

MAKERERE



UNIVERSITY

**QUANTITATIVE EVALUATION OF ANTI-BIOTIC RESIDUES IN RAW MILK
SAMPLES FROM KIBOGA DISTRICT, CENTRAL UGANDA**

BY

IRENE MBATIDDE

BVM (MAK)

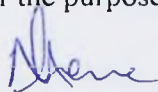
**A RESEARCH DISSERTATION SUBMITTED TO THE DIRECTORATE OF
RESEARCH AND GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE AWARD OF A DEGREE OF MASTER OF SCIENCE IN
VETERINARY PREVENTIVE MEDICINE OF MAKERERE UNIVERSITY**

JAN, 2017

DECLARATION

I **Mbatidde Irene**, hereby declare that to the best of my knowledge, this dissertation is my original piece of work and has never been submitted to any University or any other institution of higher learning for the purpose of award of a degree.

Signed



Date

23rd/02/2017

Mbatidde Irene

Reg. No. 2013/HD17/3056U

The dissertation has been submitted with the approval of the following Academic Supervisors;

Assoc. Prof. Frank N. Mwiine (BVM, MSc, PhD)

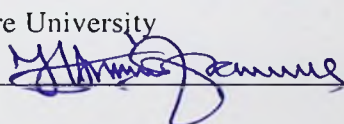
Dean of students

School of Biosecurity, Biotechnology and Laboratory Science (SBLS)

College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB)

Makerere University

Signed



Date

23/02/2017

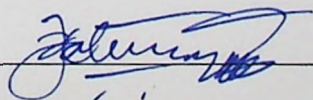
Dr. John N. Kateregga (BVM, MSc, PhD)

Senior Lecturer, Veterinary Pharmacy, Clinics and Comparative Medicine,

College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB)

Makerere University

Signed



Date

23/02/2017

DEDICATION

I dedicate this work to my Dad; Dr. Emmanuel Benjamin Lule and Mum; Mrs Jane Aliyinza Lule, thank you for your support and endless efforts to give me a meaningful education.

ACKNOWLEDGEMENT

I would like to express my profound gratitude to my supervisors Ass. Prof. Frank N. Mwiine and Dr. John N. Kateregga for the technical advice you offered me during my research work. Special thanks to National Agricultural Research Organisation, (NARO) administration for giving me an opportunity to upgrade my academic qualifications as well as meeting my tuition fees for the course. I also wish to sincerely extend my gratitude to the Agricultural Share (AgShare) project that funded all my research work and paper publication.

I highly appreciate my husband, children and Ms Rachel Nangobi for the support you offered me during my study period. I also wish to thank my parents, brothers, sisters and in-laws for the love and support you gave me and the children during my study period. I thank my course mates Dr. Katali Benda and Dr. Abulhamed Kateregga for the support you offered as well as the entire Masters of Veterinary Preventive Medicine, (MVPM) team.

I thank my lecturers and workmates at Mbarara Zonal Agricultural Research and Development Institute (MBAZARDI) for the support and guidance offered. I thank Dr. Paul Sajjakambwe, Dr. Patrick Vudriko for the guidance and Dr. Charles Lagu for the continued professional guidance. Last but not least, I wish to thank Dr. Moses Dhikusooka, for stimulating discussions, professional criticisms, and technical advice. I sincerely thank you all and pray for God's blessings upon you as we build for the future.

TABLE OF CONTENTS

DECLARATION	i
DEDICATION	ii
ACKNOWLEDGEMENT	iii
TABLE OF CONTENTS.....	iv
LIST OF TABLES	vi
LIST OF FIGURES	vi
LIST OF ABBREVIATIONS AND ACRONYMS	viii
ABSTRACT.....	ix
CHAPTER ONE	1
INTRODUCTION	1
1.1 Background	1
1.2 Problem statement.....	3
1.3 Study objectives	4
1.3.1 General Objective	4
1.3.2 Specific Objectives	4
1.4 Research questions.....	4
1.5 Significance of the study.....	5
1.6 Justification of the study	5
Figure 1: Conceptual frame work	6
CHAPTER TWO	7
LITERATURE REVIEW	7
2.1 The importance of the Dairy Sub-sector.....	7
2.2 Knowledge, attitude and practices of farmers concerning antimicrobial residues in milk	9
2.3 Antimicrobial residues and risks they pose to human health.....	10
2.4 Factors associated with the occurrence of drug residues in milk.....	13
2.5 Methods for detection of antibiotic residues.....	14
CHAPTER THREE	16
MATERIALS AND METHODS.....	16
3.1 Study design.....	16
3.2 Study area.....	16

3.3 Sample and data collection	17
3.4 Sample preparation	18
3.5 Sample analysis.....	19
3.6 Data and statistical analysis	19
CHAPTER FOUR.....	20
RESULTS	20
4.1 Demographic characteristics of the participants	20
4.2 Farmers' level of knowledge and perception on drug residues in milk	20
4.3 Drug administration on the farms	21
4.4 Antibiotic residue levels in milk samples	22
4.5 Factors influencing occurrence of antibiotic residues in milk	24
CHAPTER FIVE	27
DISCUSSION	27
CONCLUSIONS AND RECOMMENDATIONS	32
6.1 Conclusions.....	32
6.2 Recommendations	32
REFERENCES	33
APPENDICES	39

LIST OF TABLES

Table 1: Demographic characteristics of the respondents	20
Table 2: Farmers' level of knowledge and perception on occurrence of drug residues in milk ...	21
Table 3: Mean antibiotic concentrations and percentages of the positive milk samples	23
Table 4: Factors influencing occurrence of antibiotic residues in milk.....	25
Table 5: Significant factors for the occurrence of antibiotic residues in milk.....	26

LIST OF FIGURES

Figure 1: Map showing the study area	17
Figure 2: Distribution of antibiotic residues based on personnel administering the drugs.....	22
Figure 3: Chromatogram showing oxytetracycline peak	23
Figure 4: Chromatogram showing amoxicillin peak	24

LIST OF ABBREVIATIONS AND ACRONYMS

BVM	Bachelor of Veterinary Medicine
BMCC	Bulk Milk Collection Centre
CAC	Codex Alimentarius Commission
COVAB	College of Veterinary Medicine Animal Resources and Biosecurity
CE	Capillary Electrophoresis
DDA	Dairy Development Authority
ELISA	Enzyme-linked Immunosorbent Assay
FAO	Food and Agriculture Organisation
FR	Factor of Response
GDP	Gross Domestic Product
GIT	Gastro Intestinal Tract
HPLC	High Performance Liquid Chromatography
IDF	International Dairy Foundation
MAAIF	Ministry of Agriculture , Animal Industry and Fisheries
MAK	Makerere University
MBAZARDI	Mbarara Zonal Agricultural Research and Development Institute
MSc	Master of Science
MVPM	Masters of Veterinary Preventive Medicine
MRL	Maximum Residual Limits
NARO	National Agricultural Research Organisation
NDS	National Dairy Strategy
OIE	Office International des Epizooties
PhD	Philosophy Doctorate
SPSS	Statistical Package for Social Scientists
TLC	Thin Layer Chromatography
UHT	Ultra High Temperature
U.S.A	United States of America
WHO	World Health Organisation

