed in Kiambu County, Kenya.

1.5 Study purpose

The study aims to identify factors that contribute to the presence of antibiotics, which compromise the safety of milk to the ultimate consumer.

1.6 Objectives

1.6.1 Main objective

To determine dairy farming practices, prevalence and intake of antibiotic residues in raw milk produced and consumed in Kiambu County, Kenya

1.6.2 Specific objectives

i. To establish dairy farming practices that compromise milk quality and safety in Kiambu County.

ii. To determine the prevalence of antibiotic residues in raw milk sold and consumed in Kiambu County, Kenya.

iii. To evaluate the quantitative risk assessment of penicillin intake through consumption of milk in Kiambu County, Kenya.

Research questions

i. What is the relationship between dairy practices and the presence of antibiotics?

ii. What is the prevalence of antibiotic residues in milk sold and consumed in Kiambu County, Kenya?

iii. Does consumption of milk contaminated with antibiotics residue pose any risk to the consumers?

CHAPTER TWO: LITERATURE REVIEW

2.1 Kenya dairy sector and dairy farming practices

The dairy sector in Kenya is one of the largest and most sophisticated in Africa. It has an estimated herd of 3.5 million dairy animals, 9 million zebus, 12 million goats, and 900,000 camels. Cattle account for 88 % of the milk produced. In contrast, camel and goats account for the rest (KDB, 2015). With an estimated 5 billion liters of milk produced in the country per annum, the dairy industry is an essential player in the economic and nutritional aspects of the Kenyan population. Of the total produced, 60 % of the milk reaches the market through traders, cooperatives, hotels, and kiosks. An estimated 84 % of the milk produced is sold in raw form to consumers ranging from rural to urban dwellers, according to Regional Dairy Centre of Excellence (RDCoE, *2013*). The sector faces numerous challenges despite immense opportunities ahead.

The sector has adopted various practices which are not without challenges due to its subsistence nature. The mains practices that act as drivers of successful dairy farming include health management, milk hygiene practice, nutritional management, and animal welfare. The farmers need to put in place programs that are geared toward effective health care. Milk should be harvested and stored under hygienic conditions. The equipment should be suitable and well maintained to avoid contamination of milk during handling. To have safe and quality milk, animals should be fed and watered with quality, reliable feeds. A conducive environment should be provided to cater for animal welfare; these include the "five freedoms."

- Freedom from thirst, hunger, and malnutrition
- Freedom from discomfort
- Freedom from pain, injury, and disease
- Freedom from fear
- Freedom to engage in the relatively normal pattern of animal behavior

According to a report by the United States Agency for International Development (USAID) on consumer milk quality perception, "Kenya dairy sector competitiveness program" released in

2010, a majority of Kenyans have a high degree of positive opinion of milk, with 62 % of respondents associating milk with high protein content. The positive perception and the fact that Kenya is one of the highest consumers of milk in developing countries provide an opportunity for growth in the dairy sector.

2.2 Dairy sector input in economy and nutrition

The dairy industry accounts for 14 % of agriculture GDP and 6-8 % of the country's GDP. According to USAID, the industry generates an estimated 1 million jobs at the farm level and an additional 500,000 indirect waged employment and 750,000 jobs in support services. (Food Business Africa, 2014). The dairy sector is vital in poverty alleviation in rural and urban areas as it contributes to food and nutritional security and increased household incomes. Kenya's 1 million stockholders keep the largest dairy herd in Africa (larger than *S. Africa*), according to Jimmy Smith, Director (ILRI, 2007*)*. According to USAID, the sector contributes USD 2 billion to the country's GDP; this includes farmers, traders and vendors, collections centers, and retailers (Food Business Africa, 2014).

A huge variety of dairy products includes cheese, cream, butter, and yogurt. The nutritional composition of milk is highly complex, and it contains. The nutritional values are as follows: calories (149), water (88%), proteins (7.7grams), carbohydrates (11.7grams), sugar (12.3 grams), fiber (0 grams) and fat (8 grams). Milk is considered to be an immunity-boosting food due to the presence of zinc and vitamin D. It is suitable for the heart; having a glass of milk can improve cholesterol levels in the blood. It also enhances the overall metabolism of the body. The milk protein (casein) can increase the absorption of minerals, such as calcium and phosphorus. (Holt et al., 2013)

2.3 Challenges facing the dairy industry in Kenya

There is seasonal fluctuation in the production of milk. This has a significant effect on the processors' ability to absorb all the milk availed to them at any time during the year, with a period of surplus followed shortly by that of low milk volumes. Companies like Brookside Dairy and New KCC have powder plants that convert the surplus milk into powder milk, reconstituted during the dry period. (Kenya Dairy board, *2016*).

Poor quality of milk is due to the sector's domination by small scale holders; hence quality and food safety aspects become a big hurdle. This is characterized by poor milking practices, a fragmented small scale dairy farming system, and a lack of cooling facilities and storage facilities at the farm level. The poor quality reduces the acceptability and shelf life of processed milk and has affected the ability of dairies to sell to some export markets. Incidences of milk adulteration are evident, with unscrupulous farmers and traders adding chemicals and water to increase quantities delivered to dairies (Afmass, 2017).

Due to expensive feeds and supplements, the farmer is faced with the challenge of buying quality feed, hence buying cheap feeds of low-quality products from small feed manufacturers. The farmers opt for hay grass, which is traded freely at the doorstep and used in a fresh folder, especially in central Kenya. Due to the ever-changing weather pattern, the incidence of disease infections is very high, which can wipe out cattle's stock. Diminishing veterinary services is another hurdle in the dairy sector. The costs are quite high, unlike when government officials did rounds in the farms. The artificial insemination (A.I.) services have also become quite costly.

The farmer lacks proper storage facilities after milking leading to the high incidence of milk spoilage in case of delay in delivery to processing plants. There is a lot of milk wastage, especially during peak periods. In recent years, this has been witnessed in central Kenya due to inadequate capacity in the milk processing factories. There is the poor infrastructure that leads to milk wastage, especially in the wet season. Most of the high yielding areas are still underserved by proper roads. Delayed payments to farmers have resulted in milk hawking, especially in counties near Nairobi city. Control of milk in terms of quality and safety is a significant challenge in the dairy sector. (Owango *et al.*, 2015).

Informal milk trade dominates the sector, accounting for 60 % of the total, according to RDCoE. It is marketed through traders, cooperatives, hotels, and kiosks. Of the volume produced, 84% is consumed unprocessed due to the consumers providing instant cash or higher prices to the farmers.

2.4 Preventive measures against livestock diseases

Diseases in animals play a significant negative role in livestock production. They lead to reduced productivity and constrained market access and eradication of entire flocks or herds, with eventual loss in terms of biodiversity and treasured genetic resources. Some emerging diseases can be transmitted to humans. Combating livestock diseases in developing countries can make a substantial contribution to lowering morbidity and mortality. Proper control measures can promote: poverty alleviation by creating employment, revenue generation for education and training, cultivating avenues for trade in livestock and animal products, and industrial raw materials supplies (FAO, 2012).

The World Organization for Animal Health (OIE) has always been aggressively involved in the prevention and control of zoonosis diseases concerning safe food production. The main objectives being the development of international standards to promote secure international trade in animals and animal products, and prevent and control animal diseases, including zoonosis, and promote animal health and welfare. These standards detail the OIE's requirements to avoid pathogenic biological agents' transmission to animals, humans, and the environment, based on biosafety and biosecurity principles. (OIE, 2018).

Progressive control pathways (PCPs) are increasingly used to control several animal diseases, including foot and mouth disease, brucellosis, and rabies. Consequently, it encourages the improvement of national control plans that support strategies and the use of the various tools that promote disease control, including improved surveillance, vaccination programs, public awareness campaigns and, in particular, enhanced biosecurity, the latter to be implemented through control of animal and product movements by quarantine, reduced mixing of different groups of animals, improved hygiene and sanitation practices, particularly about the animal examination and treatments and the handling of potentially contaminated food. (Diall *et al.*, 2017).

According to *Shitandi* and Stemesjo (*2004*), lack of education and training in antibiotics use, and their effects among farmers have been considered one of the main reasons for antibiotic residue in Kenya's small scale farm milk.

In antibiotic residue screening studies, the E.U. (European Union) and Codex regulation for Maximum Residue Levels (MRLs) are mainly followed. The sum of sulfonamides should not exceed 100 µg/kg (EUR LEX, 2010). The MRL for tetracycline is 100 µg/kg (EUR .LEX 2010 AND Codex 2012). The MRLs for beta-lactam vary by compounds, but mainly below sulfonamides (0.01 ppm) and tetracycline (0.1 ppm) limits.

2.5 Commonly used antibiotics in livestock

The most frequently used antimicrobials associated with milk are antibiotics, employed to combat mastitis-causing pathogens in the dairy cow. The pharmacokinetics of antibiotics in lactating cows are mainly revealed that antibiotics are the drugs mostly used or prescribed for lactating animals; among them related to the route of administration, the disease, some treatments, specific physicochemical properties of the incipient, the milk production and the condition of the udder (Bavani *et al*., 2007). The percentage of the dose excreted as residues in milk after intramuscular administration is 0.001 % for Benzylpenicillin, 0.02 % for Neomycin, 0.07 % for oxytetracycline, 0.08 % for Ampicillin, 1.45 % for Lincomycin, 2.6 % for Tylosin and 3.8 % for Erythromycin. (Bavani *et al*., 2007). These are the most commonly used drugs for treatment in food animals.

In Kenya, approximately 36% of produced milk is consumed by the households or fed to calves, and the remaining 64 % is marketed to cooperatives, processors, and directly to consumers. Approximately 8 % of the marketed milk is sold raw in the unregulated informal market, leading to public concerns about hygiene and safety (Omore *et al*., 1999). Drug residues are also of interest, more so in processed milk products and meat products (Colak *et al*., 2007). This is a result of inadequate monitoring of the withdrawal period after administration. If the withdrawal period is well observed, drugs are well metabolized and clear from the animal's body system. If the withdrawal period is not observed, the minimum residual level will be above the tolerance limits. There are various reports from all parts of the world; the USA, Europe, India, and Africa. In Kenya, multiple studies have been carried out on milk safety and quality. It was found that the presence of milk inhibitory substances exceeds the CODEX Alimenterias standard beta Lactam 4 micro g/kg. Antibiotics are introduced as a result of the treatment of cow's udder infected by mastitis. It is defined as inflammation of the udder. The residue ends up in the milk system hence posing a risk if the withdrawal period is not adhered to (Ahlberg *et al.*, 2016).

Other sources of contaminants may result from milk handlers and types of equipment. Milking staff plays a crucial role in milk hygiene and contamination. Dust and dirt, as a result of poor milk handling, bring immense microbial contaminants such as *Staphylococcus aureus*, *Coliforms*, and *Escherichia coli*. Microbial risks are relatively higher in hand milking as compared to machine milking. The cow's coat serves as a vehicle through which microbes are directly introduced to milk. The hairs around udder, flanks, and tail contribute to the higher bacterial count in milk. The coat may indirectly add microbes into air, especially *Bacillus* sp.

Subsequently, contamination may be induced by poor practices in pest control such as Acaricid*e* used in ticks control at the farm level. This may end up in milk and meat hence the risk to the consumers. Proper administration practices are essential to curb the transfer of contamination to the ultimate consumer. (Collins, 2004).

The dairy sector in Kenya is amorphous; hence proper guidelines and regulations are not felt at the grass-root level. The poor economic status of producers leads them to sell off their produce to vendors for ready cash, to have daily bread. (Muriuki *et al.*, 2007) These have contributed to the subsistence nature of the dairy sector. The milk ends up in urban centers such as Nairobi city and its periphery.

.The whole value chain poses a risk to the consumer due to poor handling practices hence a need to carry out research on milk quality and safety. The study will show the milk sector well-coordinated with the emergence of policies and regulations at the county level (ACOG, 2012).

2.6 Occurrence of antibiotics in milk and their control

Antibiotics and antimicrobial residues in milk occur regularly due to their use in therapeutics in dairy animals, such as the frequent use of drugs to treat mastitis and other infectious diseases, the residues resulting from the improper use of antimicrobials. Residues also from such improper practices as extra-label drug use and unscrupulous practice of some producers, who dilute residue-containing milk with untainted bulk milk (Collins, 2004) did a survey and found out that 45 % of the milk sampled in the United States contained sulfonamide residues, compared to 30 % in Canada. The occurrence of antibiotic residues in milk may have economic, technological, and even human health implications. Most dairy companies also use rapid tests to monitor all incoming milk for the presence of ß-lactam antibiotics.

The solution to the problem of drug residues in milk lies in the application of general principles of "Good Farming Practices" which include: Good farm management should in the first place be directed towards the prevention of infectious diseases, such as (sub) clinical mastitis, to limit the use of veterinary drugs; In the process, farmers must keep their animals in sound physical condition by ensuring hygiene and good housekeeping practices and implementing sound farming management; In preventing mastitis, the use of proper functioning milking machines is of primary importance (FAO, 2011).

The use of veterinary drugs remains necessary, but this option should only be exercised after a correct diagnosis by a veterinarian. Only registered pharmaceuticals with a known depletion pattern should be used; Correct administering the drugs is also very important in terms of prescribed dose, route, and frequency of administration; the keeping of reliable records of such medications used is also essential (Shitandi, 2004).

It is the responsibility of the milk producer to respect the withdrawal periods. In the process, the treated animals need to be marked clearly to allow for correct identification; treated cows need to be milked last. During the withholding period, the milk must be discarded properly. The milking equipment should also be adequately cleaned after contact with the contaminated milk. Also, special care should be taken with milk of cows that have been treated with long-acting dry cow products or with milk from cows that have been purchased recently (Layada *et al*., 2016).

Records of all animal treatments can be used as the first measure to control or reduce residues of medication in milk (Muriuki *et al.*, 2007; and Charlebois and Haratifar, 2015).

Non-adherence to withdrawal periods or use of extra-label fashion, that is, different doses, different routes, or frequency of administration, can result in volatile levels of antibiotics entering milk supply. In the United States, the Animal Drug Use Clarification Act of 1994 allows veterinarians to prescribe extra-label uses of certain approved animal drugs and recommend human medicines for animals under certain conditions. An extra label use algorithm has been made available through the American Veterinary Medical Association (AVMA) website as a guide to veterinarians when extra-label use is contemplated (AVMA, 2003).

Milk obtained from lactating animals that have been treated with veterinary medicinal drugs must not contain drug residues, which may pose health hazards for consumers (Hou *et al.*, 2014). Hence testing milk for antibiotic residues of ß-lactam antibiotics has become mandatory (US FDA, 2003).

2.7 Antibiotic residues in milk and human health

The occurrence of antibiotic residues in milk intended for human consumption is undesirable for some reason. As recently as 30 years ago, the presence of antibiotic residues in milk was primarily a manufacturing problem related to inhibition of cheese and yogurt starter culture (Stead *et al*., 2008). More recently, the presence of antibiotics in milk has been prohibited because of concerns about public health. Initially, public health officials desired to protect hypersensitive individuals from exposure to a specific antibiotic.

2.8 Common diseases in livestock

The antibiotics are manufactured for treatment and prevention of animal diseases such as mastitis, arthritis brucellosis, and gastrointestinal diseases, plus other infections (Tollenfson and Miller, 2000). To some extent, antibiotics have been found useful to improve animal production as well as fattening (Nisha, 2008). In the treatment of diseases, the drug is rapidly assimilated into the body tissues and fluids. Over 90% bind to plasmic proteins and reach a high level between the 3rd

and 6th hours of administration (Sulejmani *et al*., 2012). The liver metabolizes the drug and is filtered in the glomerular. If the withdrawal period is not observed correctly, the drugs end up in large amounts of milk and meats (Richelle, 2007). They end up in the supply chain and unto the ultimate consumer.