ABSTRACT

Milk and milk products are among the most consumed food products all over the world. However, ensuring that milk consumed by humans especially in developing countries is of the best quality without adulterations is still a problem. In Uganda there are few documented studies that quantify drug residues in milk. A study to establish the presence of antibiotic residues in raw milk samples was carried out in March 2015. The study aim was to establish and quantify presence of antibiotic residues in milk from two pastoralist sub counties in Kiboga district. Fifty one milk samples, including 47 on-farm samples and four samples from community bulk milk collection centres were randomly collected from Kapeke and Bukomero in Kiboga district. The milk samples were collected using sterile Bijou bottles and at the same time, a structured questionnaire on factors associated with presence of residues in animal products was administered to participants. The milk samples were analyzed for residues of 3 antibiotics namely Gentamycin, Oxytetracycline and Amoxicillin by high performance liquid chromatography (HPLC). The questionnaires were analysed using SPSS version 20. All the three antibiotics were detected in the milk samples. All samples from the bulk milk collection centres were found positive with Oxytetracycline and Amoxicillin, while 25% were positive for Gentamycin. From the individual farm milk samples, 59% were positive for Gentamycin, 74% for Amoxicillin and 100% for Oxytetracycline. The mean concentration of the antibiotics were; Gentamycin (0.098 ± 0.098), Amoxicillin (0.173 ± 0.125) and Oxytetracycline (0.35 ± 0.1). However, concentrations of all the three types of antibiotics residues in milk were found to be below the recommended limits set by the World Health Organization (WHO) in milk. The factors that were associated with the occurrence of drug residues in milk included; non observance of drug withdraw periods, lack of records, lack of knowledge of the grazing system and the number of milking animals. The government should enforce legislations regarding use of antibiotics to avert the likely side effects of such antibiotics in the milk value chain.

CHAPTER ONE

INTRODUCTION

1.1 Background

Milk and milk products are among the most important daily consumed foodstuffs. They are nutritious food items and are highly recommended in the diet of most individuals since they offer a balanced ration that provides most of the vital nutrients required by the body (Haug, *et al* 2007). Milk is an excellent source of calcium, phosphorus, riboflavin, and vitamin D and a good source of protein, vitamin A, potassium, and several B vitamins that are essential for growth and development of all age groups. Owing to its composition, milk is highly recommended for the vulnerable groups including; infants, young children and the elderly (Haug *et al.*, 2007). As such, provision of quality milk without adulteration to the public should be a priority in the country.

Antibiotics have for long been used in veterinary practice as curative drugs, prophylactic drugs and to a lesser extent to improve feed efficiency Marshall & Levy (2011). Their use in animals should however be restricted and they should be administered under strict guidelines of the veterinarians. The antibiotics used in veterinary medicines belong to six major classes including; Beta-lactams (e.g. penicillin, ampicillin, cephalexin, cephaprin etc), Aminoglycosides (e.g. gentamycin, neomycin, streptomycin etc), Tetracyclines (e.g. oxytetracycline, chlortetracycline etc), Macrolides (e.g. erythromycin, spironomycin), Quinolones (e.g. enrofloxacin, norfloxacin), and Sulphonamides (e.g. sulfadiazine, sulfadimethoxine). Any of the drugs belonging to these groups can appear in milk (Syt, 2009). Several types of antibiotics are commonly used in food animals Marshall & Levy (2011). They are normally administered parentally via intramuscular, intravenous, or subcutaneous injections. Other means of administration include orally, topically, as intra mammary or as intrauterine infusions (Syt, 2009).

Antibacterial drugs such as oxytetracycline and penicillin G are routinely used in veterinary medicine for prevention and control of diseases. Oxytetracycline is used as a broad spectrum antibiotic active against many infections and is usually applied for the purpose of prevention or treatment of conditions such as bronchopneumonia, mastitis and metritis in cows (Syt, 2009).

Gentamycin is used against most gram positive and gram negative bacteria including staphylococcal and streptococcal species. There is injudicious use of these antibiotics by the farmers. Farmers get these drugs over the counter without prescription by a Veterinary doctor. Further still, farmers do not observe the withdrawal periods. The regulatory policies for enforcing withdrawal periods compliance is also very weak. As a result, there are concerns that antibiotic residues of these compounds may be present in the milk and milk products hence posing a public health risk of antibiotic resistance (Syit, 2009)

The use of antibiotics therapy to treat and prevent udder infections in milking cows is a key component of mastitis control in many countries, Uganda inclusive. As a result, much effort and concern should be directed towards the management and monitoring of antibiotics used in such treatment in order to prevent contamination of raw milk (Kebede *et al.*, 2014).

Studies carried out in different parts of the world indicate occurrence of drug residues in milk. In Croatia, a study on presence of chloramphenicol, sulfonamides, tetracycline, gentamycin, streptomycin, dihydrostreptomycin, flumequine and enrofloxacin in milk showed varying levels of residues of these drugs, although in quantities that were below the Maximum Residual Limits (MRLs) based on the Office International des Epizooties (OIE) requirements (Bilandžić *et al.*, 2011). In Hyderabad city, 36% of the collected milk samples were positive for beta-Lactams mean concentration ($59.53\mu\text{g/L}$) (Khaskheli *et al.*, 2008).

In Ghana, 35.5% of the collected milk samples indicated high contaminations with one or more of the screened antimicrobial drugs (Aning *et al.*, 2007). In Kenya, 16% of the screened samples from market outlets and consumer households had beta-lactams and tetracyclines (Kang'ethe *et al.*, 2005), while in Uganda, 13% of raw cow milk samples contained penicillin residues above the MRL in a cross-sectional study that investigated the occurrence of penicillin G residues in Masaka and Mbarara districts (Sasanya *et al.*, 2013).

Regulatory measures have been put forth by the World Health Organisation (WHO) and Food and Agriculture Organisation (FAO), specifying the Maximum Residual Limits of antibiotic residues that can occur in food substances. Whereas these quality standards have been successfully implemented in the developed countries, they have largely failed in most of the

developing world where raw milk sales predominate (Omore et al., 2005). In Uganda these measures have not been enforced. There is also still less information depicting the occurrence of drug residues in milk in the country. Only one documented study has been carried out to establish the occurrence of Penicillin G in cow milk and edible tissues in Mbarara and Masaka (Sasanya *et al* 2013). However the status of other antibiotic residues in milk in Uganda still remains unknown.

This study was therefore conducted out to determine the occurrence and quantities of amoxicillin, oxytetracycline and gentamycin residues in cow milk. The study also assessed the farmers' level of knowledge and perception about drug residues in milk as well as the factors associated with occurrence of antibiotic residues in milk using a structured questionnaire.

1.2 Problem statement

In Uganda, there are numerous reports of indiscriminate use of antibiotics for treating disease conditions in livestock. Some livestock products that have been exposed to these drugs (antibiotics) are sold for human consumption unknowingly. Most farmers treat their animals without even following the recommended dose rates and observing the drug withdrawal periods. This is escalated by the costs and difficulties in accessing veterinary services from qualified veterinary doctors especially in the rural livestock keeping households countrywide.

As a result, farmers never observe the drug withdrawal periods before marketing their livestock products more so the milk that is consumed directly in the raw form. Besides, the absence of strict regulations towards the veterinary drug shops operators has led to farmers to treat their animals without proper guidance on the correct usage of these drugs.

It was reported that some unscrupulous traders adulterate raw milk with antibiotics, sodium bicarbonate, calf-urine and formalin to try and extend its shelf life (Kang'ethe et al., 2005). However there are limited documented studies concerning the amounts of antibiotic residues in milk sold directly from farms and bulk milk collection centres Uganda. This poses a big risk to the milk consumers, the milk processors, and the milk traders in the entire milk value chain.

Consumption of milk adulterated with antibiotic residues poses public health threats including; development of bacterial resistance, hypersensitivity reactions, gastrointestinal disturbances and possible development of carcinogenic conditions in human beings. Antibiotics in milk also inhibit starter cultures for yoghurt making and they have a negative effect on local and international trade in processed milk products (Olufemi *et al* 2016)

Recently, Uganda has become the first exporter of dairy products in the region, with dairy exports valued at USD 24.6 million in 2013 both regional and international market (Bingi & Tondel, 2015). These sales could be affected if antibiotic residues occur in milk. Results of the study will inform farmers, consumers, veterinary extension workers and policy makers on measures for averting the likely public health and trade effects of antibiotic contamination in milk. This study therefore quantified the amount of antibiotic residues in milk from individual farms and four bulk milk collection centres in Kiboga district

1.3 Study objectives

1.3.1 General Objective

To determine the occurrence and levels of antibiotic residues and assess factors associated with their presence in raw cow milk from Kiboga district

1.3.2 Specific Objectives

- To assess the farmers level of knowledge and perception about drug residues in milk
- To determine occurrence and quantity of amoxicillin, oxytetracycline and Gentamycin residues in milk from individual farms and bulk milk collection centres
- To determine the factors associated with occurrence of antibiotic residues in milk

1.4 Research questions

1. What is the perception and level of knowledge regarding the occurrence and effects of antibiotic residues in milk among pastoralist farmers in Kiboga district?

2. What are the actual drug residual levels of oxytetracycline, gentamycin and amoxicillin in milk?

3. What are the factors associated with occurrence of antibiotic residues in milk?

1.5 Significance of the study

This study will reveal the levels of antibiotic residues in milk to guide policy makers and technical officers in regulating antibiotic use. This will promote better milk trade locally and regionally hence increased revenues from the dairy sector to the country.

1.6 Justification of the study

Levels of antibiotic residues in milk above the Maximum Residual Limits (MRL) pose a threat to the general public leading to hypersensitive reactions, antibiotic resistance, and toxicity to body organs such as the liver and the kidneys. Some of the residues are potentially harmful to human health and they affect processing and marketing of milk products (Takele Beyene, 2015).

Milk for human consumption should always be tested to ensure its safety. Kiboga district being one of the leading raw milk producing districts in Uganda never had any documented studies on the occurrence of antibiotic residues in raw milk. Oxytetracycline, amoxicillin, and gentamycin are in the classes of the most commonly used antibiotics of public health importance. Previous studies in Uganda have used semi quantitative techniques to access the presence of antibiotic residues in milk, this study will determine the occurrence and quantities of antibiotic residues in milk using High Performance Liquid Chromatography which is a more sensitive and specific method.

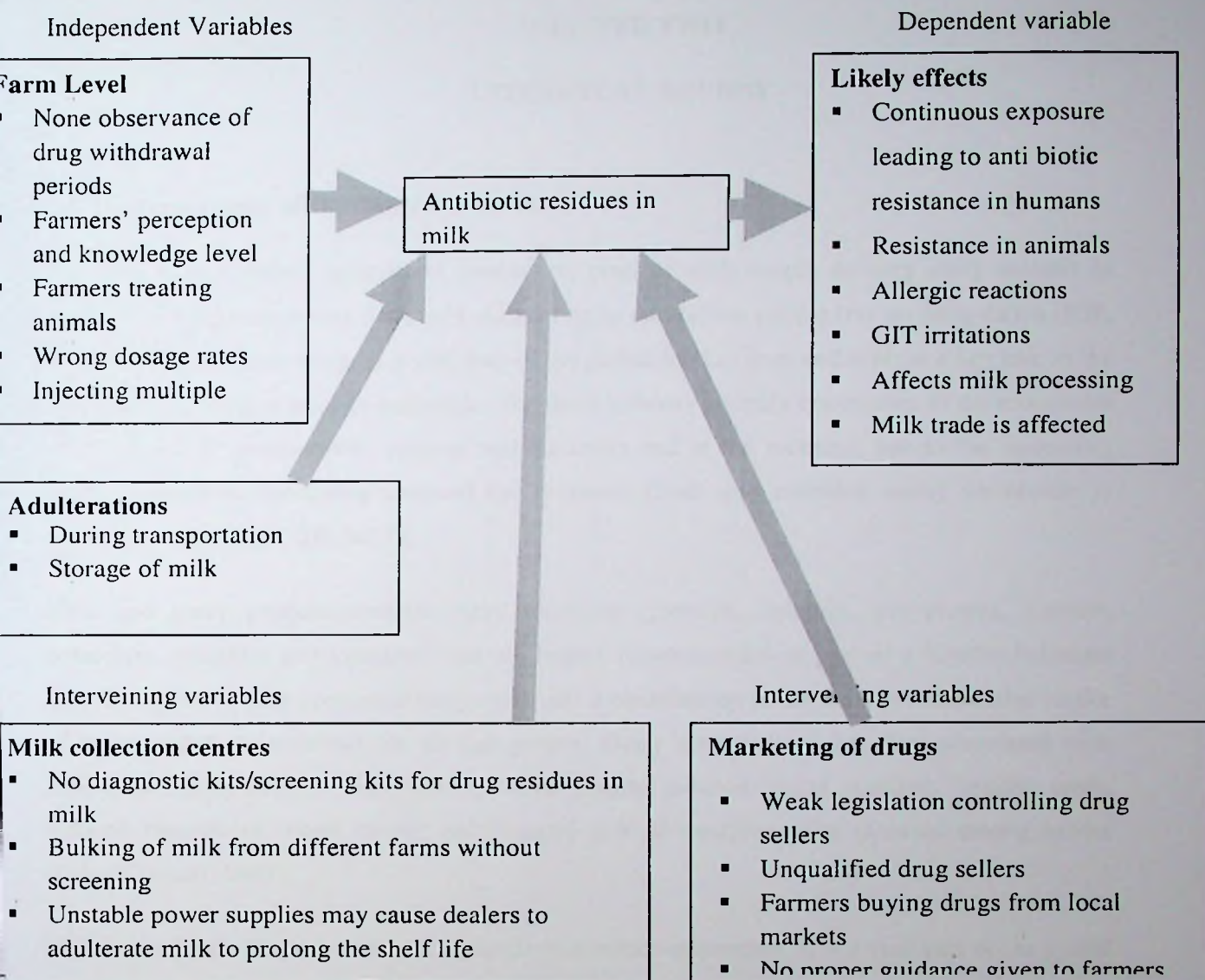


Figure 1: Conceptual frame work

CHAPTER TWO

LITERATURE REVIEW

2.1 The importance of the Dairy Sub-sector

Dairying is a universal agricultural production practice with people milking dairy animals in almost every country across the world. Almost up to one billion people live on dairy farms (IDF, 2013). The Dairy Sub-sector is a vital part of the global food system and it plays a key role in the sustainability of rural areas in particular. The dairy industry actively contributes to the economies of a number of communities, regions and countries and at the moment, due to the increasing world population, increasing demand for balanced foods and nutrition safety worldwide is noticeably emerging (IDF, 2013).