2.9 Antibiotics use in livestock health management

Antibiotics usually work in one of two ways; kills (bactericidal) bacteria by interfering with the formation of the cell wall or its cell content. It can also act as bacteriostatic, stopping the multiplication of bacteria. Antibiotic residues are remnants of antibiotics, or their active metabolites that are present within tissues or products such as meat, milk, and eggs from treated animals (International Dairy Federation., 1995, Codex Alimentarius Commission., 1998, Collins and Wall, 2004b) and levels of the drugs and their metabolites may persist at unacceptable levels. Consumers can be exposed to them (Kabrite *et al*., 2019), most of which occur due to dairy animals being treated for mastitis through intra-mammary or intravenous infusions (Kopp *et al*., 2010). According to the Centers for Disease Control (CDC, 2013), dairy cattle treated with antibiotics produce milk containing antibiotic residue for a while after treatment. Milk from treated cows should be excluded from the supply chain; the proper withdrawal period should be addressed (CDC, 2013). The residues' presence may result from failure to observe the mandatory withdrawal periods, illegal or extra-label use of drugs and incorrect dosage. Many drugs are also retained in the animal's body for longer times than indicated by the label discard times (Ivona *et al*., 2002). Trace levels of antibiotics and antimicrobial drugs occur in food products of animal origin and are consumed by the public (Mathew, 2004). Residues in milk have been the subject of considerable public debate. The overall question of the biological significance of antibiotics and antimicrobial residues in milk has been debated for over 20 years and remains the subject of considerable scientific and political activity.

In Kenya, Shitandi (2001) showed that 21 % of the 1109 samples analyzed were positive for antibiotics, while Kang'ethe *et al*., 2005) found a 16% prevalence of antibiotic residues in Kenya. In Nakuru district, Kenya, Sternesjo, and Shitandi (2001) demonstrated that 21 % of the milk sampled contained antibiotic residues, of which 14.9% of these contained Penicillin-G type residues. However, Ombui (1994) failed to detect any antibiotic residues in bulked milk received at Dairy Cooperatives in Kiambu district, Kenya, probably due to the high level of awareness on withdrawal periods among farmers delivering the milk.

2.9.1 Commonly used antibiotics food production

Penicillin G is the most frequently used treatment of bovine mastitis, followed by other ß-lactam antibiotics and oxytetracycline (Collins and Wall 2004b, Mitchell *et al.,* 1998). The pharmacokinetics of antibiotics in lactating cows are mainly related to the route of administration, the disease, some treatments, specific physicochemical properties of the incipient, the milk production, and the condition of the udder (Bavani *et al.,* 2007). It was reported that; the percentage of the dose excreted as residues in milk after intramuscular administration is 0.001 % for Benzylpenicillin, 0.02 % for Neomycin, 0.07 % for oxytetracycline, 0.08% for Ampicillin, 1.45 % for Lincomycin, 2.6% for Tylosin and 3.8 %f or Erythromycin. (Bavani *et al.,* 2007).

In Kenya, an estimate of 36 % of produced milk is consumed by the households or fed to calves, and the remaining 64% is marketed through cooperatives, processors s and directly to consumers. Approximately 8 % of the marketed milk is sold raw in the unregulated informal market, leading to public concerns about hygiene and safety (Omore *et al.,* 1999) how antibiotics may occur in milk and their control. Antibiotics and antimicrobial residues in milk occur regularly due to their use in therapeutics in dairy animals, such as the frequent use of drugs to treat mastitis and other infectious diseases, the residues resulting from the improper use of antimicrobials. The occurrence of antibiotic residues in milk may have economic, technological, and even human health implications. Most dairy companies also use rapid tests to monitor all incoming milk for the presence of ß-lactam antibiotics. Some of these companies claim compensation for the costs of disposing of milk of a contaminated tanker load from the responsible farmer (Layada *et al*., 2016). The solution to the problem of drug residues in milk lies in the application of general principles of "Good Farming Practices" which include: Good farm management should in the first place be directed towards the prevention of infectious diseases, such as (sub) clinical mastitis, to limit the use of veterinary drugs;(Bion *et al.,* 2015) In the process, farmers must keep their animals in sound physical condition by ensuring hygiene and good housekeeping practices and implementing sound farming management; In preventing mastitis, the use of proper functioning milking machines is of primary importance. (CDC, 2017) The use of veterinary drugs, nevertheless, remains necessary, but this option should only be exercised after a correct diagnosis by a veterinarian. Only registered pharmaceuticals with a known depletion pattern should be used; Correct administering the drugs is also very important in terms of prescribed dose, route, and frequency of administration; the keeping of reliable records of such medicines used is also essential. This remains the responsibility of the milk producer to respect the withdrawal periods. In the process, the treated animals need to be marked clearly to allow for correct identification; treated cows need to be milked last, and during the withholding period, the milk must be discarded the proper way (Baur *et al*., 2017). The milking equipment should also be adequately cleaned after contact with the contaminated milk. Also, special care should be taken with milk of cows that have been treated with long-acting dry cow products or with milk from cows that have been purchased recently. Records of all animal treatments can be used as the first measure to control or reduce residues of medication in milk (Muriuki *et al.*, 2007). Non-adherence to withdrawal periods or use of extra-label fashion, that is; different doses, different routes, or frequency of administration, can result in volatile levels of antibiotics entering milk supply.

In the United States, the Animal Drug Use Clarification Act of 1994, allows veterinarians to prescribe extra-label uses of certain approved animal drugs and approve human drugs for animals under certain conditions. An extra label use algorithm has been made available through the American Veterinary Medical Association (AVMA) website as a guide to veterinarians when extra-label use is contemplated. Milk obtained from lactating animals that have been treated with veterinary medicinal drugs must not contain drug residues which may pose health hazards for consumers. Hence testing milk for antibiotic residues of ß-lactam antibiotics has become mandatory (US FDA, 2003).

2.9.3.0 Antibiotics derivatives

2.9.3.1 Tetracycline

The tetracycline discovered in the 1940s is a family of antibiotics that inhibit protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor site (Chopra, 1985). Tetracycline are broad-spectrum agents, exhibiting activity against a wide range of gram-positive and gram-negative bacteria, typical organisms such as chlamydiae mycoplasmas, and rickettsiae, and protozoan parasites (Chopra, 1994). They are also used prophylactically for the prevention of malaria caused by mefloquine resistant *Plasmodium falciparum*. Moreover, in some countries, including the United States, tetracycline is added at sub-therapeutic levels to animal feeds to act as growth promoters. Although the tetracycline retains essential roles in both human and veterinary medicine, the emergence of microbial resistance has limited their effectiveness (Nelson *et al.*, 1993; Borass *et al.,* 2011). Undoubtedly the use of tetracycline in clinical practice has been responsible for the selection of resistant organisms. Nevertheless, as we enter the new millennium, the use of tetracycline and other antibiotics as animal growth promoters is becoming increasingly controversial because of concerns that this practice may be contributing to the emergence of resistance in human pathogens (Smilack, 1999). The increasing prevalence of bacterial resistance to tetracycline has, in turn, occasioned efforts to establish the mechanisms by which genetic determinants of resistance are communicated between bacteria and the molecular basis of the resistance mechanisms themselves. The enhanced understanding of tetracycline resistance mechanisms realized by this work has provided openings for the latest unearthing of a new generation of tetracycline, the glycylcyclines (Figure 2.1). Further study, already underway, is instituting approaches by which inhibitors of tetracycline resistance mechanisms potency be developed for use in combination with earlier tetracycline to reestablish their antimicrobial activity (Nelson and Levy 1999, Stead *et al*., 2008).

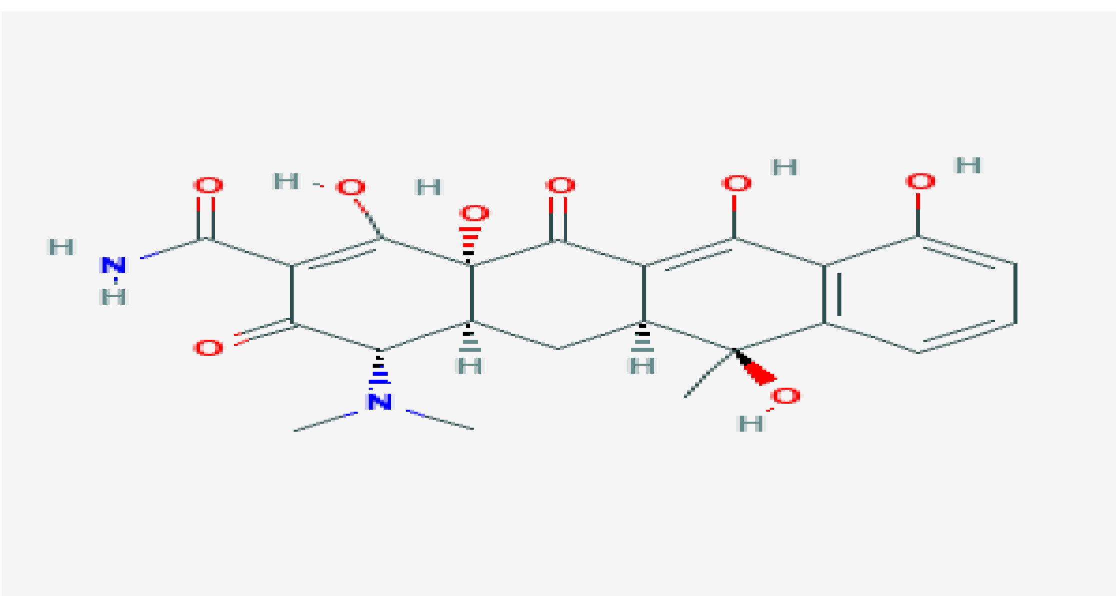

Figure 2. 1: Skeletal formula of tetracycline

2.9.3.1.1 Evolution of the tetracyclines

Chlortetracycline and oxytetracycline, both discovered in the late 1940s, were the first members of the tetracycline group to be defined. These molecules were products of *Streptomyces aureofaciens* and *S. rimosus*, respectively. Other tetracyclines were identified later, either as naturally occurring molecules, for instance, tetracycline from *S. aureofaciens, S. rimosus,* and *S. viridofaciens* and demethylchlortetracycline from *S. aureofaciens*, or as products of semisynthetic methodologies, for example, tetracycline, doxycycline, and minocycline (Kwon *et al.*, 2000). Despite the early tetracycline achievement, analogs were sought with enhanced water solubility either to allow parenteral administration or to heighten oral absorption (Kucer and Benett, 1987). These approaches resulted in the development of the semisynthetic compounds rolitetracycline and lymecycline. The most recently discovered tetracyclines are the semisynthetic group referred to as glycylcyclines, for example, 9-*(N*, *N*-dimethyl glycyl amido)-6-dimethyl-6-deoxytetracycline, 9-(*N*, *N*-dimethyl glycyl amido) - minocycline, and 9-*t*-(butyl glycyl amido)-minocycline. These compounds possess a 9-glycylamido substituent (Finch, 1997).

2.9.3.2 Beta-lactam antibiotics

The beta-lactam antibiotics are mostly considered to be a group of antibiotic consisting of an agent containing a beta-lactam ring (Figure 2.2 and Figure 2.3). This includes penicillin derivatives, cephalosporins, monobactams, carbapenems, and carbacephems. Most of them act by inhibiting cell wall biosynthesis in bacterial organisms. Its discovery originated from Alexander Fleming in 1928, with the isolation of Penicillium produced form the molds; therefore, antibacterial penicillin (Fleming 1928). He carried out several studies on the antibacterial activity of penicillin in vitro using the filtrate from Penicillium's liquid cultures (Kabrite *et al.*, 2019). He also showed that the culture filtrate appeared to be non-toxic when injected into mice and rabbits. However, he did not carry out any studies of penicillin against experimental infections in animals. He thus failed to demonstrate penicillin's essential property, namely its ability to overcome bacterial infection when administered systemically (Matthew, 2004).

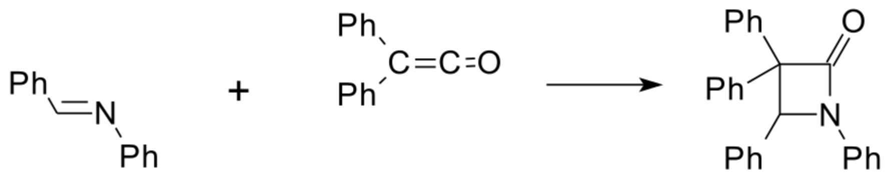

Figure 2. 2: Skeletal formula of the beta-lactam ring

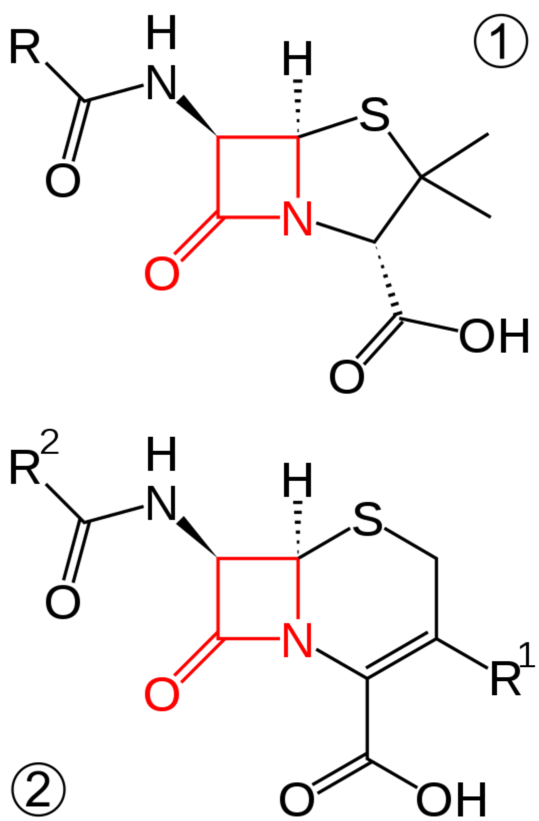

Figure 2. 3: Core structure of penicillin

2.9.3.3 Sulfonamides

Sulfonamide is an antibiotic that has a functional group, which is the base for several groups of drugs; sulfa drugs or sulpha drugs (Figure 2.4). It has been prescribed in diabetes treatment and pain relief. It was first invented in 1906 and made way into usage in the 1930s. Its mode of action is through bacteriostatic and not bactericidal; it inactivates cell activity by stopping the synthesis of folic acid. The synergistic action with specific diaminopyrimidines renders these drugs more effective than sulfonamides alone. It has an N4 amine group; therefore, its allergic effects during administration (Bion *et al.,* 2015).

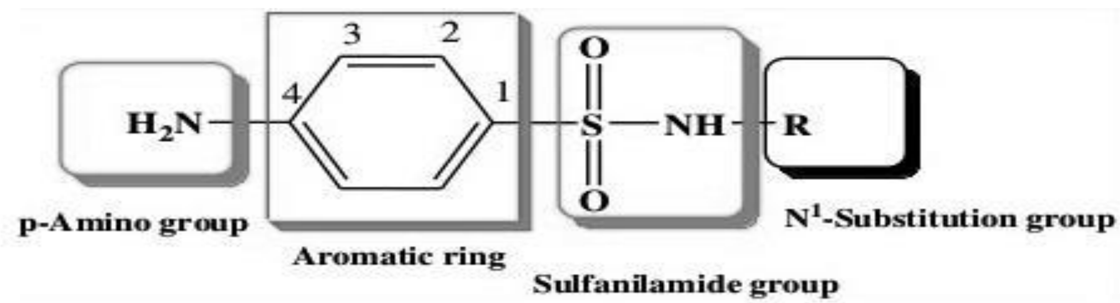

Figure 2. 4: Sulfonamide chemical structure

2.9.4 Antibiotics control by E.U. regulation

The Maximum Residue Limits (MRLs) the level at which the concentration should not exceed. (E.U. Regulation 2377/90):

- 0.004µg penicillin, amoxicillin, or ampicillin 1ml
- 0.1µg oxytetracycline or sulfamethazine 1ml
- 0.04µg erythromycin 1ml
- 1.5µg neomycin in 1ml
- 0.2µg streptomycin in 1ml
- 0.05µg trimethoprim in 1ml

The Kenya Bureau of Standards (KEBS) has adopted E.U. (European Union) and Codex standards on the antibiotics residue. The sulfonamides MRLs should not exceed 100µg/kg or 0.1µg/ml), while MRLs for tetracyclines are 100µg/kg(EU-LEX 2010 and CODEX 2012), The MRLs for beta-lactams varies with respective compounds; are mainly bellow the limits for sulfonamides and tetracyclines (EU-LEX 2010)

2.9.5 Common diseases in livestock

The antibiotics are manufactured for treatment and prevention of animal diseases such as mastitis, arthritis brucellosis, and gastrointestinal diseases, plus other infections. To some extent, antibiotics have found useful to improve animal production as well as fattening. (Hou *et al*., 2014) In the treatment of diseases, the drug is rapidly assimilated into the body tissues and fluids. Over 90% bind this bind to plasmic proteins and reach a high level between the 3rd and 6th hours of administration. (Sulejmani *et al*.; 2012) The liver metabolizes the drug and is filtered in the glomerular. If the withdrawal period is not observed correctly, the drug ends up in milk and meats in large amounts. (Richelle, 2007) They end up in the supply chain and unto the ultimate consumer. There is a substantial body of evidence that the use of antibiotics for veterinary therapy, prophylaxis, and animal growth promotion results in the selection of resistant animal pathogens and commensals (Khachatourians 1998, Borras *et al*., 2011). The critical impact of resistance to antibacterial in terms of veterinary medicine is the failure of empiric treatment of bacterial septicity, which courses an increase in sickness and death and hence persistent suffering of infected animals; untreatable infections do occur but are rare and are not yet a significant problem in veterinary medicine **(**Borass *et al.,* 2011).