Milk and dairy products contain many nutrients (proteins, calcium, phosphorus, sodium, potassium, selenium and vitamins) that are highly recommended as part of a healthy balanced diet. One glass of milk consumed daily can make a contribution to the daily recommended intake of many important nutrients for all age groups. Dairy consumption has been associated with numerous health benefits that include: bone health, reduced blood pressure, healthy teeth, reduced chances of breast cancer and reduced risk of cardiovascular diseases among others (Dairy Council, 2007).

Milk is considered to be a universal agricultural production product. It is a vital part of the global food system and it plays vital role in the sustainability of rural areas in particular (IDF, 2013). Dairy business is considered the number one agricultural business in many states in the USA such as California, Wisconsin, New York, Pennsylvania, Idaho, Michigan, New Mexico, Vermont, and Maine. In California alone, dairy is a \$31 billion industry employing more than 400,000 people (Geoff & Are, 2008). In Netherlands, the sector is one of the most important engines of the Dutch economy with a total production value of 12 billion Euros and is responsible for 45,000 direct jobs (Lint, & Krachtcentrale, 2013).

In West Africa, the dairy subsector has been earmarked as one of the most important economic entity with a high demand for milk and milk products (Dieye *et al.*, 2004). In East Africa the

dairy sector is one of the fastest growing agricultural sub-sectors highly crucial for rural development, poverty reduction and food and nutrition security (Bingi & Tondel, 2015). The sector has generated significant economic returns and employment opportunities along the dairy value chains (Bingi & Tondel, 2015).

Eastern Africa has been identified as the leading milk-producing region in Africa, with 68% of the continent's milk output (Agriterro, 2012). Ethiopia, Kenya and Tanzania are among the biggest dairy producers in Africa. Rwanda's milk production according to the 2013 National Dairy Strategy (NDS) has been rising rapidly and continues to rise, from 51.5 million litres in 2000 to 445 million litres in 2012 (Agriterro, 2012).

In Kenya, annual per capita milk consumption estimates vary between 80 and 100 litres, which is the highest in Africa. The per capita milk consumption in Rwanda is relatively low, estimated at 40 litres per year. According to the 2013 NDS, consumer demand for both raw and processed milk is not increasing fast enough to clear projected raw milk supplies. In Tanzania milk consumption is also lower than that in Kenya, with people consuming 40 litres of milk per capita and per year (FAO, 2011).

In Uganda, the dairy sub-sector is one of the critical contributors to the economy. Dairy production is an important livelihood option of many rural Ugandans with an estimated annual milk production of 1 billion litres. The dairy sector in Uganda contributes 40-50 percent of the livestock Gross Domestic Product (GDP), which in turn contributes 17-19 percent of the overall agricultural GDP (TechnoServe Uganda, 2008).

Over 2.5 million households in Uganda engage in milk production and the national herd is estimated at m 7.5 million head of cattle of which 6.2 million are indigenous cattle and 1.3million are exotic and crossed cattle (MAAIF, 2008). The leading districts in dairy production are; Kiboga, Mbarara, Bushenyi, Ntungamo, Kabale, Mukono and Kiruhura (Rastogi *et al.*, 2016). The dairy sector is said to be growing at an annual rate of 8-10 percent per annum with the processing milk capacity growing at an estimated 11% per annum. The per capita consumption of milk products is 58 litres/person/year, far lower than the 100 litres/person/year in

neighboring Kenya or the 200 litres/person/year recommended by FAO. Nonetheless, the market has the potential to consume more milk (Agriterro, 2012).

Uganda's milk is marketed through; the formal segment which deals with the processed milk, and includes various products such as (pasteurized milk, UHT, yoghurt, ice-creams, powder milk and cheeses) and the informal segment which accounts for 90% of the total milk produced (Rastogi *et al.*, 2016). Therefore there is need to put in place measure that ensure production of quality and safe milk for consumption.

2.2 Knowledge, attitude and practices of farmers concerning antimicrobial residues in milk

Farmers play a key role in the occurrence of antimicrobial residues in milk. Therefore, knowledge, attitude and practices can influence the situation either positively or negatively since they are the primary producers and handlers of the milk.

A study conducted among dairy farmers in Michigan revealed that 90% of the farmers adhere to recommended drug withdrawal periods. The study also showed that the occurrence of residues was reduced in herds owned by dairy farmers who are aware that antibiotic residues in milk presented a major public health problem (Kaneene & Ahl, 1987).

However, 14% of the dairy farmers in the same study had no opinion about the public health implication of drug residues in milk while 76% of the farmers in the same study reported that drug residues in milk was more of an economic than a public health concern to the industry (Kaneene & Ahl, 1987). The study concluded that the occurrence of drug residues in milk is associated with, insufficient knowledge about drug withdrawal periods, errors due to hired help, insufficient records of treatment, and identification of treated animals by farmers.

Another study carried out in Pennsylvania, USA showed that 50% of the farmers kept written or computerized records of antibiotic treatment. The study also revealed other on farm practices by farmers to avoid occurrence of drug residues in milk like having a written plan for treatment of animals, seeking veterinary advice on the use of antibiotics on the farm (Sawant *et al.*, 2005). In Ethiopia, a study by Syit (2009) attributed the occurrence of antibiotic residues to absence of use

of post milk teat dipping, milking by contract laborers and poor record keeping systems on farms.

In Kenya, reports suggest that antimicrobial residues in marketed milk most likely originate at the farm level (Omore *et al.*, 2005). Still in Kenya, Shitandi & Sternesjö, (2004) suggested poor or no treatment records and lack of a monitoring system as major risks for contamination. It was therefore concluded that intensification of the education among small-scale dairy producers would greatly reduce the occurrence of antimicrobial residues in milk.

In a survey conducted in Masaka and Mbarara districts of Uganda by Sasanya *et al.*, (2013), 90% of the respondents acknowledged use of antibiotics on their animals. Ninety six percent of the respondents did not observe drug withdrawal periods in animals or animal products sold from their farms. Only 1% of the respondents were aware of the human health risks associated with exposure to residues of drugs through consumption of contaminated animal products. Eighty five percent of the respondents agreed that strict government regulations on the use of veterinary drugs as well as improved education on use of drugs by veterinary extension officers could be the key to limiting residues in foods. Twelve percent of the respondents affirmed that strict market demands would compel cattle farmers to institute proper drug management practices on their farms (Sasanya *et al.*, 2013)

These studies indicate that there is a gap in the knowledge, attitudes and practices regarding the occurrence of drug residues in milk. There is therefore need to articulate issues of drug residues in milk at national level so that everyone is made aware of their occurrence and repercussions.

2.3 Antimicrobial residues and risks they pose to human health

It is undesirable for antibiotic residues to occur in milk for human consumption. This is because of the various effects on human health and the environment. Thirty years ago, the presence of antibiotic residues in milk was considered primarily a manufacturing problem related to inhibition of cheese and yoghurt starters (Ruegg, 2005).

More recently, the presence of antibiotics in milk has been prohibited because of negative concerns on public health. The effects of public health importance associated with the

consumption of antibiotic residues in milk include; allergic reactions in sensitive people, toxicity to body cells and carcinogenic effects on people (Ruegg, 2005).

Beta-lactam antibiotics especially penicillins, cephalosporins and carbapenems have been associated with allergies in penicillin allergic persons. It is reported that penicillin is not inactivated by pasteurization or drying and levels as low as 0.03 IU/ml has caused skin rashes (Shitandi, & Sternesjö, A. 2004). Chloramphenicol has been reported to cause disruptions like aplasia of the bone marrow. Tetracycline residues have the potential to stain teeth of young children and can also react with nitrite to produce nitrosamine which is a carcinogenic compound (Shitandi, & Sternesjö, A. 2004).

Furthermore, continuous exposure to low levels of antibiotics can lead to a buildup of an antibiotic resistant population. It has been suggested that use of antibiotics in animals can contribute to resistance in human populations in a number of ways including; the direct transfer of resistant pathogens from animals to human via the food supply or via the environment (DeVincent, 2006).

McEwen (2006) states that resistance to antibiotics can occur through the transfer of resistance genes to human bacteria and chronic exposure to low levels of antibiotics in the form of food product drug residues. The development and spread of antibiotic resistance represents a serious threat to the population and the health sector. The dissemination of resistance traits could narrow the line of defense against bacterial infections to only a few antibiotic agents and this increases health care costs individuals and the government (Nampoothiri & Dominique, 2014).

Development of antibiotic resistance in bacteria is mediated by selective pressure due to antibiotic use and the presence of resistance genes. Resistance to antibiotics is by four major mechanisms including; i) alterations in the target site of the antibiotic, such as changes in penicillin binding proteins ii) drug degradation and enzymatic inactivation of the antibiotic (e.g. penicillins), iii) changes in cell wall permeability that prevent access to antibiotics and iv) increases in the activity of efflux pumps in the cell wall which prevent accumulation of antibiotic within the cell (Nampoothiri & Dominique, 2014).

Scholars have suggested several factors that are thought to influence the development of resistance and these include; drug concentration, long-term exposure, and organism and antibiotic type as well as host immune status. Low level long-term exposure to antibiotics may in particular have a greater selective potential than short term, full-dose therapeutic use. The judicious use of these drugs is thus of great global concern if we are to reduce anti biotic resistance in the population (Shitandi & Sternesjö, 2004).

The other effects of antimicrobial residues include immune-pathological effects, autoimmunity and carcinogenicity, which have been identified especially with sulphamethazine, oxytetracycline and furazolidone (Nampoothiri & Dominique, 2014). Nephropathy has been reported with gentamycin. Hepatotoxicity, reproductive disorders, bone marrow toxicity has been noted with chloramphenicol (Nampoothiri & Dominique, 2014). Other reports have noted that antimicrobial residues cause gastrointestinal tract (GIT) irritations through inhibition of lactic acid production (Ruegg *et al.*, 2013).

Antibiotic residues are of toxicological significance for the consumer and influence the technological properties of milk used for manufacturing fermented products. For example, antibiotic residues in milk are also said to inhibit the starter culture for yoghurt making thus causing financial losses to the manufactures (Ruegg *et al.*, 2013).

Concerns have been raised that antibiotic resistant bacteria may be transferred from animals to humans through; direct contact with affected animals, the environment (e.g., water, manure) or contaminated milk products (CAC., 1999). Research has generally showed that active metabolites of antibiotics may be excreted by animals through urine and faeces and reach the soil and water (Douglas *et al.*, 2013).

The most prevalent antibiotics found in the environment (surface waters) belong to the macrolide and the sulfonamide groups (Thorsten *et al.*, 2004). Tetracyclines, penicillins or fluoroquinolones have only been found in some cases and at low concentrations. Some commonly used anti-infectives such as erythromycin, cyclophosphamide, sulfadimidine, and tetracycline can persist in the soil and remained in surface waters and soils for over a year. These

are feared to cause water pollution through surface drainage into the lakes and rivers and cause water contamination (Thorsten *et al.*, 2004).

2.4 Factors associated with the occurrence of drug residues in milk

Antibiotic residues are remnants or metabolites of antibiotic drugs used in the treatment or as food additives that occur within animal tissues or product like meat, milk and eggs. Levels of the drug and their metabolites may at times persist at unacceptable levels and consumers are exposed to them (CAC, 1999).

There are many factors that could lead to the occurrence of antibiotics in milk including; wrong dosage rates by farmers, non-observance of drug withdraw periods, lack of knowledge concerning the occurrence of drug residues in milk or their effects as well as poor farm management practices like lack of records. Some scholars also suggest that lack of proper prescription on the administration of these drugs by the veterinarians and drug shop operators could also be a contributing factor to the occurrence of drug residues in milk (Redding *et al.*, 2014).

In order to safeguard human health, the World Health Organisation (WHO) and the Food Agriculture Organisation (FAO) have set standards for acceptable daily intake and maximum residue limits (MRL) in foods. Although these guidelines on the MRLs of drugs in food substances exist, not all national governments follow them, even where the MRLs are adapted, they are rarely enforced, especially when food products are sold on the informal market (Redding *et al.*, 2014).

A variety of antibiotics are used in livestock healthcare in Uganda today with tetracyclines and penicillins among the most common antibiotics for veterinary use in the country (Sasanya *et al.*, 2013). Unfortunately, there is no national antibiotic residue monitoring programme in Uganda. Furthermore, there is limited data on the occurrence and trends of residues of antibiotics in foods of animal origin in the country. This is a big gap since data on residues of antibiotics in foods of animal origin is essential to inform policy, so as to identify problems related to antibiotic usage, and to put forth necessary intervention.

Following economic policy reforms in Uganda, the marketing and distribution of veterinary inputs, including antibiotics, were liberalized and privatized. This policy shift, coupled with a weak regulatory mechanism, has resulted into enormous challenges in the management of veterinary drugs, particularly antibiotics, in the country (Sasanya *et al.*, 2013).

It is necessary for a country to have a national monitoring programme to check the trend and pattern in the occurrence of veterinary drug residues in animal products. This should be supported by a well-informed population that demands quality products free from drug residues.

It is also important for the leaders to have political will to fight any loophole in the system that can lead to the occurrence of drug residues in milk. This should be done along the entire dairy value chain from the dairy farms, milk transportation, at the collecting centres/bulking centres, at processing level and marketing level. Milk that does not meet the required standards should be rejected and the quality milk should be taken up at a higher cost to act as an incentive for farmers to produce quality milk.

2.5 Methods for detection of antibiotic residues

Different kinds of methods have been developed to detect antibiotic residues. These consist of; screening methods and chromatographic techniques. The screening methods are based on the susceptibility of bacteria to different antibiotics. The antibiotic residue detection assays that are currently available use different methods and test microorganisms (Kebede *et al.*, 2014). Microbiological assays for the detection of antibiotic residues utilize bacteria such as *Bacillus stearothermophilus* because of its high sensitivity to the majority of antibiotics.

Both microbiological and chromatographic methods can be used to monitor antibiotic residues in milk and animal tissues. Although the microbiological assay techniques have been recommended as official and conventional methods because of their simplicity, the bioassay methods lack specificity and provide only semi-quantitative measurements of residues detected and sometimes produce false positives (Kurittu *et al.*, 2000).