2.9.6 Risk assessment

A well-coordinated process has been developed through world organizations such as WHO, FAO, AND OIE. Globally the food safety process has been championed through CODEX and end every county passed to have its own quality regulatory body (CAC, 2004). A good structure process is followed to achieve risk assessment, as shown below (Figure 2.5). To start with, a hazard is identified as the causative agent, which can adversely affect human health when ingested. It can be categorized as a biological, chemical, or physical hazard. It then characterized through quantitative/ qualitative analysis to determine the nature of adverse health effects associated with biological, chemical, or physical risks, which may be present in food. For chemical hazard, dose-response assessment is performed. While for biological and physical hazards, the dose-response assessment should be performed if the data are attainable. Consequently, exposure assessment follows through the quantitative or qualitative determination of likely intake of biological, chemical, or physical agents via food as well as from other sources if relevant. Finally, the risk is characterized by the probability of occurrence or severity of known or potential advance effect in a given population based on hazard identification, risk characterization, and exposure assessment. (FAO and WHO, 2011)

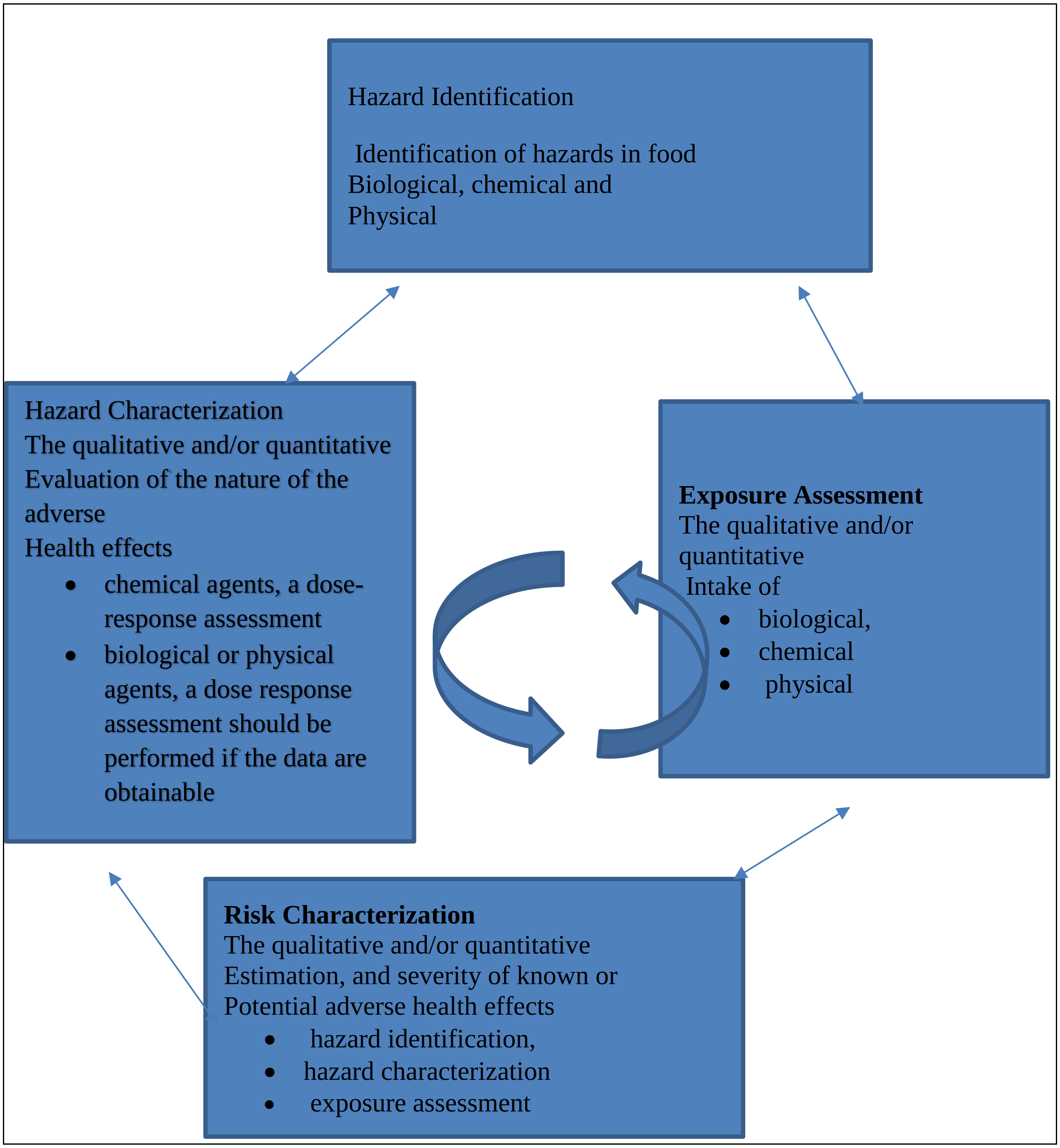

Figure 2. 5: Steps in risk assessment process

2.9.7 Determination of antibiotics

Several methods have been developed for the detection of antibiotic residues in milk: Delvo P or SP® test, Lac tet ß-lactam® test, the penzym® test, Twin sensor® test, trisensor® test, Charm II® Assay, SNAP ®Test, Beta STAR® test, liquid Layer Chromatography, among others. (Anon *et al*., 1998) Some of this test is rapid and is carried out at the platform level while others are long and mostly used for research purposes. The quick test may not give the exact amount of antibiotic present or type present in the milk, but they have minimum sensitivity levels set for their detection. The quantitative analysis can be carried out by the use of high-performance liquid chromatography HPLC or the use of ultra-high-performance liquid chromatography LC-MS/MS.

2.10 Gaps in knowledge

Despite various research conducted to discover the presence of antibiotics in food products, the aspect of the risk-based analysis is not yet addressed in developing countries. The ever increasing demand for food and maximization of production in line with modern technology, have compromised food safety plan. The cost factor becomes a more significant hindrance in addressing food safety issues hence shortfall in documented evidence. It has been a challenge to alleviate risks to the ultimate consumers. Despite the presence of food regulatory agencies, risks level across the supply chain is yet to be established. The implications associated with the risk factors in terms of morbidity and mortality remain unknown.

CHAPTER THREE: EFFECTS OF DAIRY FARMING PRACTICES ON RAW MILK CONSUMED IN KIAMBU COUNTY, KENYA

3.0 Abstract

Kenya has witnessed unanticipated growth in the urban population over the past decade. Food safety has been a subject of concern in the recent past; due to an increase in chronic illnesses. The present study aimed to find out how safe the raw milk produced and consumed in Kiambu County is. The unregulated trade of milk prompted this study by hawkers and the presumed low level of safety awareness among the farmers.

A cross-sectional study was carried out using a structured questionnaire. To determine awareness of food safety practices among dairy farmers. It covered Gatundu south sub-county (1°1'0" South, 36°56' 0" East), which covered three regions, namely: Kiganjo, Gatei and Gatundu town. Purposive sampling technique was used to select 100 participants. From each of them, samples were picked in the morning and the evening. The study showed that small scale farmers accounted for (98 %) who keep 2 to 3 cows.

The use of equipment and quality control revealed that 65 % of farmers use aluminum cans, while 35% still use plastic cans in milk handling. Manual milking is still major adopted (97 %) as a milking method as opposed to machine use at 3%. The storage of milk still wanting where most adopt cold bath (45 %) in cooling milk, 12 % freezer, while 43% do not use any form of cooling milk to curb spoilage. Health management at the farm is not conducted with the seriousness it deserves, although a considerable number of farmers have encountered the use of antibiotics (45 %). The study revealed that various quality control parameters scored poorly, such includes, quality monitoring (23 %), corrective action (0 %), extension services (3%), hygiene improvement (3%), and use of health records 12 %. All this contributed to 64 % of the farmers who encountered milk rejection at their collection centers. This means that hygiene standards and safety aspects among dairy farmers in Kiambu County are compromised. Consequently, there is a need to enforce controls in informal marketing channels, besides training, infrastructural development, code of practice, and inspections to enhance the quality and safety of dairy products along the supply chain.

3.1 Introduction

Dairy farming in Kenya approximates 4.5% of Gross Domestic Product (GDP) *(*National Livestock Policy, 2008). According to the Kenya Dairy Board (KDB, 2013), milk production has exceeded 4.6 million tonnes a year; of this, 1.5 million are produced by small scale holders. Officially the total herd size is about 3.5 million heads of dairy cattle (Muriuki *et al*., 2011). Today, the actual herd is seemingly more significant than reported by the Kenya Dairy Board (KDB, 2013). The pattern of rains has changed due to the effect of global warming, seasons have changed; commencement of long rains seasons as well as short rains seasons at times delay. There is, therefore, fluctuation in the quantity of milk supply and hence unstable milk price. KDB has come on board with marketing tools to assist farmers though much has not been realized in terms of benefits.

During the long rains, April to June, the quantity and quality of pasture increases hence increase in milk production. Due to high volume, the supply exceeds the demand leading to low prices. Companies such as Brookside Dairy and Kenya Co-operatives Creameries (New KCC) process excess of the supply to powder milk. During a dry spell, most of the powder is reconstituted into liquid and sold in the form of pasteurized fresh milk.

Dairy farming in Kenya is characterized by small scale farmers having 2-3 cows, which account for 70 % of milk production from dairy farming. According to Smallholder Dairy Research and Development Project (2010), 70 % of jobs in the dairy sector are informal. The informal sector is characterized by milk hawking, milk bars, and upcoming milk ATMs (Automated vending machines) (KDB, 2013). The milk ATMs have taken the markets by storm, with minimal safety precaution to the ultimate consumer (KBD, 2003). The traceability of milk has been an issue due to the influx of unscrupulous traders. These have led to adulteration of milk with additives such as water and unapproved chemical preservatives. The milk sold in these places is unpasteurized, and in some instances, it is chilled, and at times it is not chilled. This has triggered the issue of milk preservation; hence the safety aspect of this product to the consumers is not assured (Mwangi *et al.,* 2000). The Kenya Dairy Board has recently championed campaigns on milk safety and control of milk hawking countrywide. They have drummed up support on the use of milk for a healthy nation, especially in school feeding programs (KDB, 2016).

In Kenya, sulfonamides, beta-lactam, aminoglycosides, and tetracycline are the most used antibiotics in the treatment of livestock (Aboge *et al.,* 2000). Noncompliance with the drug residue limits has increased since market liberalization in Kenya. In 1978 noncompliance with the penicillin residue limit was found to be 1% of the milk samples analyzed while in the year 2000, it was 16% (Kangethe *et al.,* 2005). Shitandi and Stemesjo 2004 studied farm practices related to veterinary drug usage; only 22% of small scale farmers documented drug usage. According to Orwa *et al.* (2017), tetracycline was the most used drug (55% of farmers) followed by sulfonamides (21%) and beta-lactam (6%). According to *Shitandi* and Stemesjo (2004), lack of education and training in antibiotics use, and their effects among farmers are among the main reasons for antibiotic residue occurrence in Kenya's small scale farm milk.

In antibiotic residue screening studies, the EU (European Union) and Codex regulations for MRLs (Maximum Residue Levels) are mainly followed. The sum of sulfonamides should not exceed 100 µg/kg (EUR LEX -2010). The MRLs for tetracycline are 100 µg/kg *(*EUR .LEX 2010, Codex 2012). The MRLs for beta-lactam vary by compounds, but mainly below sulfonamides (0.01 ppm) and tetracycline (0.1 ppm) limits. In Kenya, various studies have been carried out on milk safety and quality. It was found that the presence of milk inhibitory substances exceeds the CODEX Alimenterias standard and beta-lactam (4 micro g/kg). Antibiotics are introduced as a result of the treatment of cow's udder infected by mastitis. The residue ends up in the milk system hence posing a risk if the withdrawal period is not adhered to (Ahlberg *et al.,* 2016).

The dairy sector in Kenya is amorphous; hence proper guidelines and regulations are not felt at the grass-root level. The poor economic status of producers leads them to sell off their produce to vendors for ready cash, to have daily bread. (Muriuk*i et al.,* 2007) These have contributed to the subsistence nature of the dairy sector. The milk ends up in urban centers such as Nairobi city and its periphery.

The dairy industry in Kenya operates mostly in small scale holdings, which accounts for 70-80% of the milk production. The small scale is characterized by keeping 2 to 3 cows and milking 5 to 10 liters of milk/day/cow. They lack tools such as strip cup for mastitis testing at milking site. Contamination may result from milk hawkers and middlemen who are associated with adulteration incidences. Due to harsh economic conditions, farmers only allow one day withdrawal period hence pose a higher risk to the consumer. Therefore risk factor becomes a matter of importance to food safety regulatory authority (Mitem*a et al*., 2001)

The dairy farming management for small scale farmers becomes more complicated due to lack of record-keeping; and follow-up in operations. Due to the high cost of veterinary doctors, farmers sometimes resort to self-treatment of their animals based on past treatments. Therefore they don't have proper administration of drugs, and withdraw periods are not followed to the letter (Omore *et Al.,* 2005).Successful farm management entails proper coordination of various management functions at the farm level; this includes: forage production, feeds and nutrition, housing, health management, fertility management, young stock, and hygiene (Figure 3.1).

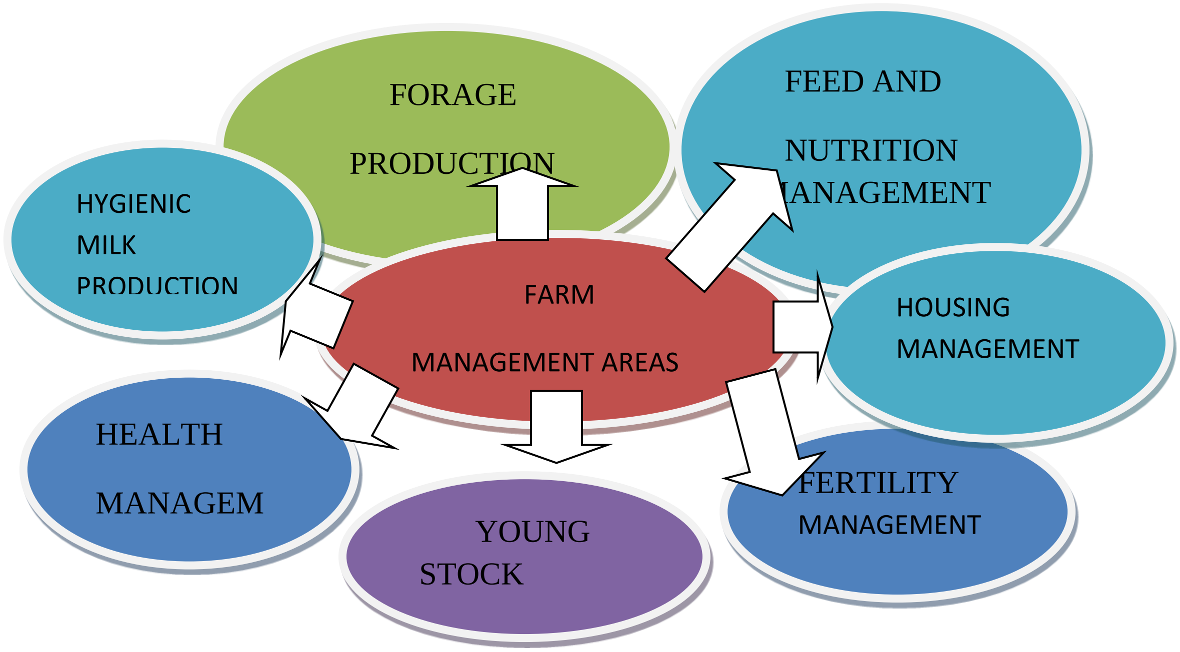

Figure 3. 1: Farm management areas

3.2 Study design and methodology

A cross-sectional study with an analytical component was carried out to establish the effect of dairy farming practices on the quality and safety of raw milk produced in *Kiambu* County and to quantify antibiotic residue in the milk.