As a result, chromatographic techniques, such as, Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), and Capillary Electrophoresis (CE), have been developed to replace microbiological assays (Cinquina *et al.*, 2003).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

This was a cross-sectional study in which farmer practices that promote occurrence of antibiotic residues in cow milk were assessed through collection of data from farmers using questionnaires. Cow milk samples were collected from farms and milk collecting centres in Kiboga district of Uganda. Quantities of amoxicillin, oxytetracycline and gentamycin residues in these samples were determined by high performance liquid chromatography (HPLC).

3.2 Study area

Kiboga district is located in central Uganda between 010⁰N, 3146¹E (Latitude: 1.0000; Longitude: 31.7667). It is one of the leading producers of marketed milk in Uganda and is located in Uganda's 'cattle corridor' belt, an area of high livestock density (MAAIF, 2008). Two sub counties of Kapeke and Bukomero in Kiboga district were selected because they areas of high milk production located in a pastoral area with livestock cooperative societies that have BMCC.

A total of 51 milk samples were hence collected. Each sample was well labeled using indelible ink with necessary coded identification information including farm, farmer name, location, number of milking cows. The samples were then kept at 4-8°C, transported to Government Analytical Laboratory (GAL) and stored at -20 degrees until analysis. A structured interview guide (Appendix 1) was designed and administered to dairy farmers that belonged to Kiboga dairy cooperative residing in Kapeke and Bukomero subcounties in Kiboga district. This was through a face to face interview to determine the level of knowledge, perception and factors associated with occurrence of antibiotic residues in milk.

The questionnaire captured data on general farm management practices, herd structures and herd dynamics, use and management of antibiotics, observance of drug withdrawal periods, and awareness of human health risks associated with presence drug residues in milk and knowledge of regulatory mechanisms for safety of animal products each questionnaire was corresponded with the milk sample picked from that particular farm.

3.4 Sample preparation

Analysis for drug residues was carried out as described by Khaskheli *et al.*, (2008). Five (5) mls of each milk sample was mixed with 400 μ l of 10% aqueous solution of acetic acid in a 10 ml sterilized Pyrex screw-cap centrifuge tube and vortexed briefly for 1 minute. This mixture was then centrifuged at 3500 rpm for 10 minutes at 4°C in the Beckman centrifuge machine (USA). A clear supernatant was then drawn using a disposable syringe to avoid scooping of the upper fat layer and filtered through a 0.45 μ m nylon filter (13mm diameter) with the back pressure of the pump.

The filtered extract was then transferred into a 2ml sterilized vials and 5 μ mls of the same extract injected into the HPLC machine (Shimadzu Class VP 10). Each sample was prepared in duplicate. Stock standard solutions at concentrations of 100 μ g/ml of each analyte were prepared individually in deionized water, previously filtered in 0.45 μ m filter paper. A mixed standard working solution (100 μ l) of the three antibiotics to be analyzed (amoxicillin, oxytetracycline, and gentamycin) was prepared in advance from the stock solution.

3.5 Sample analysis

The quantification of the antibiotic residues was carried out by injecting the standard stock solution, blank sample and milk samples to be analysed. For each drug residue analyzed the factor of response (FR) consisting of the ratio between the height of analyte peak and height of internal standard was verified. The antibiotic residue amounts were then entered in Microsoft Excel spreadsheet and computed to give the drug residue concentration per sample.

3.6 Data and statistical analysis

Antibiotic residue levels of each sample analysed were compared to WHO/FAO maximum residue limits (MRLs) for each antibiotic in milk. The interview guide was coded, information entered into an Excel® and cleaned. The sheet was exported to SPSS version 20 and analyzed. Descriptive statistics were obtained and tables and graphs indicating frequencies and percentages were drawn. Pearsons' correlation analysis was carried out between the independent variables that were captured in the interview guide and the laboratory results for occurrence of antibiotic residues. The factors that showed a high correlation coefficient (p values <0.05) were considered significant.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of the participants

Out of the 47 respondents, 42 (89%) were male while 5(11%) were female. Of the respondents, 29.8% had no formal education while 29.8% had primary education. Majority of the respondents were aged between (18-35) years. Table 1 summarizes the demographic characteristics of the respondents.

Table 1: Demographic characteristics of the respondents

Item	Frequency	Percentage(%)
<i>Respondents' gender</i>		
<i>Males</i>	42	89.4
<i>Females</i>	5	10.6
<i>Age Group</i>		
18-35	25	55.3
36-59	17	34.1
60 and above	5	10.6
<i>Education Level</i>		
No formal education	14	29.8
Primary	14	29.8
Secondary	14	29.8
Tertiary education	5	10.6

4.2 Farmers' level of knowledge and perception on drug residues in milk

The study revealed that 66% of the respondents followed the recommended dosage rates. Of the respondents, 46.8% read labels supplied on the bottles bearing the manufactures' advice on drug usage while 38.3% milk animals following treatment (observed drug withdrawal periods). Only

14.9% of the respondents kept treatment records. Up to 48.9% of the respondents thought that antibiotics could end up in milk while 68.1% did not mind about the occurrence of drug residues in milk. Table 2 below shows the level of knowledge and perception of the respondents on the occurrence of drug residues in milk.

Table 2: Farmers' level of knowledge and perception on occurrence of drug residues in milk

	Farmers' response (%)					
	Yes	No	Sometimes	Never	I don't know	Don't care
Observed recommended dose rate	66	19.1	14	-		
Read labels on bottles	46.8	34	12.8	6.4		
Kept records	14.9	85.1	-	2.5		
Milk treated animals alone	36.2	61.7		2.1		
Observed drug withdrawal periods	38.3	55.3	4.3	2.1		
Gave combination of drugs	34	55.3	10.6	-		
Never minded about having antibiotics in milk	23.4	68.1	-	-	8.5	
Thought that antibiotics could end up in milk	48.9	23.4	-		19.1	8.5

4.3 Drug administration on the farms

It was observed that a higher number of samples had drugs residues on the farms where the farmers and herds men administered the veterinary drugs to the animals compared to when the veterinary doctors administered the drugs. This is showed in figure 2 below.