3.2.1 Study area

Kiambu County is a vast area that constitutes 12 sub-counties (Figure 3.2). These are further divided into 60 wards of its capital. It is a county in the former Central Province in Kenya. Its capital is Kiambu, and its largest town in Thika. Gatundu South Sub-County was purposively selected as a representative study area. Kiambu County has uniform social-economic dynamics, vibrant dairy production, and proximity to Nairobi suburbs.

Figure 3. 2: Map of Kiambu County in central Kenya

Source: www.kara.or.ke

There is a total population of 2,417,735 of which 1,187,146 are male and 1,230,454 are female. Agriculture account for 17.4% of the county's population income. It contributes significantly to food security, employment, food security, and income earnings. The average farm size under small scale farming is 0.36 Ha and 69.5 Ha under large scale farming. The areas under small landholdings are mostly found in the upper parts of Gatundu North, Gatundu South, Kiambu, Limuru, and Kikuyu constituencies. According to the 2009 population and housing census, the number of livestock were as follows; 230,294 cattle, 120,056 sheep, and 89817goats. Milk produced in 2010 was 267.5 million kgs valued at 5.0 billion. The growth has been encouraged by ready markets in Thika, Ruiru, Kiambu, and Nairobi.

3.2.2 Methodology

3.2.2.1 Study instrument

A questionnaire was prepared and pretested in the Gatundu South Sub-County and administered across other sub-counties. The questionnaire helped to substantiate; type of dairy farming practice (zero-grazing or free grazing), mode of feeding and storage of feeds, how drugs are administered, type of drugs and treatment, records keeping in treatment, mode of disposal of milk from the treated animal, and the withdrawal period. Also, it addressed milk handling and storage at the farm;

and mode of quality parameter monitoring, deviation, cause of rejection, and corrections. Also included were the evolution of experience and educational level, the farmer's experience in dairy practice, level of formal education, level of knowledge of farm records management, and how to trace the source of milk within the farm **(**Appendix 2**)**

3.2.2.2 Sample size determination

The study population consisted of intensive dairy farmers. A simple random sampling technique was used to get the farmers who participated in the study. To determine the sample size, Fischer's formula (Fischer *et al*., 1991) was used:

Where n = desired sample size

p=proportion expected to have the features under study; in this case, those who sell milk through dairy co-operative societies (50%) =0.5

Z=standard deviation set at 1.96 (95% confident interval)

q=1-p i.e. proportion not expected to have the features under study (50%) =0.5

d=degree of accuracy/sampling error-±10% = 0.1

Using the formula

Four farmers were added to this number *n* to cater for attrition, giving a total of 100 farmers.

3.2.2.3 Data collection

The questionnaire was administered to the consenting randomly selected dairy farmers who were present in the respective milk collection centers during the interview period (Appendix 2). Those who could read and write were requested to fill the questionnaire on their own while those who could not be interviewed by three well-trained enumerators who translated the questions from English to Kiswahili and filled the responses given by the respondents.

3.2.2.4 Sampling procedure

The simple random sampling was used in picking samples from the three stratified areas; namely Gatei, Gatundu, and Kiganjo. From each, a uniform number of samples were picked Gatei (34), Gatundu (33), and Kiganjo (33).

3.2.2.5 Study ethics

Before answering any question, consent was sought from the respondents. The respondents were taken through the purpose of the study, asked to participate in the study voluntarily, and assured of the confidentiality of their responses. They were then asked to sign a consent form to show that they agreed to participate in the study (Appendix 1).

3.2.2.6 Data analysis

Data obtained were coded and entered into SPSS for Windows software (IBM version 21) and analyzed. Descriptive statistics, namely, percentages and frequencies, were used to express the socio-demographic characteristics of the study population and the different milk handling practices. A chi-square test of significance was used to test for any existing significant associations between the various variables understudy with a p-value= 0.05 being set as the level of significance.

3.3 Results

Among the farmers, 65 % have adopted aluminum containers, while 35 % still use plastic containers. The storage methods devised varied from refrigeration (7 %), cold bath (45%), while 43% have adopted none. The majority of the farmers interviewed still use the manual milking method, which accounted for 97 %, while only 3 % use machine milking. At the farm level, it was found that 45 % have improvised cold bath dip storage, while 7 % were using refrigeration methods. Quite a substantial number (43 %) did not have any form of milk preservation (Table 3.1).

Table 3. 1: Equipment use and quality control at the farm level

Characteristic		frequency	**% (n=100)**
Lactating cows	Milking	1	45
	dry cows	2	55
milking equipment			
	aluminum	65	65
	plastic	35	35
milking method			
	machine	2	3
	manual milking	97	97
Milk storage and preservation			
	Cold bath	45	45
	Fridge	7	7
	Deep freezer	5	5
	none	43	43

The milk rejection and quality control at the farm level are wanting; the results show that milk rejection accounted for 64 %, which resulted from poor storage and lack of quality monitoring equipment at the farm. The quality monitoring and correction of deviation were strictly observed by only 3 % of the respondents (Table 3.2). Implementing mitigation measures against losses through proper storage, hygiene improvement, improved feeding, and prompt corrective action against quality deviation would uphold milk quality at the farm

Table 3. 2: Milk rejection and quality control at farm level

Quality control checks		Respondent % (n=100)
Quality monitoring	Yes	23
	No	77
Correction of deviation at farm level	None	0
Feeding concentrate	Yes	1
	No	99
Seeking extension services	Yes	3
	No	97
Milk rejection	Yes	64
	No	36
Hygiene improvement	Yes	3
	No	97

As shown in Table 3.3, nearly half of the farmers interviewed have encountered antibiotic treatment (45 %), while 74% practice record-keeping at their farmer. A considerable number of farmers (95 %) understand about withdrawal period, which varied from 48hrs (9 %) to 72hrs (52 %), while 31 % adhered to vet instructions. Among them, 7% did not have any information on the withdrawal period upon cattle treatment during lactation.

Table 3. 3: Antibiotic and treatment practices at the farm

Health practices	frequencies	Percentage % (n=100)
Use of antibiotics in animal treatment	45	45
Use of dairy records in the farm	74	74
Milk Handling From Treated Cow		
Experience in use of antibiotics	20	20
Use of health records	12	12
Knowledge in withdrawal period for sick animal		95
Practice on withdrawal period		
48hours	9	9
72hours	52	52
vet instruction	31	31
None	7	7

It was found that most of the farmers milked at around 4.30 am and 6.00 am (78 %), while 22 % milk three times, 80 % have adopted to cold bath storage before delivery to buying stations, 84 % sold to society while 14 % to brokers, 2 % sells to their neighbors (table 3.5). The farmers deliver milk to the buying centers within 2 hours hence minimal milk spoilage due to microbial growth. The farmers who have experienced spoilage due to poor handling of evening milk delivered in the morning. Those farmers who do not deliver evening milk due to low volumes have adhered to separation of evening and morning milk to avoid spoilage of entire morning delivery. The rejection was due to off-smell and alcohol positive (85 % alcohol v/v) results. Other factors included low density which was attributed to poor feeding and suspected adulteration incidence (table 3.4)

Table 3. 4: Milk handling and selling to the co-operative society

Handling and selling practices	PERCENTAGE %
EQUIPMENT RINSING DURING MILKING	94
MILKING TIME	
4.30 to 6.00am	78
Mid-day/evening	22
MILK PRESERVATION BEFORE SELLING	
Use of cold bath	80
Others means of preservation	20
MILK BUYERS	
company/societies	84
Brokers	14
Local	2

Table 3. 5: Statitical output of dairy farming practices at farm level, confidence interval at 95% CI (p<0.05 level of significance)

SOURCES	t	Df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
How many cows are you milking presently?	13.074	99	.000	2.320	1.97	2.67
Which method of milking does you use, machine or manual?	114.508	99	.000	1.990	1.96	2.02
Do you sanitize milking equipments before use?	29.305	99	.000	1.470	1.37	1.57
Do you monitor any quality parameter at farm level?	49.939	99	.000	1.840	1.77	1.91
Do you have any formal training in dairy farming?	40.612	96	.000	1.763	1.68	1.85
Do you keep any farm records?	27.935	97	.000	1.367	1.27	1.46
Are you able to trace source of milk?	28.052	97	.000	1.388	1.29	1.49

The dairy practices significantly correlate ($p<0.05$) to each other hence improvement in one variable will considerably have effect the outcome of quality and safety at the farm level. Dairy practice are significant at farm level in determination of quality milk produce (Table 3.5)

Table 3. 6: Statistical output (ANOVA) of various dairy practices at farm level

SOURCES		Sum of Squares	df	Mean Square	F	Sig.
Which method of milking does you use, machine or manual?	Between Groups	1.633	11	.148	9.625	.000
	Within Groups	1.357	88	.015		
	Total	2.990	99			
Do you sanitize milking equipment before use?	Between Groups	3.589	11	.326	1.347	.213
	Within Groups	21.321	88	.242		
	Total	24.910	99			
Which type of milking equipment do you use plastic or aluminum?	Between Groups	.951	11	.086	.357	.969
	Within Groups	21.049	87	.242		
	Total	22.000	98			
Do you have any formal training in dairy farming?	Between Groups	2.504	10	.250	1.431	.180
	Within Groups	15.043	86	.175		
	Total	17.546	96			
Have your milk ever rejected?	Between Groups	2.946	11	.268	1.070	.394
	Within Groups	22.014	88	.250		
	Total	24.960	99			
Do you monitor any quality parameter at farm level?	Between Groups	2.092	11	.190	1.475	.155
	Within Groups	11.348	88	.129		
	Total	13.440	99			
Do you keep any farm records?	Between Groups	1.807	10	.181	.750	.676
	Within Groups	20.969	87	.241		
	Total	22.776	97			
Do you have withdrawal period for treated cows?	Between Groups	.461	11	.042	.713	.723
	Within Groups	5.179	88	.059		
	Total	5.640	99			

The difference between the practices have no significance $P>0.05$, while F-ratio revealed variation in dairy practices hence justifying null hypothesis. While the method of milking has significance ($p<0.05$) in outcome of produce quality. The mean between the groups are not equal (Table 3.6)

3.5 Discussion

3.5.1 Treatment and antibiotics use at the farm.

From the present study, quite a substantial number of participants understood the use of antibiotic, but a need to take it seriously in terms of monitoring and understanding dangers that comes with its presence in milk is paramount. They understood the use of records, but their use is minimal; hence change is inevitable. Disposal of milk from treated animals varied across the participants, therefore, showing a minimal understanding of dangers that come with antibiotics in milk. Findings in the present study partly agree with the earlier study on smallholders, which showed a poor understanding of antibiotic residues in milk (Ndugu *et al.,* 2016*).* The farmers do not understand the risks associated with antibiotics since rejected milk is either given to pets or given for feeding the calves by secretly selling back to brokers. They have minimal know-how on drugs administration hence solely depend on veterinary guidance on their use. All farmers should know health hazards exist when it comes to selling milk during antibiotic treatment, and maybe they do, but the problem could be an economic one. Most smallholder farmers operate subsistence farming, not farming as a business, making it understandable why a farmer is forced to sell the milk. According to Shitandi and Sternesjö *(*2004*)*, the farmers' most common reason for not discarding milk from recently treated cows was limited food supply and poverty, which differs partly from the present study. An earlier study shows that the origin of antibiotic residues is mostly at farm-level, which partly agrees with the findings of the present study, though farmers have little knowledge about it and the necessary control measures at farm level (Omore *et al.*, 2005). Currently, big players such as Brookside Dairy Company have implemented rapid antibiotic detection at their cooling collection stations all over the country.

3.5.2 Farm management and records keeping at the farm

Farms have not adapted to records; they find them tedious since they do not take farming as a business. From previous studies, the finding was similar to the present study in that only the large scale farmers were serious in record keeping (Shitandi, 2004). This helps in monitoring the productivity and the prospects of dairy farming as the business. Farmers are encouraged to adopt records keeping to enhance dairy management and promotion of productivity in terms of economic gain. This also helps to deter misuse of material and health management of the herd and improve in breeds (FAO, 2011). In case of future treatment by a different veterinarian, it eases the retrieval of medical history. Efficient record keeping enhances accountability and prospective future growth of a farming business. The records enhance knowledge in ancestry origin, heat period dates, breeding, pregnancy, bulls used, vaccinations, and milk production performance growth rates (Moran, 2017).

3.5 Conclusion

The current study demonstrated that farming practices and dairy management are lacking at the farm level; this has been contributed to by the nature of the dairy sector, which is characterized by its small scale holding. The study depicted the use of antibiotics at the farm in the treatment of sick animals; the farmers, despite having withdrawal knowledge, there is laxity in implementation hence posing a risk to consumer health.

3.6 Recommendations

The farmers should also be trained on the risks associated with the milk of animals under treatment. The farmers should be sensitized on quality control checks at the farm and the need to produce safe milk. The study also recommends further research on the risk assessment of antibiotics in dairy products.

CHAPTER FOUR: PREVALENCE OF ANTIBIOTIC RESIDUES IN RAW MILK CONSUMED IN KIAMBU COUNTY, KENYA

4.1 Abstract

Food safety has been wanting in the recent past leading to an increase in chronic illnesses, which leads to morbidity and mortality in the developing world. These challenges have prompted research in the milk production sector, focusing on quality control, regulation of trade in milk, and maximum level of food safety awareness among the farmers. The present study aimed to find the prevalence of antibiotics residue in raw milk produced and consumed in Kiambu County, Kenya. A cross-sectional study was carried out to examine the level of drug residues in milk produced by dairy farmers in Gatundu South Constituency, segmented into three regions: Kiganjo, Gatei, and Gatundu town. Purposive sampling technique was used to select 33 participants in each locality. The samples were screened using BIOEASY rapid testing kit (CODE YRM1007-40). The positive samples were further subjected to quantitative analysis using UHPLC-MS/MS 6460 (QuEChERS KIT LC-MS/MS).

The study revealed the presence of 10% antibiotics in the samples. Across the study area, 10% of the milk samples tested positive for beta-lactam on screening through the rapid test. The three regions performed as follows: Gatei (11.7%), Gatundu (6%), and Kiganjo (12.1%). Tetracycline and sulphonamides tested negative on rapid test screening using bio easy, rapid kit. Eight drug residues were identified and quantified as follows: Ampicillin (0.007±0.0µg/ml), Amoxicillin (0.020±0.022µg/ml), Cloxacillin (0.008±0.004µg/ml), Dicloxacillin (0.007±0.0µg/ml), Nafcillin (0.010±0.004µg/ml), Oxacillin (0.009±0.002µg/ml), Penicillin G (0.016±0.017µg/ml) and Phenoxymethyl-penicillin (0.009±0.005µg/ml). Food safety among dairy farmers in Kiambu County is wanting. Consequently, there is a need to put in place controls in health management besides training, food safety surveillance, infrastructural development, code of practice, and inspections to enhance the quality and safety of dairy products.

4.2 Introduction

Alexander Fleming discovered penicillin in 1928 as the first natural antibiotic. Antibiotics either kill or slow the growth of bacteria (Brady and Katz, 1988). When used correctly, antibiotics are powerful medicines in fighting infections; they stop bacterial growth or destroy them. Antibiotics are extensively used in disease control and safeguarding the nutritional well-being of livestock (FAO, 2017). The aspect of food safety in milk has, in the recent past, received much attention due to health liability in society (Baur *et al.,* 2017). The social-economic development in a nation can be realized when the health of the nation is prioritized. Milk contributes a large portion of the diet; it is used as a supplementary feed. If the milk is consumed regularly, it may cause a build-up of antibiotics in the body; hence resistance to drugs and allergic reactions may occur (Muriuki *et al*., 2011). This may lead to challenges in disease control, therefore, impacting negatively on the economic development of the nation. (Kosgey, Shitandi, and Marion 2018). Antibiotic residues also cause inhibitory effects during the manufacture of fermented products in the dairy industry . The incidence of antibiotic residues in milk can be managed through proper health management and a quick check of fresh lactating cows, identification, and isolation of affected herd. Antibiotic residues are remnants of antibiotics or their active metabolites that are present in milk from treated animals (Codex Alimentarius Commission, 2015), and the drugs and their metabolites may persist at unacceptable levels, and consumers can be exposed to them (CAC, 2004), most of which occur due to treatment of dairy animals for mastitis through intra-mammary or intravenous infusions (Batavani *et al*., 2007). The residues' presence may result from failure to observe the mandatory withdrawal periods, illegal or extra-label use of drugs and incorrect dosage. Many drugs are also retained in the animal's body for longer times than indicated on the label. (Ivona *et al., 2002*).

In Kenya, Shitandi (*2001*) showed that 21% of 1109 samples were positive for antibiotics, while Kang'ethe *et al. (2005*) found a 16% prevalence of antibiotic residues. In Nakuru County, Kenya, Sternesjo, and Shitandi *(2001)* demonstrated that 21% of the milk sampled contained antibiotic residues, of which 14.9% of these contained Penicillin-G type residues. However, Ombui *(1994)* failed to detect any antibiotic residues in bulked milk received at Dairy Cooperatives in Kiambu County, Kenya, probably due to a high level of awareness on withdrawal periods among farmers delivering the milk (Orwa *et al.*, 2017). According to the Centers for Disease Control (CDC, 2013), dairy cattle treated with antibiotics produce milk containing antibiotic residue for a while after treatment. Milk from treated cows should be excluded supply chain; proper withdrawal period should be addressed (CDC, 2013). The most common groups of antibiotics are tetracyclines (tetracycline), beta-lactams (penicillin), aminoglycosides (streptomycin), and sulphonamides (sulfamethazine) (Brandy *et al*., 1988).

4.3 Materials and methods

4.3.1 Study area and study design

A cross-section study was carried out across Gatundu South Sub-County, altitude 1.0500 (1° 1'0" S), longitude 36.9200 (36°56' 0" E). The study area was selected based on an increase in milk demand in the peri-urban centers, which are within the proximity. Three main areas were mapped for the study, namely Gatei, Kiganjo, and Gatundu town (Figure 4.1).

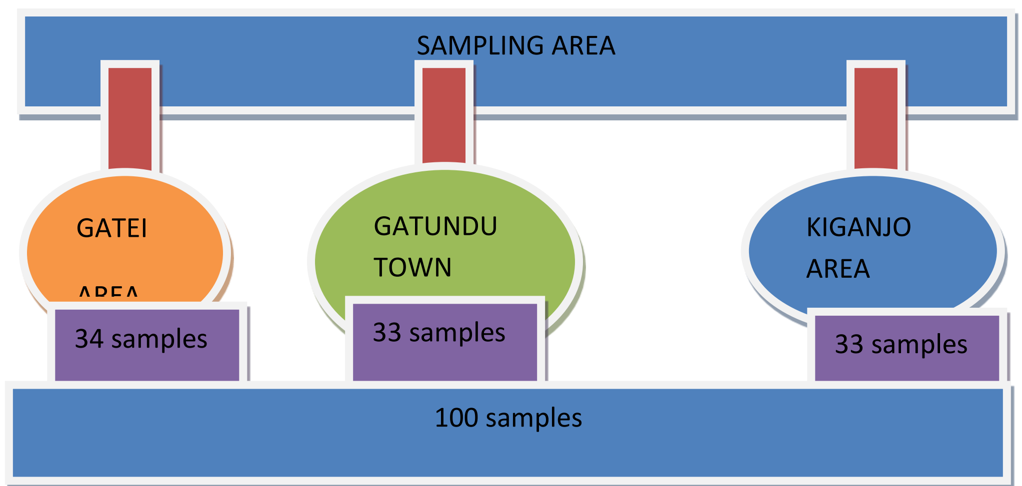

Figure 4 1: Sampling area and samples

4.3.2 Sampling procedures

The randomized sampling process was used in the three mapped areas. The three areas being, Gatei, Gatundu town, and Kiganjo. From each area, milk samples were taken from an average of 33 farmers (Figure 4.1), two samples from each morning and evening. They were labeled and preserved in cool boxes for further analysis.

4.3.2 Qualitative analysis (samples preliminary screening)

The screening of the sample was done using a bio easy testing kit. The kit comes with an incubator, 40±2c°, and a micro-dispensers (figure 4.2). The detection and discrimination are based on the colloidal gold immune-chromatography technology (Brendan, 2013). The test kit (CODE YRM1007-40) constituted 12 test tubes, each containing one strip of 8 red reagent micro-wells and eight dipsticks, 1pc pipette 200µl 100pcs pipettes tips, positives standards, and negative standards, plate holder and a timer.

Figure 4 2 : An incubator and µ dispenser

4.3.3 Test procedures

Two hundred milliliters (200µl) milk samples were drawn into the reagent micro-well and mixed by pipetting up and down 5-10 times. It was incubated 3mins at 40±2°C. Dipstick was inserted into the micro-wells after first incubation and was incubated another 5 minutes at 40±2°C (Figure 4.2). The dipstick was taken out from the micro-well, and it was removed from the sample pad at the lower end and was interpret the result. The dipstick was interpreted (visually) by checking the top control line (C-line) is present. If it was found, it was compared with the color intensity of the test line (T-line) and C-line and interpret based on the chat (Table 4.1)

Table 4. 1: Test interpretation on sample screening using bioeasy rapid kit

Test line vs control line	Result interpretation	Result analysis
T>C	NEGATIVE	No antibiotics or low level below detection limits
T=C	WEAK POSITIVE	The sample contains antibiotics or Close to the detection limit
T>C OR NOT	POSITIVE	The sample contains antibiotics above detection limits

4.3. Quantitative analysis

4.3.1 LC-MS/MS Conditions

Chromatographic analysis was performed using Agilent Ultra High-Pressure Liquid Chromatography UHPLC (ACES/EQU/O9 MODEL 75004250 THERMO SCIENTIFIC, USA), using 6460 mass spectrometers (Triple Quadrupole Tandem MS Agilent Technology). With an injection volume of 5.00 L/h and a quat pump 0.2ml/min. The mobile phase constituted of 0.1% formic acid in water and CAN ACETONIC NITRATE. It was subjected to a pressure varied from 200.00bars (min) to 1300.00bars (max) with a stop time of 23 minutes. The separation was achieved using Column ZORBRAX eclipse plus C18 Rapid resolution HD 2.1X50MM

1.8MICRO (Agilent Technology, USA), oven column temperature 40 °c, with a flow rate of 0.2Ml/min.

As shown in Table 4.2, the injection volume was defined with 20 µl. The gradient started with 98 % mobile phase A ($H2O$, CAN ACETONIC NITRATA 0.1%) holding for 15 min, decreasing to 98 % within 1.6 min, followed by a further decrease to 5 % the next 5 min. This composition was held for 2 min, followed by an increase to 100% mobile phase B (CAN ACETONIC NITRATE, 0.1 %) within 1 min, held for 2 min. For column equilibration, initial mobile phase composition (95% solvent A) was reached within 5 min. According to this gradient, the complete separation was fulfilled within 20 min.

Table 4. 2: Mobile phase for water (A)% and CAN Acetonic 0.1 (B) %

TIME (MIN)	WATER A%	CAN ACETONIC 0.1 B%
0.00	98	2
1.5	98	2
1.6	98	2
15	5	95
20	5	95

4.3.2 Analysis using Agilent bond elute

The analysis was based on the method described by Jank *et al.* (2015). 15ml of the sample (milk-free fat) was measured into a 50ml centrifuge tube and was spiked. Then 15ml solution of 1% acetic acid in acetonitrile was added to each tube then shook (vortex) for 1 min. Then 4g of sodium sulfate and 1g of sodium chloride were added (Bond elute extraction salt packet P/N5982-0032). It was vigorously shacked for 1 min and centrifuged at 5000rpm for 10 min at 4°c. Aliquot of upper acetonitrile layer 8ml was transferred into 15 ml PTFE centrifuge tube containing 50mg of PSA and 150mg C18EC and 900mg of anhydrous NA_2SO4 (Bond elute dispersive SPE >VET drugs in foods P/N 5982-4950) –cap and vortex for 1 min extract, centrifuge at 500rpm for 10min. The extract was transferred into another tube for drying by use of nitrogen flow at 40°c. The extract was resolved into 1ml of MeOH/H_2O (1/9, V/V), then filter residue through a 0.45µm membrane, analyzed by LC-MS/MS.

4.3.3 Data analysis

The data was analyzed using SPSS Version 23 software (IBM Corp., 2015). Descriptive statistics were generated to describe variables based on dairy practices concerning food safety knowledge—such descriptive includes; mean± standard deviation, frequencies, range minimum, and maximum score.

ANOVA was used to test the mean difference within antibiotics derivatives analyzed. The Tukey test was used to compare the mean within antibiotic derivatives. Statistical significance was tested at $p>0.05$.

4.4 Results

4.4.1 Antibiotic residues screening results

The screening results revealed that 10 % of all the samples from the three areas of Gatundu South Sub-County were positive for beta-lactam on screening. The proportion of the samples that tested positive for beta-lactam in each of the three areas was: Gatei (11.7 %), Kiganjo (12.1 %), and Gatundu (6 %). The tests for tetracycline and sulfonamides were negative in all the samples from the three areas (Table 4.3).

Table 4. 3: Antibiotic residues screening results

AREA	BETA-LACTAM	T-CYCLINE	SULFONAMIDES	PERCENTAGES
GATEI	4+	NIL	NIL	11.7%
GATUNDU	2+	NIL	NIL	6%
KIGANJO	4+	NIL	NIL	12.1%
TOTAL	10	NIL	NIL	10%

4.4.2 Quantitative levels of antibiotic residues in milk samples

The survey conducted in the Gatundu South sub-county showed the presence of antibiotics residue in milk. Through quantitative analysis, it is evident that antibiotic residues were present in the samples that were positive for beta-lactam (Table 4.4). The analytical standards were varied in concentration (ppb) of 5, 10, 20, 100, and 150 for solvent standard and in the calculation of standards' matrix; British Standard (BS) 2019 (Appendix 3). The various derivatives of beta-lactam found in the samples were: Amoxicillin, Ampicillin, Cloxacillin, Dicloxacillin, Nafcillin, Oxacillin, Penicillin G, and Phenoxymethylpenicillin. All sample showed no significant difference ($p>0.05$) in concentration on the beta-lactam in morning and evening milk (Table 4.5) The mean for respective antibiotics were calculated as follows: 0.020±0.22, 0.070±0, and 0.016±0.017 µg/ml for Amoxicillin, Ampicillin, and Penicillin G, the codex MRLS being 0.004 µg/ml (CODEX, 2015) which were above the recommended MRLs, while cloxacillin, dicloxacillin, oxacillin, nafcillin, and phenoxy methyl showed 0.080±0.004, 0.0070±0, 0.009±0.002, 0.010±0.004, and 0.009±0.005 µg/ml respectively, were below codex MRLs being 0.030 µg/ml (CODEX, 2015).

Table 4. 4: statistical output of eight antibiotic residues from the analyzed samples (µg/ml) mean, at confidence interval at 95% and the p-value.

	Time			Statistic	Std. Error	p-value
Amoxicillin	Morning	Mean		.01340000	.004334513	
		95% Confidence Interval for Mean	Lower Bound	.00136546		
			Upper Bound	.02543454		
		Std. Deviation		.009692265		

		Minimum		.005200		0.366
		Maximum		.029400		
	Evening	Mean		.02658000	.013043711	
		95% Confidence Interval for Mean	Lower Bound	-.00963515		
			Upper Bound	.06279515		
		Std. Deviation		.029166625		
		Minimum		.006600		
		Maximum		.077400		
Ampicillin	Morning	Mean		.00712000	.000058310	
		95% Confidence Interval for Mean	Lower Bound	.00695811		0.391
			Upper Bound	.00728189		
		Std. Deviation		.000130384		
		Minimum		.007000		
		Maximum		.007300		
	Evening	Mean		.00730000	.000189737	
		95% Confidence Interval for Mean	Lower Bound	.00677321		

			Upper Bound	.00782679		
		Std. Deviation		.000424264		
		Minimum		.007000		
		Maximum		.008000		
Cloxacillin	Morning	Mean		.00644000	.000823772	0.123
		95% Confidence Interval for Mean	Lower Bound	.00415284		
			Upper Bound	.00872716		
		Std. Deviation		.001842010		
		Minimum		.005100		
		Maximum		.009600		
	Evening	Mean		.01032000	.002096521	
		95% Confidence Interval for Mean	Lower Bound	.00449912		
			Upper Bound	.01614088		
		Std. Deviation		.004687963		
		Minimum		.006200		
		Maximum		.018300		
Dicloxacillin	Morning	Mean		.00672000	.000124097	

		95% Confidence Interval for Mean	Lower Bound	.00637545		0.180
			Upper Bound	.00706455		
		Std. Deviation		.000277489		
		Minimum		.006400		
		Maximum		.007000		
	Evening	Mean		.00672000	.000182757	
		95% Confidence Interval for Mean	Lower Bound	.00621259		
			Upper Bound	.00722741		
		Std. Deviation		.000408656		
		Minimum		.006400		
		Maximum		.007300		
Nafcillin	Morning	Mean		.00840000	.001012423	
		95% Confidence Interval for Mean	Lower Bound	.00558906		
			Upper Bound	.01121094		
		Std. Deviation		.002263846		
		Minimum		.006000		

		Maximum		.011800		0.457
	Evening	Mean		.01174000	.002034355	
		95% Confidence Interval for Mean	Lower Bound	.00609173		
			Upper Bound	.01738827		
		Std. Deviation		.004548956		
		Minimum		.008500		
		Maximum		.019700		
Oxacillin	Morning	Mean		.00820000	.000423084	0.376
		95% Confidence Interval for Mean	Lower Bound	.00702533		
			Upper Bound	.00937467		
		Median		.00830000		
		Variance		.000		
		Std. Deviation		.000946044		
		Minimum		.007100		
		Maximum		.009600		
	Evening	Mean		.00914000	.001125877	
		95% Confidence Interval for Mean	Lower Bound	.00601406		

			Upper Bound	.01226594		
		Std. Deviation		.002517538		
		Minimum		.006900		
		Maximum		.013200		
Penicillin	Morning	Mean		.00738000	.002002598	0.519
		95% Confidence Interval for Mean	Lower Bound	.00181990		
			Upper Bound	.01294010		
		Std. Deviation		.004477946		
		Minimum		.000000		
		Maximum		.011500		
	Evening	Mean		.01662000	.009646834	
		95% Confidence Interval for Mean	Lower Bound	-.01016390		
			Upper Bound	.04340390		
		Std. Deviation		.021570976		
		Minimum		.001000		
		Maximum		.054500		
	Morning	Mean		.00800000	.001197080	

Phenoxymethylpenicillin		95% Confidence Interval for Mean	Lower Bound	.00467637		0.516
			Upper Bound	.01132363		
		Std. Deviation		.002676752		
		Minimum		.006400		
		Maximum		.012700		
	Evening	Mean		.01012000	.002905065	
		95% Confidence Interval for Mean	Lower Bound	.00205425		
			Upper Bound	.01818575		
		Std. Deviation		.006495922		
		Minimum		.006300		
		Maximum		.021600		

All the antibiotic residues under the current study were detected in the sampled milk regardless of the sampling time (Table 4.4). There were no significant differences ($p>0.05$) between the morning and evening samples among all the drug residues. There was no association ($p>0.05$) between the levels of the antibiotics and the time of milking. Therefore the risk of exposure to antibiotics intoxication would not be influenced by the time of the day the milk .was obtained. The levels of most antibiotics were observed to be lower than the recommended levels (Table 4.5); therefore, within safety levels.