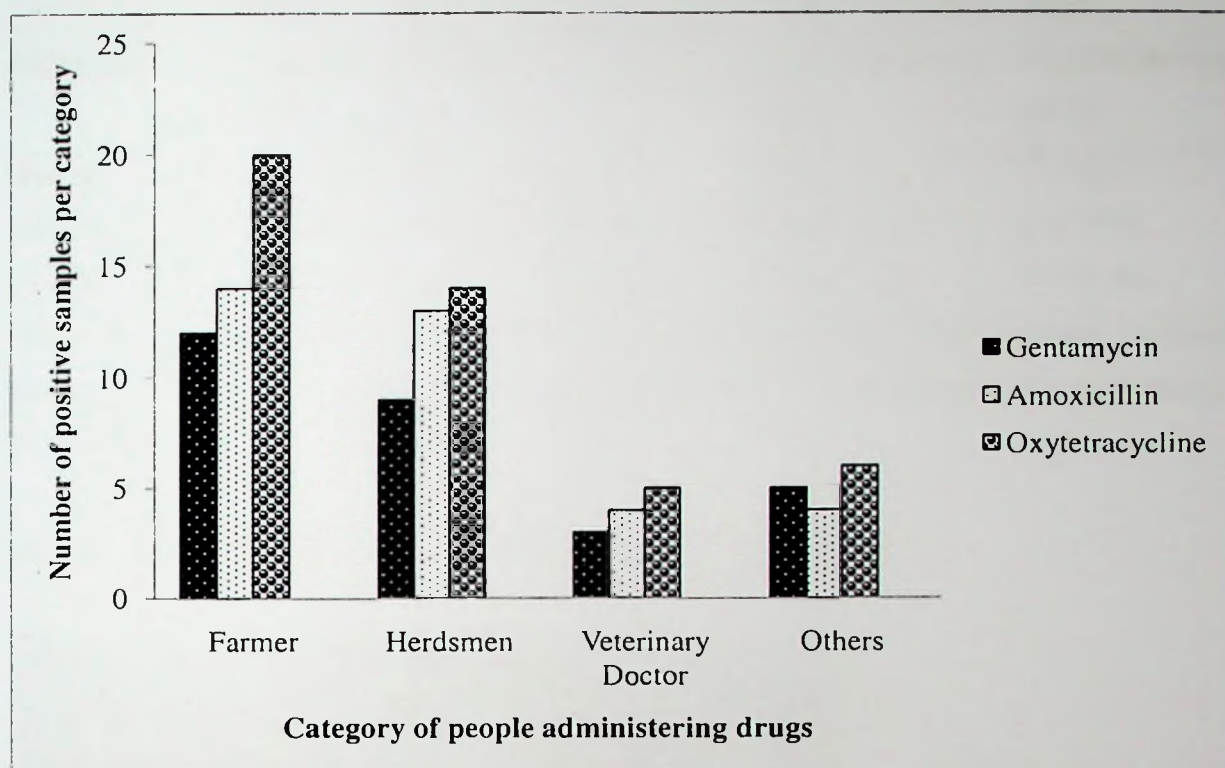


Figure 2: Distribution of antibiotic residues based on personnel administering the drugs

4.4 Antibiotic residue levels in milk samples

The mean antibiotic residue concentration for the three antibiotics in milk samples from BMCCs was highest for oxytetracycline ($0.35 \mu\text{g/kg}$) and lowest for Gentamycin ($0.098 \mu\text{g/kg}$). For the milk samples from individual farms, antibiotic residue concentration was highest for Gentamycin ($40 \mu\text{g/kg}$) and lowest for amoxicillin ($0.134 \mu\text{g/kg}$) as shown in Table 4 below. All concentrations of antibiotic residues from both farms and collection centres were below WHO recommended levels.

Table 3: Mean antibiotic concentrations and percentages of the positive samples

Antibiotic	Mean antibiotic conc. in milk from BMCC ($\mu\text{g/kg}$)	BMCC (%) +ve) (n=4)	Mean antibiotic conc. from farms ($\mu\text{g/kg}$)	Farm samples(%+ve) (n=47)
Gentamycin	0.098 (+0.098)	1 (25%)	40 (+0.25)	28 (59%)
Amoxicillin	0.173 (+0.125)	4 (100%)	0.134 (+0.17)	35 (74%)
Oxytetracycline	0.35 (+0.1)	4 (100%)	0.52 (+0.34)	47 (100%)

NB. The recommended Maximum residual limits for drug residues in milk and milk products by WHO are $200\mu\text{g/kg}$ for gentamycin, $4\mu\text{g/kg}$ for amoxicillin and $100\mu\text{g/kg}$ for oxytetracycline (Nisha, 2008).