Table 4. 5 Level of antibiotics residue in morning and evening milk μg/ml

SOURCES	TIME OF THE DAY		
	Morning	Evening	Total
Amoxicillin	0.013±0.010	0.027±0.029	0.020±0.022
Ampicillin	0.007±0.0	0.007±0.0	0.007±0.0
Cloxacillin	0.006±0.002	0.010±0.005	0.008±0.004
Dicloxacillin1	0.007±0.0	0.007±0.0	0.007±0.0
Nafcillin	0.008±0.002	0.012±0.005	0.010±0.004
Oxacillin	0.008±0.001	0.009±0.003	0.009±0.002
Penicillin G	0.016±0.015	0.017±0.022	0.016±0.017
Phenoxymethylpenicillin	0.008±0.003	0.010±0.006	0.009±0.005

Table 4. 6 Correlations between antibiotics residue level and milking time. Confidence Interval (CI) 95% with Pearson Correlation

SOURCES	Time of the day	
	Pearson Correlation	P-value
Amoxicillin	-0.32	0.37
Ampicillin	-0.31	0.39
Cloxacillin	-0.52	0.12
Dicloxacillin	0	1
Nafcillin	-0.46	0.18
Oxacillin	-0.27	0.45
Penicillin G	-0.03	0.51
Phenoxymethylpenicillin	-0.23	0.52

There was no significant difference ($p>0.05$) among the various antibiotic residues quantified in different samples. Dicloxacillin indicated that there is no linear relationship ($r=0$) with other antibiotic residues, all others indicates decrease in linear relationship ($r<0$) among the variables (Table 4.6)

4.5 Discussion

The present study has revealed the presence of antibiotics residue in milk (Table 4.4), posing a great risk to the health of ultimate consumers. Previous findings have concurred with the present in spearheading stringent measures in food safety and quality campaigns (FOA, 2011). From this study, the challenges of food safety in developing countries are wanting; there is loose adherence to regulation and guidelines that promote food safety; this has also be revealed in a previous study (Van Boeckel *et al*., 2015). Additionally, the presence of antibiotics has shown that consumers are exposed to great health risks (Aboge *et al*., 2000; ILRI, 2018). The subsistence nature of dairy farming, coupled with social-economic challenges, has greatly contributed loss safety guidelines. The farmers tend to use a shortcut to evade losses, hence compromising food safety (Kang'ethe *et **al***., 2005).

The amorphous nature of the dairy industry in Kenya, the majority being small scale farmers, accounts for more than 90% of milk production, and most farmers keep between one and five cows. It has been revealed that poor infrastructure and inadequate skills have significantly contributed to compromising food safety (KBD, 2016).

The prevalence of antibiotics in milk samples may be as a result of poor farming practices with farmers most likely injecting the drugs to the animals at any time of the day. Furthermore, the excretion rate of the drugs through milk could most likely be slow and lasting for several days and, therefore, detect milk. These may be as a result of overdosing the animals or lack of observation of an adequate withdrawal period of the milk from the markets after treatment. However, the levels of Ampicillin, Amoxicillin, and Penicillin G were much higher than the recommended safety levels in fresh milk. From the previous studies, it was revealed that beta-lactam-based antibiotics had been widely used in cattle therapy, particularly for the treatment of mastitis (Sachi *et al.,* 2019)

From the results in Chapter 3 of this dissertation, quite a substantial number of participants understood the use of antibiotics (Table 3.5), but there is a need for serious monitoring and understanding of the dangers that come with its presence in milk. They understood the use of records, but their use is minimal; hence change is inevitable (Table 3.5). Disposal of milk from treated animals varied across the participant, therefore, showing a minimal understanding of dangers that come with antibiotics in milk. Findings in the present study partly agree with the earlier study on smallholders, which showed a poor understanding of antibiotic residues in milk (SDP, 2005). The risk-based approach to food safety is the most effective way to curb risk associated with antibiotics residue, farm-to-fork tactic emphases on economic prevention and controls in the supply chain, and support capacity building and providing market incentives for food safety management (Unnevehr, 2015). Policy and legislative framework for food safety and quality, sufficient infrastructure, and properly trained inspectors are required if responsible authorities are to function efficiently. This would provide a coordinated and preventive approach to food safety management along the milk value chain (Kenny, 2013). Acclaimed good farming practices, which include: animal health management, milking hygiene, feeding management, animal welfare, environment, and socio-economic management are important management tools toward an effective food safety system. The focus is to minimize contamination at the farm-level by control of microbial contaminants in feed, facility hygiene, cleanliness of cows, good animal health management, effective cleaning and disinfection procedures of milking equipment, and use of efficient, suitable milk handling equipment (Giffel and Wells-Bennik, 2010)

Disease prevention lessens the usage of antibiotics in the farm; it entails good health management, good husbandry practices, biosecurity at the farm level, proper diagnosis and prompt treatment of diseases, and stringent measures on withdrawal periods for the treated animals. Consequently, following recommended milk-withdrawal period and use of feed for the intended animals with considerably lowers drug residues in milk. The proper method of testing should be put in place to ascertain the well-being of the milk from treated animals (Bohm et al., 2009).

Appropriate record-keeping of the treatment program in the farm should be adhered to; they should take account of; bio-data, dates and time of treatment, the antibiotic used, its dosage, rate of recurrence and route of administration, the milking withdrawal period of the antibiotics used and regular monitoring of the treatment records. It is also important to keep milk from cows that are receiving oral, intramuscular, udder infusion, or intrauterine antibiotic treatments out of the milking line. Milk all treated animals last and discard milk from all the four quarters of the udder, even if only one quarter was treated. Test all purchased herd replacements before introducing them into the milking herd (Giffel *et al*., 2010).

4.6 Conclusions

The current study shows antibiotic residue in cow milk above the MRLs set by Codex; this is a potential health hazard to the consumer. The results suggest that the farmers do not follow stipulated guidelines for alleviating antibiotic residue in milk. The quantified antibiotics identified were mainly beta-lactams; of these, with concentrations above MRLs were penicillin G, ampicillin, and amoxicillin. Poor farm management practices directly contribute to veterinary drugs in milk and milk products.

4.7 Recommendations

A rapid test in the cold chain should be done to avert compromise in food safety.. Food safety and quality practices should be carried out continously to avert disease spread. The revenue and resources should be geared towards improvement in farm management, infrastructure, quality surveillance, control, and support services., A well-coordinated risk analysis system should be put in place to help in upholding food safety and quality

CHAPTER FIVE: QUANTITATIVE RISK ASSESSMENT OF PENICILLIN G. INTAKE THROUGH CONSUMPTION OF MILK IN KIAMBU, KENYA

5.1 Abstract

The amorphous structure in Kenya's dairy sector wants in terms of quality controls. Farmers lack proper guidance at the grass-root, in terms of quality control of their produce. This brings with it the emergence of uncoordinated milk trade in urban and peri-urban areas. The issue of food safety has not been given attention along the milk supply chain, hence putting consumers at risk. This study used primary data from milk produced in Gatundu South sub-County through a cross-section survey and secondary data from re-known scientific journals such as Science Direst journals, Elsevier, and Spring. Also used were reports from world organizations such as FAO, Codex, and WHO.

The results showed that the most common antibiotic was beta-lactams, including ampicillin, amoxicillin, cloxacillin, dicloxacillin, nafcillin, penicillin G, and phenoxymethylpenicillin. The amount of milk consumed daily was found to be 514 ml per person, which agreed with other studies 438.3 - 657.5 ml per person. The risk assessment model based on penicillin G revealed that daily intakes were 15 µg/person/day, amoxicillin (18.30 µg/person/day), cloxacillin (7.34 µg/person/day), ampicillin (6.12 µg/person/day), dicloxacillin (6.12 µg/person/day), nafcillin (8.57 µg/person/day), oxacillin (7.34 µg/person/day), and phenoxymethylpenicillin (8.57 µg/person/day); taking the average weight of adult consumers of 61kg bodyweight. For penicillin G, this was much low compared to standard ADI set at 30 µg /person/day as well as other which had low ADI below the set standard. The population under study was safe as per the set standards by the CODEX. The enforcement of already laid infrastructure in terms of legislation should be taken seriously, and sensitization on danger caused by antibiotic residues. This can be accomplished through farmer's field day seminars and short dairy farming courses to farmers by agricultural extension officers.

5.2 Introduction

Ensuring food safety to protect public health and promoting economic development in both developed and developing countries remains a big challenge. The provision of safe food to ever-growing world population becomes a significant hurdle; ensuring its safety is being prioritized to reduce the incidence of foodborne illness and accrued economics loss (CAC, 2017) To reduce food safety challenges, a science-based approach has been developed in a well-structured manner for appropriate response where necessary. Risk analysis is being adopted to caution food safety challenges. It allows the use of data on food hazards associated with the foodborne disease epidemiological data, making it easier to determine risk in human health (CAC, 2017). Risk assessment is a vital tool in ascertaining the severity of food safety hazards. An unregulated food vendor plays a significant role in the day to day food supply to the urban/ peri-urban population (Moran, 2015). In the developing world, unscrupulous food traders contribute significantly to the food supply in an informal settlement (Muriuki, 2007).

Risk analysis has been an essential tool in improving food safety along the value chain. An effective food safety system is crucial in maintaining consumer confidence, and it provides an infrastructure for sound domestic and international trade. Risk analysis has been proved as an essential tool in improving food safety decision-making processes. It offers a structure through which governments effectively assess, manage, and communicate food safety risks in cooperation with the various stakeholders involved. Also, it provided a process to establish realistic, science-based targets to reduce the incidence of food-borne disease. Through proper planning and implementation of interventions, and monitoring of the outcomes, either successful or unsuccessful, risk analysis contributes to continuous improvements in food safety (CAC, 2004).

The increase in food demand due to globalization of the food trade and changing consumption patterns have called for the stringent measure in promoting food safety across the world (FAO, 2011). To match the demand, agriculture has been intensified to maximize production against ever reducing agricultural land. Additionally, increased travel and tourism, and new types of production and manufacturing systems are among the trends calling for improved food safety system. As much as food safety measures are put in place, the number of existing and new food safety hazards is increasing; among them are; veterinary drugs residue, fertilizers, mycotoxins, and feed additives others. Other challenges are also emerging of new pathogens and evolving of existing ones. The primary concern is the increase in resistance of food-borne pathogens to anti-microbial agents (WHO, 2012)

The presence of antimicrobial in food production has been a big hurdle in food safety and quality assurance. When humans ingest antimicrobial-resistant microbes in food, they may result in illness, while others can be sources of transferable resistance microbes. In 2006 FAO established a task force to develop a science-based approach to managing human health risks associated with Antimicrobial resistance organisms (AMR) from food and animal feeds (FAO, 2019). Food safety systems are a vital tool in improving public health and increase consumer confidence upon food systems. The Sanitary and Phytosanitary Agreement (SPS) is geared towards protecting life and upholding people's health internationally (CAC, 2017). The agreement ensures that countries enforce SPS measures based on an assessment of risk to human health, animal, or plant life. These measures foresee legitimate in food trade operations globally. It also obliges developed to assist developing countries to improve their food safety systems (FAO and WHO, 2012).

The first step in identifying food safety problems is articulating its nature and characteristic. It may require more information, or the information present may be sufficient to conduct a risk assessment. Food safety regulators get information on food safety through; inspection, environmental monitoring, laboratory, epidemiological, clinical, toxicological studies, disease surveillance, outbreak investigations, monitoring of contaminants in foods, permit application. The risk manage a designed model synchronized in a way an identified problem is scientifically resolved in a structured way. First and foremost, the problem is identified, a risk profile is generated, and the problem is ranked to give its severity. The best option to deal with the problem is identified and implemented under the risk manager's guidance. It evaluated to review the result and achievement on the set goals (FAO, 2004).

5.3 Materials and methods

5.3.1 Sampling, sample size, and sample collection

The antibiotics data obtained in chapter four was used to conduct a qualitative risk assessment for this chapter. All eight antibiotics derivatives identified were used to establish risk factors.

The use of Fischer's formula determined the sample size. A simple random sampling technique was used to administer the questionnaire consumers Ruiru town who participated in the study. To determine the sample size, Fischer's formula (Fischer *et al.*, 1991) was used. Where standard deviation was set at 95% confidence interval, with a degree of accuracy set at ±10%, those under study were estimated to be 50% of milk consumers from vender while those who were not 50%. The output was 96 consumers with an addition of four to cater for attrition, hence a total of 100 participants.

The study targeted the dweller of roar town being the primary market Centre of Gutundu South sub-county. Simple random techniques of sampling were used to administer the questionnaire using simple structured questions for descriptive purposes. The data was documented for further analysis using statistical

5.3.2 Study instrument

A questionnaire on consumption pattern was prepared and pretested in the Ruiru town. The questionnaire helped to determine; the age of the consumer, level of education, body weight, amount of milk consumed/day/week, estimate height, and occupation (Appendix 4).

5.3.3 Risk assessment tool

The milk consumption data was obtained based on daily consumption of milk(Appendix 5) and was calculated by dividing the weekly intake of milk (ml/person) by respondents' body weights and dividing again by seven days WHO/FAO (2011) to obtain the amount of milk consumed per kg body weight per day. The Penicillin G distribution in milk was obtained by dividing the levels of the antibiotic per ml of milk while the intake levels were calculated by multiplying the amount of Penicillin G and the milk consumption to obtain the amount consumed per kg body weight per day. The average exposure and 95th percentile (P95) were obtained to estimate margins of exposure (MOE) for risk characterization. Monte Carlo simulation for exposure using 1,000, 000 iterations was performed to determine variability.

5.3.4 Data analysis

SPSS version 23 was used to calculate the descriptive statistics of the antibiotics analyzed. The risk estimate was generated using a Monte Carlo simulation on a scale of 0-1,000,000 iterations.

5.4 Results

Mean Milk Consumption By Gender

Table 5: 1 Group statistics on milk consumption between male and female

	Sex	N	Mean	Std. Deviation	95% C.I)	Std. Error Mean	P-value
Milk_ Consumption	Male	47	368.0851	123.54612	331.81-404.35	18.02105	0.232
	Female	53	336.7925	135.21740	299.52-374.06	18.57354	

No significant difference between gender and milk consumed by gender (p>0.05)

Using the Kolmogorov-Smirnov test to test for uniformity and normality of milk consumption, there milk intake per day is not uniform (normal) (p<0.05). Looking at the mean and median in this scenario means it is not the best measure of milk consumption viz a viz Median is the best.

Table 5. 2: Statistical output of mean weight and milk consumption based on ml/kg BW

			Statistic	Std. Error	p-value
Weight	Mean		61.1515	1.08649	0.015**
	95% Confidence Interval for Mean	Lower Bound	58.9954		
		Upper Bound	63.3076		
	Std. Deviation		10.81039		
	Minimum		44.00		
	Maximum		94.00		
	50th Percentile (Median)		58.00		
	95th Percentile		82.95		
Ml/kgBW	Mean		5.9762	.23197	0.072
	95% Confidence Interval for Mean	Lower Bound	5.5158		
		Upper Bound	6.4365		
	Std. Deviation		2.30810		
	Minimum		1.41		
	Maximum		10.23		
	50th Percentile (Median)		6.02		
	95th Percentile		10.0		

Statistics for Weight and Ml/kg BW is the Kolmogorov-Smirnov test. Since 50th percentile for weight and Ml/kg BW is close to the mean, it's reliable.

The result showed average milk consumption ranging from 0 to 18ml/kg body weight per day, with an average of 8.47ml/kg body weight (Table5.3). The samples showed an averaged 0.01 units/ml of the analyzed milk samples. The intake from consuming contaminated milk ranged from zero to a maximum of 0.35 units/ml, the maximum intake of Amoxicillin (0.35μg/kg BW/day) while the minimum was Ampicillin and Dicloxacillin (0.06μg/kg BW/day). (Table 5.5).

The results revealed from the model output showed a likelihood of intakes, highest being amoxicillin (18µg/person/day), with dietary exposure of 0.30µg/kg bwt/day. The lowest was ampicillin and dicloxacillin with 6.12µg/person/day and dietary exposure of 0.10µg/kg bwt/day. All the results were based on the margin of exposure (MOE) from the respondent, with an average weight of 61.2kg body weight (Table 5.7)

Table 5. 3: Results for distribution fitting and simulation used for quantitative risk assessment milk consumption

	Mean output	90% CI	
		Min	Max
Milk Consumption ml/kgbw/day	8.47	-0.37	18.38

CI-Confidence interval, Bwt-body weight, Min-minimum intake, Max-Maximum P95-95th percentile, Min-minimum intake MOE- Margin of Exposure. The MOE was based on the respondents' average weight, 61.2 kg.

Table 5. 4: Estimated margins of dietary exposure to antibiotics through milk consumption

Antibiotics	Mean consumption of antibiotics µg/ml/kgBw/day	90% CI		MARGIN OF EXPOSURUE BASED ON 61.2KGS (p95)
		MIN	MAX	
Amoxicillin	0.17	-0.02	0.35	18.30
Cloxacillin	0.07	0.00	0.14	7.34
Ampicillin	0.06	-0.10	0.13	6.12
Dicloxacillin	0.06	-0.10	0.13	6.12
Nafcillin	0.08	-0.01	0.17	8.57
Oxacillin	0.07	0.00	0.14	7.34
Penicillin G	0.14	-0.01	0.30	15.30
Phenoxymethyl	0.08	-0.01	0.17	8.57

P95-95th percentile, Min-minimum intake MOE- Margin of Exposure. The MOE was based on the respondents' average weight, 61.2 kg

Table 5. 5: Summary of distribution fitting and simulation, mean intakes, mean output(90% CI), estimated dietary exposure to antibiotics at p95-95th percentile.

ANTIBIOTCS	mean µg/ml	MEAN µg/ml/kgBW/day	Margin Of Exposure (MOE) (61.2kg)		
			µg/person/day	mean-out (µg/kg) 90%CI	p95
Amoxicillin	0.01999	0.17	10.40	0.30	18.30
Cloxacillin	0.00838	0.07	4.28	0.12	7.34
Ampicillin	0.00721	0.06	3.67	0.10	6.12
Dicloxacillin	0.00672	0.06	3.67	0.10	6.12
Nafcillin	0.01007	0.08	4.90	0.14	8.57
Oxacillin	0.00867	0.07	4.28	0.12	7.34
Penicillin G	0.0162	0.14	8.57	0.25	15.30
Phenoxymethyl	0.00906	0.08	4.90	0.14	8.57

5.4.1 Exposure assessment

The milk consumption was characterized by Triangular distribution with the average milk consumption ranging from 0 to 18ml/kg body weight per day (Table5.3). The amount of milk consumed averaged 514 ml per day, which is in agreement with a study by Ahlberg *et al.* (2018), who reported that the average amount of milk in low and middle-income countries ranges from 438.3 - 657.5 ml per person. The mean exposure based on body weight varied from 0.10 µg/kg to 0.30 µg/kg (90% CI). All the derivatives showed mean-output (90% CI) below the ADI, ranging from 0.10 µg/kg to 0.30 µg/kgs (CAC, 2013). While the exposure output was varied between 6.12 µg/kg BW/person/day and 18.3 µg/kg BW/person/day(Table 5.4)

5.4.2 Risk Characterization

The current study had a mean intake of 8.57 μg/person/day, assuming an average weight of 61.2 kg for the participants under study. However, if consumers took uncontaminated milk, there was nonexistent exposure to the harmful effects of the presence of the antibiotic. The model output for amoxicillin revealed an exposure of 0.30 μg/kg, which is within the ADI 0- 0.7 μg/kg per day. Accordingly, the model output indicated a likelihood of 6.12 μg/person/day to 18.3 μg/person/day for the current study (Table 5.5). The recommended acceptable daily intake (ADI) for The ADI set by FAO for amoxicillin (0-0.7 μg/kg BW), ampicillin (3 μg/kg BW), oxacillin (200 μg/kg BW), nafcillin (30g/kg BW), and penicillin G (30 μg/person /day) (CAC, 2017).

5.6 Discussion

The current study showed the presence of antibiotics in milk. The acceptable daily intakes for all the antibiotic derivatives were below the acceptable levels, about codex guidelines (CAC, 2013). The margin of exposure (MOE) is within acceptable limits under the current study. The consumers under the current study may be safe from consuming the sampled milk. Although taking into consideration the weight distribution, the study was biased since the consumer was only adults; hence the consequence of exposure to the children may be at high risk. Loose farming practices act as contributing factors to the presence of the antibiotics in milk hence exposing milk consumers to the risk of intoxication (CDT,2017). There is, therefore, a need to for creating awareness among dairy farmers to ensure proper antibiotic application practices to protect the consumers given that these are chemical and with potential usually withstand all the processing conditions ending up in the consumer bodies and have a high potential for future disease burdens and complications (Sachi *et al*., 2019). The intake from consuming contaminated milk ranged from zero to a maximum of 0.30 units/ml., the EU, and USA MRL level set at 3-4 and 3.6, respectively (Kang'ethe *et al.,* 2015). This is, therefore, likely to expose consumers to food safety concerns from consuming such milk. This also indicates poor agricultural practices in which the farmers may not be observing the correct withdrawal periods after cattle treatment with antibiotics (Layada *et al*., 2016).

5.6 Conclusion

The current study revealed that due to the minimal level of consumption below the ADI, the consumers under this study are safe against intoxication.

5.7 Recommendation

Farmers should be trained on safe usage of farm drugs and strict adherence to withdrawal periods. They should also be sensitized on the dangers of consuming drug residue in milk. Additionally, quality control checks along the supply chain should be put in place to lower the drug residue risks to the consumers.

CHAPTER SIX: GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1: Conclusions

The current study showed that, despite the demand in food production, the improvement in dairy farming practices is still wanting. Adherence to stringent measures in upholding good farming practices will alleviate and ease compromise to food safety and quality at the farm level. Due to small scale holding, the farm management practices are disregarded, hence inadequate quality control measures and compromised food safety. Adoption in the use of modern equipment such as milking machines would considerably improve on the quality of the dairy produce, consequently lowering incidence on contamination at the farm level. Adequate milking will lead to depletion of milk in teats cistern lowering contamination to cow's udder hence inflammatory disease e.g., mastitis. Proper observation of various critical practices pointed out in this study will considerably improve on quality and safety. The tightening of government regulatory measures, which are already in place, on good farming practices will lead to a working dairy farming sector. Regular training on good dairy farming practice will be of great importance in dairy farm growth.

From the present study, it was evident that milk is contaminated with antibiotics. It was depicted that due to loose farming practices, there was the presence of antibiotics in milk at the farm level. Their presence would expose consumers to intoxication hence compromise on food safety. The penicillin G, amoxicillin, and ampicillin level was above the MRLs (0.004µg/ml). These warrants strict measures in upholding food safety at the farm level. The social-economics factor acted as the main contributing factor toward relaxed measures on health management at the farm, hence the adoption of small-scale dairy holding. It was found that the most common antibiotic use by the dairy farmers is the beta-lactams due to the treatment of mastitis for treatment.

The risk assessment test under the current study revealed that milk consumed in Ruiru town was safe, based on ADI set by Codex. For instance, penicillin G (15µg/person/day) was below the set standard (30µg/person/day). Despite MRLs for amoxicillin, ampicillin and penicillin G being above the set standard (0.004µg/ml), the ADI was within the safe limits. Additionally, for Cloxacillin, Dicloxacillin, Nafcillin, Oxacillin, and Phenoxymethyl-penicillin, were within the safe limits for MRLs and ADI. Having confirmed the presence of antibiotic residues in milk, consumers are exposed to intoxication. If daily consumption goes higher than the current, therefore a need for intervention against drug residue in milk.

6.2 Recommendations

This study suggests proper improvement in farm practices to deter antibiotic residue in milk and milk products. Additionally, farmers should embrace the use of veterinary specialists in livestock treatment, avoid unnecessary antibiotics administration, and intermittent withdrawal periods. Collectively, using these practices would lower the incidence of antibiotic residue hence lowering food safety risks. The stack holders in the dairy sector should champion the change of attitude toward embracing good farming practices for the sector. Enforcement of already documented legislation on good farming practices, coupled with training, would be of great importance in food safety and quality improvement.

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Appendices

turnitin

TURNITIN REPORT

18/08/2020

Turnitin Originality Report
Effect of dairy farming practices on intake of antibiotic residues in milk consumed in Kiambu County, Kenya by Stephen Kimani Njoroge
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APPENDIX 1: CONSENT FORM

University of Nairobi Department of Food Science, Nutrition and Technology, Food Safety and Quality Assurance Program.

Stephen Kimani Njoroge is a student from the University of Nairobi studying MSc. in Food Safety and Quality Assurance. He is carrying out a study on dairy practices that compromises food safety in Kiambu County. To get this data, I am delighted to have you take part in this study.

The study involves responding to a few questions, with the answers you give being filled in a questionnaire and a checklist to be filled regarding your farming. The data will assist in ascertaining the safety aspects of milk with regards to antibiotics.

The data you will provide is confidential and in as much, no names will be included will be included in the report. There is no way any data will be directly associated with you. I encourage you to partake in the study and your assistance is highly appreciated.

Kindly sign below if you agree to take to be part of the study

Name of Interviewer……………………………………………..

Signature of interviewer………………………………………

Date………………………………..

In case of any problem,

Contact

STEPHEN KIMANI

0722582182

Appendix 2 QUESTIONNAIRE

Kindly answer the following question by giving a brief answer or ticking in the boxes provided in the respective questions.

FARMERS

Handling and hygiene during milking

1. How many cows do you have? ________________________
2. How many cows are you milking presently? ----------------------------------
3. Which method of milking do you use, machine or manual? ---
4. Where do you milk your cows from; crush or open space? --
5. Do you wash udder with warm or cold water; with detergent or without? --
6. Do you rinse the udder? Yes [ ] or no [ ]
7. If yes do you dry and how? ---
8. Do you use milking gel? Yes [ ] or no [ ], if yes which brand? --
9. Which type of milking equipment do you use plastic or aluminum?
10. Do you sanitize milking types of equipment before use, yes [ ] or no [ ] if yes how? ---
11. Do you sieve the milk after milking? Yes [ ] or no [ ]
12. How do you preserve milk after milking? --

Antibiotic

13. How many dairy cows do you have? ---
14. Have you ever administered antibiotic treatment, if yes which one? ---
15. Is there a cow currently under treatment? Yes [ ] or [ ] what type of treatment? --
16. Do you keep any dairy management record? If yes which once? --
17. Do you sell milk from treated cows? Yes [ ] or no.[ ]
18. If nowhere do you dispose of it? ____
19. Do you have a withdrawal period for treated cows? Yes [ ] or no [ ] If yes how many days? ----------------

Handling and milk storage

1. How do you ensure the general hygiene of staff before handling milk? --
2. How do you ensure milking equipment hygiene of equipment before milking? ---
3. How do you store milk after milking? ---
4. Do you preserve your milk if yes how? Yes [ ] or no [ ]
5. At what time do you milk? ---
6. At what time you sell your milk? ---
7. Whom do you sell to? ---

Quality Controls at farm level

1. Do you monitor any quality parameter at the farm level? If yes which once? Yes [ ] or no [ ]
   ----
2. How do you handle quality deviations? -------------------------------------
3. Has your milk ever rejected? Yes [ ] or no [ ]
1. What caused rejection? --
2. How had do you correct the deviation? ---

Experience and education

1. For how long have you been in dairy farming? --
2. Do you have any formal training in dairy farming? Yes [ ] or no [ ]
3. Do you keep any farm records if yes which once? Yes [ ] or no [ ]
   ----
4. Are you able to trace the source of milk? Yes [ ] or no [ ] if yes how
   ----

APPENDIX 3: UHPLC MS/MS DATA ON SAMPLES SPIKING (ppb)

Amoxicillin						
Data File	**Compound**	**RT**	**Response**	**Final Conc**	**Exp Conc**	**Accuracy**

BS2019 cal in matrix std 1.d	Amoxicillin	11.448	2486	6.9146	5.0000	138.29
BS2019 cal in matrix std 2.d	Amoxicillin	11.448	5514	8.3665	10.0000	83.66
BS2019 cal in matrix std 3.d	Amoxicillin	11.398	5245	22.9382	20.0000	114.69
BS2019 cal in matrix std 4.d	Amoxicillin	11.406	15777	45.6912	50.0000	91.38
BS2019 cal in matrix std 5.d	Amoxicillin	11.414	31983	76.6946	100.0000	76.69
BS2019cal in matrix std 6.d	Amoxicillin	11.414	46475	151.0896	150.0000	100.73
sample 1A.d	Amoxicillin	11.398	14	5.2882		
sample 1B.d	Amoxicillin	11.481	213	22.0338		
sample 2A.d	Amoxicillin	11.515	236	15.6833		
sample 2B.d	Amoxicillin	11.481	142	8.2956		
sample 3A.d	Amoxicillin	11.423	40	7.0497		
sample 3B.d	Amoxicillin	11.498	53	6.6730		
sample 4A.d	Amoxicillin	11.498	175	10.4092		
sample 4B.d	Amoxicillin	11.481	193	18.7367		
sample 5A.d	Amoxicillin	11.506	229	29.3048		
sample 5B.d	Amoxicillin	11.481	164	77.4561		
CAL IN SOLV STD 1.d	Amoxicillin	11.440	4546	9.2425	5.0000	184.85
CAL IN SOLV STD 2.d	Amoxicillin	11.423	81	4.7901	10.0000	47.90
CAL IN SOLV STD 3.d	Amoxicillin	11.440	7155	10.4451	20.0000	52.23
CAL IN SOLV STD 4.d	Amoxicillin	11.440	18621	22.2682	50.0000	44.54
CAL IN SOLV STD 5.d	Amoxicillin	11.440	39377	40.2478	100.0000	40.25
CAL IN SOLV STD 6.d	Amoxicillin	11.440	61973	52.5724	150.0000	35.05

Ampicillin						
Data File	**Compound**	**RT**	**Response**	**Final Conc**	**Exp Conc**	**Accuracy**

BS2019 cal in matrix std 1.d	Ampicillin	7.541	10718	8.3047	5.0000	166.09
BS2019 cal in matrix std 2.d	Ampicillin	7.566	22966	9.1188	10.0000	91.19
BS2019 cal in matrix std 3.d	Ampicillin	7.524	33470	23.4846	20.0000	117.42
BS2019 cal in matrix std 4.d	Ampicillin	7.532	94688	41.9119	50.0000	83.82
BS2019 cal in matrix std 5.d	Ampicillin	7.541	206282	72.9459	100.0000	72.95
BS2019cal in matrix std 6.d	Ampicillin	7.541	324383	152.1800	150.0000	101.45
sample 1A.d	Ampicillin	7.591	19	7.0699		
sample 1B.d	Ampicillin	7.541	40	7.4240		
sample 2A.d	Ampicillin	7.304	55	7.3256		
sample 2B.d	Ampicillin	7.759	10	7.0019		
sample 3A.d	Ampicillin	7.616	32	7.2336		
sample 3B.d	Ampicillin	7.465	37	7.1589		
sample 4A.d	Ampicillin	7.473	44	7.1681		
sample 4B.d	Ampicillin	7.625	9	7.0578		
sample 5A.d	Ampicillin	7.549	8	7.0925		
sample 5B.d	Ampicillin	7.608	20	8.2132		
CAL IN SOLV STD 1.d	Ampicillin	7.558	4731	7.6327	5.0000	152.65
CAL IN SOLV STD 2.d	Ampicillin	7.658	20	6.9671	10.0000	69.67
CAL IN SOLV STD 3.d	Ampicillin	7.558	10808	8.1924	20.0000	40.96
CAL IN SOLV STD 4.d	Ampicillin	7.549	29492	10.9138	50.0000	21.83
CAL IN SOLV STD 5.d	Ampicillin	7.541	42399	12.4015	100.0000	12.40
CAL IN SOLV STD 6.d	Ampicillin	7.549	77423	15.4615	150.0000	10.31

Cloxacillin						
Data File	**Compound**	**RT**	**Response**	**Final Conc**	**Exp Conc**	**Accuracy**

BS2019 cal in matrix std 1.d	Cloxacillin	12.290	416	5.6844	5.0000	113.69
BS2019 cal in matrix std 2.d	Cloxacillin	12.299	2458	11.9180	10.0000	119.18
BS2019 cal in matrix std 3.d	Cloxacillin	12.274	958	20.3668	20.0000	101.83
BS2019 cal in matrix std 4.d	Cloxacillin	12.290	3838	53.3128	50.0000	106.63
BS2019 cal in matrix std 5.d	Cloxacillin	12.282	7272	85.0654	100.0000	85.07
BS2019cal in matrix std 6.d	Cloxacillin	12.282	9905	158.6526	150.0000	105.77
sample 1A.d	Cloxacillin	12.097	9	5.6708		
sample 1B.d	Cloxacillin	12.299	15	9.7688		
sample 2A.d	Cloxacillin	12.341	6	5.1575		
sample 2B.d	Cloxacillin	12.357	32	7.8159		
sample 3A.d	Cloxacillin	12.366	9	6.5520		
sample 3B.d	Cloxacillin	12.382	32	9.6573		
sample 4A.d	Cloxacillin	12.299	10	5.4074		
sample 4B.d	Cloxacillin	12.215	7	6.2270		
sample 5A.d	Cloxacillin	12.256	11	9.6962		
sample 5B.d	Cloxacillin	12.391	82	183.8519		
CAL IN SOLV STD 1.d	Cloxacillin	12.290	6113	33.9915	5.0000	679.83
CAL IN SOLV STD 2.d	Cloxacillin	12.374	75	4.1510	10.0000	41.51
CAL IN SOLV STD 3.d	Cloxacillin	12.290	10142	44.0770	20.0000	220.39
CAL IN SOLV STD 4.d	Cloxacillin	12.290	25335	122.2860	50.0000	244.57
CAL IN SOLV STD 5.d	Cloxacillin	12.290	55714	253.2452	100.0000	253.25
CAL IN SOLV STD 6.d	Cloxacillin	12.290	85133	330.0014	150.0000	220.00

Dicloxacillin						
Data File	**Compound**	**RT**	**Response**	**Final Conc**	**Exp Conc**	**Accuracy**

BS2019 cal in matrix std 1.d	Dicloxacillin	12.439	10843	8.1673	5.0000	163.35
BS2019 cal in matrix std 2.d	Dicloxacillin	12.439	23158	9.2680	10.0000	92.68
BS2019 cal in matrix std 3.d	Dicloxacillin	12.430	24748	22.8064	20.0000	114.03
BS2019 cal in matrix std 4.d	Dicloxacillin	12.430	73249	42.7839	50.0000	85.57
BS2019 cal in matrix std 5.d	Dicloxacillin	12.430	156551	73.8430	100.0000	73.84
sample 1A.d	Dicloxacillin	12.380	33	6.5951		
sample 1B.d	Dicloxacillin	12.363	47	7.0738		
sample 2A.d	Dicloxacillin	12.455	85	7.0937		
sample 2B.d	Dicloxacillin	12.305	16	6.4155		
sample 3A.d	Dicloxacillin	12.355	67	7.0835		
sample 3B.d	Dicloxacillin	12.480	10	6.4132		
sample 4A.d	Dicloxacillin	12.330	20	6.4631		
sample 4B.d	Dicloxacillin	12.221	16	6.5603		
sample 5A.d	Dicloxacillin	12.397	20	6.7498		
sample 5B.d	Dicloxacillin	12.397	12	7.3445		
CAL IN SOLV STD 1.d	Dicloxacillin	12.439	26371	11.3584	5.0000	227.17
CAL IN SOLV STD 2.d	Dicloxacillin	12.430	36	6.3453	10.0000	63.45
CAL IN SOLV STD 3.d	Dicloxacillin	12.439	42327	12.8201	20.0000	64.10
CAL IN SOLV STD 4.d	Dicloxacillin	12.439	109047	26.0230	50.0000	52.05
CAL IN SOLV STD 5.d	Dicloxacillin	12.439	244374	48.5805	100.0000	48.58
CAL IN SOLV STD 6.d	Dicloxacillin	12.439	375637	61.9109	150.0000	41.27

Nafcillin						
Data File	**Compound**	**RT**	**Response**	**Final Conc**	**Exp Conc**	**Accuracy**

BS2019 cal in matrix std 1.d	Nafcillin	12.610	1696	6.3709	5.0000	127.42
BS2019 cal in matrix std 2.d	Nafcillin	12.610	3779	7.3815	10.0000	73.82
BS2019 cal in matrix std 3.d	Nafcillin	12.593	5716	24.8909	20.0000	124.45
BS2019 cal in matrix std 4.d	Nafcillin	12.602	15442	45.3199	50.0000	90.64
BS2019 cal in matrix std 5.d	Nafcillin	12.593	30603	74.3448	100.0000	74.34
BS2019cal in matrix std 6.d	Nafcillin	12.593	46001	151.0368	150.0000	100.69
sample 1A.d	Nafcillin	12.660	48	6.7934		
sample 1B.d	Nafcillin	12.610	45	8.5802		
sample 2A.d	Nafcillin	12.501	25	6.0416		
sample 2B.d	Nafcillin	12.668	226	10.5986		
sample 3A.d	Nafcillin	12.694	119	11.8330		
sample 3B.d	Nafcillin	12.710	171	11.1958		
sample 4A.d	Nafcillin	12.635	115	8.6429		
sample 4B.d	Nafcillin	12.643	62	9.4308		
sample 5A.d	Nafcillin	12.635	38	8.9889		
sample 5B.d	Nafcillin	12.618	33	19.7254		
CAL IN SOLV STD 1.d	Nafcillin	12.602	1921	6.7904	5.0000	135.81
CAL IN SOLV STD 2.d	Nafcillin	12.702	215	5.0308	10.0000	50.31
CAL IN SOLV STD 3.d	Nafcillin	12.602	2738	7.0732	20.0000	35.37
CAL IN SOLV STD 4.d	Nafcillin	12.602	6452	10.9975	50.0000	21.99
CAL IN SOLV STD 5.d	Nafcillin	12.602	10567	14.4832	100.0000	14.48
CAL IN SOLV STD 6.d	Nafcillin	12.602	18607	19.3600	150.0000	12.91

Oxacillin						
Data File	**Compound**	**RT**	**Response**	**Final Conc**	**Exp Conc**	**Accuracy**

BS2019 cal in matrix std 1.d	Oxacillin	12.251	1422	8.0921	5.0000	161.84
BS2019 cal in matrix std 2.d	Oxacillin	12.251	2517	8.5761	10.0000	85.76
BS2019 cal in matrix std 3.d	Oxacillin	12.226	4160	23.6420	20.0000	118.21
BS2019 cal in matrix std 4.d	Oxacillin	12.235	11814	42.7754	50.0000	85.55
BS2019 cal in matrix std 5.d	Oxacillin	12.235	25413	74.0211	100.0000	74.02
BS2019cal in matrix std 6.d	Oxacillin	12.235	39141	151.9145	150.0000	101.28
sample 1A.d	Oxacillin	12.251	22	7.6424		
sample 1B.d	Oxacillin	12.370	29	9.4366		
sample 2A.d	Oxacillin	12.117	32	8.3809		
sample 2B.d	Oxacillin	12.378	11	6.9444		
sample 3A.d	Oxacillin	12.050	44	9.6076		
sample 3B.d	Oxacillin	12.243	16	7.2948		
sample 4A.d	Oxacillin	12.175	13	7.1169		
sample 4B.d	Oxacillin	12.303	29	9.0719		
sample 5A.d	Oxacillin	12.268	14	8.4377		
sample 5B.d	Oxacillin	12.260	13	13.2274		
CAL IN SOLV STD 1.d	Oxacillin	12.243	661	7.3923	5.0000	147.85
CAL IN SOLV STD 2.d	Oxacillin	12.328	15	6.6320	10.0000	66.32
CAL IN SOLV STD 3.d	Oxacillin	12.235	1158	7.7086	20.0000	38.54
CAL IN SOLV STD 4.d	Oxacillin	12.243	3183	10.1519	50.0000	20.30
CAL IN SOLV STD 5.d	Oxacillin	12.251	5093	12.0337	100.0000	12.03
CAL IN SOLV STD 6.d	Oxacillin	12.243	8193	14.0739	150.0000	9.38

Penicillin G (Benzylpenicillin)						
Data File	**Compound**	**RT**	**Response**	**Final Conc**	**Exp Conc**	**Accuracy**

BS2019 cal in matrix std 1.d	Penicillin G (Benzylpenicillin)	11.425	311	6.2445	5.0000	124.89
BS2019 cal in matrix std 2.d	Penicillin G (Benzylpenicillin)	11.433	904	8.4214	10.0000	84.21
BS2019 cal in matrix std 3.d	Penicillin G (Benzylpenicillin)	11.408	880	24.9971	20.0000	124.99
BS2019 cal in matrix std 4.d	Penicillin G (Benzylpenicillin)	11.408	2256	43.9092	50.0000	87.82
BS2019 cal in matrix std 5.d	Penicillin G (Benzylpenicillin)	11.417	5077	81.4421	100.0000	81.44
BS2019cal in matrix std 6.d	Penicillin G (Benzylpenicillin)	11.417	6922	151.4277	150.0000	100.95
sample 1A.d	Penicillin G (Benzylpenicillin)	11.534	14	8.2829		
sample 2A.d	Penicillin G (Benzylpenicillin)	11.483	8	7.0579		
sample 2B.d	Penicillin G (Benzylpenicillin)	11.550	16	7.1361		
sample 3A.d	Penicillin G (Benzylpenicillin)	11.433	15	10.2427		
sample 3B.d	Penicillin G (Benzylpenicillin)	11.283	11	6.9978		
sample 4A.d	Penicillin G (Benzylpenicillin)	11.383	33	11.5311		
sample 4B.d	Penicillin G (Benzylpenicillin)	11.467	17	12.5610		
sample 5A.d	Penicillin G (Benzylpenicillin)	11.475	52	42.3549		
sample 5B.d	Penicillin G (Benzylpenicillin)	11.408	17	54.5067		
CAL IN SOLV STD 1.d	Penicillin G (Benzylpenicillin)	11.450	610	8.4886	5.0000	169.77
CAL IN SOLV STD 2.d	Penicillin G (Benzylpenicillin)	11.375	18	4.4947	10.0000	44.95
CAL IN SOLV STD 3.d	Penicillin G (Benzylpenicillin)	11.450	1007	9.8256	20.0000	49.13
CAL IN SOLV STD 4.d	Penicillin G (Benzylpenicillin)	11.450	1718	15.3178	50.0000	30.64
CAL IN SOLV STD 5.d	Penicillin G (Benzylpenicillin)	11.433	4979	34.6928	100.0000	34.69
CAL IN SOLV STD 6.d	Penicillin G (Benzylpenicillin)	11.442	8012	46.1155	150.0000	30.74

Phenoxymethylpenicillin						

Data File	Compound	RT	Response	Final Conc	Exp Conc	Accuracy
BS2019 cal in matrix std 1.d	Phenoxymethylpenicillin	7.548	2512	7.2036	5.0000	144.07
BS2019 cal in matrix std 2.d	Phenoxymethylpenicillin	7.574	5377	8.0299	10.0000	80.30
BS2019 cal in matrix std 3.d	Phenoxymethylpenicillin	7.523	8350	23.7430	20.0000	118.71
BS2019 cal in matrix std 4.d	Phenoxymethylpenicillin	7.531	24235	44.6971	50.0000	89.39
BS2019 cal in matrix std 5.d	Phenoxymethylpenicillin	7.540	50121	75.4845	100.0000	75.48
BS2019cal in matrix std 6.d	Phenoxymethylpenicillin	7.540	74809	151.3265	150.0000	100.88
sample 1A.d	Phenoxymethylpenicillin	7.565	33	6.6393		
sample 1B.d	Phenoxymethylpenicillin	7.472	62	8.9339		
sample 2A.d	Phenoxymethylpenicillin	7.876	66	7.7281		
sample 2B.d	Phenoxymethylpenicillin	7.574	34	6.3632		
sample 3A.d	Phenoxymethylpenicillin	7.700	18	6.4902		
sample 3B.d	Phenoxymethylpenicillin	7.362	61	7.2225		
sample 4A.d	Phenoxymethylpenicillin	7.531	40	6.6436		
sample 4B.d	Phenoxymethylpenicillin	7.472	17	6.6233		
sample 5A.d	Phenoxymethylpenicillin	7.632	105	12.7958		
sample 5B.d	Phenoxymethylpenicillin	7.523	58	21.6160		
CAL IN SOLV STD 1.d	Phenoxymethylpenicillin	7.574	916	6.4008	5.0000	128.02
CAL IN SOLV STD 2.d	Phenoxymethylpenicillin	7.607	15	5.8462	10.0000	58.46
CAL IN SOLV STD 3.d	Phenoxymethylpenicillin	7.565	2292	6.9700	20.0000	34.85
CAL IN SOLV STD 4.d	Phenoxymethylpenicillin	7.557	7389	10.1376	50.0000	20.28
CAL IN SOLV STD 5.d	Phenoxymethylpenicillin	7.557	10654	11.7739	100.0000	11.77

CAL IN SOLV STD 6.d	Phenoxymethylpenicillin	7.557	18186	14.5095	150.0000	9.67

APPENDIX 4: CONSUMPTION PATTERN FOR MILK QUESTIONNAIRE

A. DEMOGRAPHIC INFORMATION

Name of Interviewer		Date of Interview	
Name of Respondent			
Name of House hold head			
Relationship of Respondent to Household head			
Area/Location			
Sex: (Tick correct) applicable to all	Male=1 Female=2		
Age	Below 18 years 1 = 18- 30 years 2 = 31-40 years 3 = 41 -50 years 4 = 51 -65 years 5 = Above 65 years		
Education	1=College/University 2=Completed Secondary 3=Completed primary 4=Dropped from primary 5=In primary 6=In secondary 7=Literate e.g. Adult Education 8=Illiterate 9=Pre-primary 10= Others (specify)		
Estimated body weight(kg)			
Amount consumed/day/week			
Estimated height(m)			
Marital status	1=Married 2=Separated 3=Widowed 4=Single 5=Divorced 6=N/A		
Main occupation	1=Salaried employee 2=Farmer 3=Self-employment 4=Casual labourer		

	5=Student 6=Housewife 7=Unemployed 8=Others (specify) 9=N/A

APPENDIX 5: CONSUMPTION PATTERN OF MILK IN RUIRU TOWN

AGE	SEX	ESTIMATED BODY WEIGHT (KGS)	DAILY CONSUMPTION(ML)	ESTIMATED HEIGHT(CM)	FREQUENCY
35	F	65	200	162	1
20	F	53	150	155	2
40	M	67	250	165	2
38	F	57	100	150	3
39	F	69	150	165	1
43	M	70	500	170	2
37	F	71	300	168	2
42	M	80	500	165	2
27	M	68	450	160	2
25	F	55	0	150	0
45	F	75	500	165	1
33	F	60	450	160	2
47	M	77	300	160	1
51	F	65	500	160	1
49	F	79	200	165	2
50	M	80	250	170	2
39	M	81	300	160	2
26	M	82	300	165	1
30	F	83	500	160	1
47	M	94	200	155	2
38	F	83	600	160	1
25	F	55	450	155	1
33	F	53	250	145	2
27	M	61	450	165	1
22	M	58	450	160	2
36	M	63	450	155	1
36	F	56	500	150	1
34	M	70	200	168	2
26	F	45	450	140	1
24	F	51	300	147	2
39	F	53	250	140	1

21	M	67	450	155	2
32	F	51	150	138	1
28	M	63	450	145	1
23	M	58	200	165	2
50	F	52	450	145	2
41	F	55	450	144	2
28	F	50	300	140	2
60	M	70	300	165	1
36	M	66	300	155	1
33	F	60	250	160	3
37	M	58	450	160	2
22	F	47	250	135	2
26	F	49	450	140	1
28	F	52	450	145	1
25	F	53	300	150	2
25	M	56	450	160	1
33	F	51	450	145	1
22	M	58	450	139	2
36	M	63	450	137	1
36	F	56	500	165	1
34	M	70	200	175	3
26	F	45	450	140	1
24	F	51	300	145	2
39	F	53	250	165	1
21	M	67	450	160	2
32	F	51	250	155	1
28	M	63	500	160	1
23	M	58	200	158	2
22	M	58	450	155	2
36	M	64	500	165	1
35	F	57	450	155	1
34	M	71	100	145	3
26	F	44	450	150	1
24	F	50	300	145	2
39	F	52	250	145	1
22	M	68	500	165	2
31	F	50	150	145	1
29	M	54	450	155	1

24	M	58	250	160	2
25	F	55	450	150	1
33	F	53	250	148	2
27	M	61	450	165	1
22	M	58	450	160	2
36	M	63	450	165	1
36	F	56	500	145	1
34	M	70	200	170	3
26	F	45	450	140	1
24	F	51	300	150	2
39	F	53	250	155	1
21	M	67	450	160	2
32	F	51	150	153	1
28	M	63	450	150	1
23	M	58	200	155	2
50	F	75	450	145	2
41	F	55	450	145	2
28	F	50	300	150	2
60	M	70	300	165	1
23	M	58	200	170	2
22	M	58	450	165	2
36	M	64	500	165	1
35	F	85	450	160	1
34	M	71	100	160	3
26	F	44	450	155	1
24	F	50	300	150	2
39	F	90	250	146	1
22	M	68	500	160	1
31	F	60	150	150	1
29	M	57	450	145	1