Some of the chromatograms that were displayed during analysis

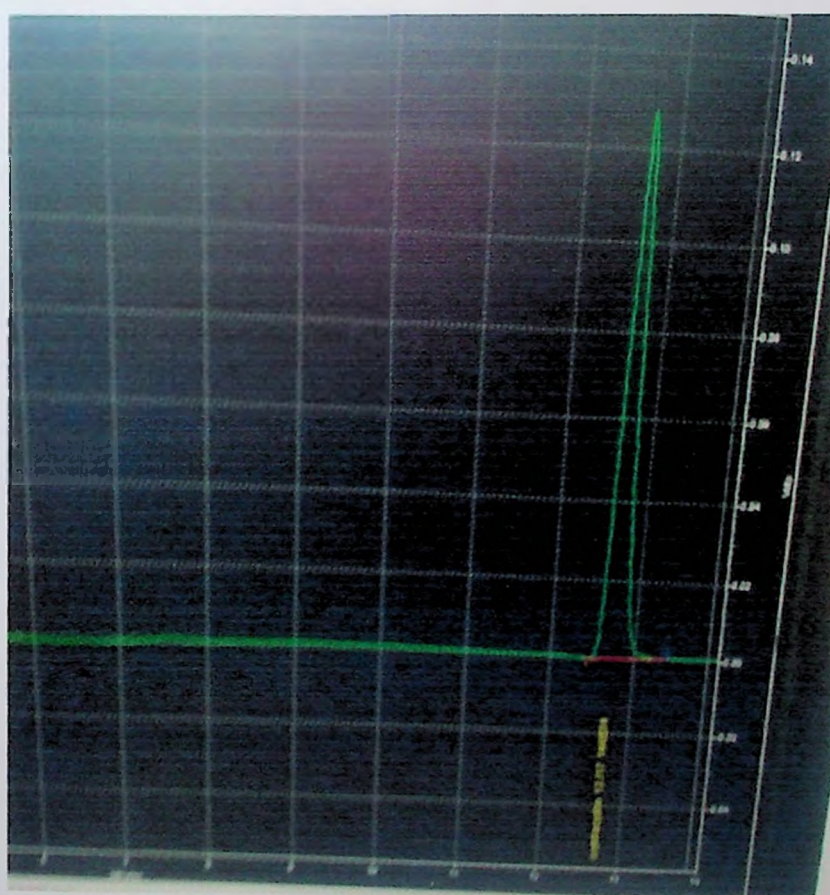


Figure 3: Chromatogram showing oxytetracycline peak

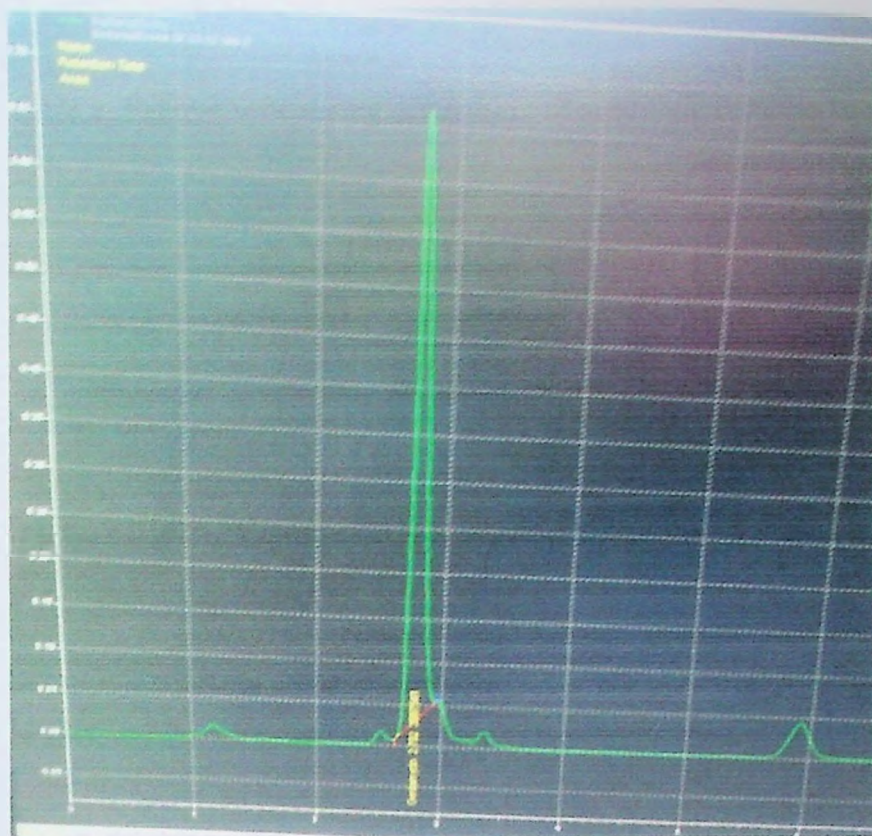


Figure 4: Chromatogram showing amoxicillin peak

4.5 Factors influencing occurrence of antibiotic residues in milk

Majority of the farmers interviewed (66%) were ranchers with 1-50 milking animals. The study further revealed that 68.1% buy veterinary drugs from licensed vet drug shops while 8.5% buy drugs from the local market. Majority of the farmers 48.9% store drugs anywhere in the house while the commonest means of transportation of milk from the farms to the milk collection center was by motorcycles 53.2%. The results are highlighted in Table 4 below.

Table 4: Factors influencing occurrence of antibiotic residues in milk

Item	Frequency(N=47)	Percentage (%)
Grazing system used		
<i>Zero grazing</i>	5	10.6
<i>Paddocking</i>	11	23.4
<i>Ranching</i>	31	66
Number of milking animals kept		
<i>1-10</i>	22	46.8
<i>20-50</i>	22	46.8
<i>60-100</i>	3	6.4
<i>>100</i>	0	0
Source of veterinary drugs		
<i>Market</i>	4	8.5
<i>Local drug shop</i>	9	19.1
<i>Venders</i>	2	4.3
<i>Vet drug shop</i>	32	68.1
Means of transportation for milk		
<i>Motorcycle</i>	25	53.2
<i>Bicycle</i>	18	38.3
<i>Foot</i>	4	8.5
Milking animals following treatment		
<i>Yes</i>	18	39.7
<i>No</i>	26	55.3
<i>Sometimes</i>	2	4.3
<i>Never</i>	1	2.1
Keeping of treatment records		
<i>Yes</i>	7	14.9
<i>No</i>	40	85.1

The factors that showed a high correlation with the occurrence of antibiotic residues in milk included; none observance of drug withdrawal periods with a $P= 0.405$, not keeping record $P= 0.31$, type of grazing system $P=0.312$ and the number of milking animals $P= 0.360$ as shown in Table 5 below

Table 5: Significant factors for the occurrence of antibiotic residues in milk

Factor	Pearsons'correlation coefficient	P value
None observance of drug withdrawal periods	0.405	0.005
Not keeping treatment records	0.31	0.031
Type of grazing system	0.312	0.033
Number of milking animals	0.360	0.013

CHAPTER FIVE

DISCUSSION

This study showed that there is relatively some level of knowledge concerning the occurrence of drug residues in milk owing to the responses by farmers as indicated in table 2. These issues include; handling of veterinary drugs, following of recommended dosage rates and reading manufactures' instructions. This level of knowledge could be attributed to the fact that most of the sampled farmers belonged to dairy cooperative societies in Kiboga that got occasional trainings on dairy hygiene and how to produce quality milk for sale by Makerere University and other public and private organizations. Similar studies in Ethiopia and Kenya also reported high levels of awareness of drug residues in milk following the treatment of sick animals (Munguba, 2001, Ombui, 1999). However, a study in Tanzania by Bukuku (2013) revealed that all the small holder farmers were aware of the occurrence of drug residues in milk, and it was reported that 97% complied with drug withdrawal periods. Other studies still in Tanzania, by Katakweba *et al* (2012) and Nonga *et al.*, (2009) have both reported 90% awareness levels of drug residues in milk while Midenge (2011) reported 72.2% awareness on drug withdrawal periods.

This level of knowledge (90%) is higher than that seen in this study in Kiboga. Bukuku (2013) attributed this to the work done by livestock extension officers who were reported to work closely with the smallholder dairy farmers as well as the publicity by media in Tanzania on the importance of quality milk. The level of knowledge in Uganda is lower. There are few extension workers covering large areas therefore farmers are not well guided on the use of veterinary drugs. There is little publicity on the occurrence of drug residues in milk and the importance of quality milk consumption; rarely do we get an advertisement on radio, television or the newspapers promoting safe milk production and consumption.

The study also showed that, majority of the respondents ,67.5% never minded about antibiotic occurring in milk at all while (60%) of the respondents thought that the antibiotics used for treatment of animals could end up in milk while. These perceptions on issues concerning the occurrence of drug residues in milk could be one of the reasons antibiotic residues are occurring in milk in most of the farms sampled. Where farmers have the notion that even if they do not adhere to the manufacturer's instructions regarding the use of antibiotics in animals, the

antibiotics cannot end up in products like milk or meat which is consumed by humans. These results are similar to those obtained by Kaneene *et al.*, (1987) where insufficient knowledge about withdrawal periods and not reading of bottle labels regarding antibiotic usage are among the factors that influenced the occurrence of drug residues in milk.

From the results of this study, there is limited knowledge on the health implications of antibiotic residues in milk, most of the farmers highlighted stomach pain and vomiting while issue like damage to body organs and antibiotic resistance were not mentioned by the farmers. This is similar to a study by Shitandi *et al.*, (2004) in Kenya which points out lack of understanding of risks related to antibiotic contamination of food and the lack of monitoring systems as a major cause for occurrence of drug residues in milk.

Kenya and Uganda both being East African countries, the level of knowledge of farmers in both countries could be comparable. This explains why the same factors can occur in both countries. Since they are all developing countries, their monitoring systems are not well established and this affects the quality of milk. For example during this study, it was observed that none of the milk collection centers carried out screening tests for drug residues in milk.

This survey also revealed that when most of the farmers and the herdsman were the ones involved in the administrations of drugs to the animals, milk from these individual farms tested positive with antibiotic residues as highlighted in Figure 2. This could be one of the leading causes of occurrence of these residues in milk since these farmers and herdsman often times overestimate the weight of their animals giving them high doses. Also farmers do not know how to read and never follow the recommended drug prescriptions and withdrawal periods during administration of those drugs.

However, a study that was carried out in Michigan State University indicated that majority of the farmers 45% would consult a veterinarian first before they administer any veterinary chemicals (Mellenburger, 1998). This is probably due to the availability of the veterinary practitioners in the USA. The ease of access to the farms could also be another factor that influences the farmers' use of veterinarian in the USA. Well as in Uganda, there is scarcity of veterinary doctors; in some cases, only one Veterinary doctor may be available to serve the whole district.

Furthermore, the poor road network in Uganda to the farms makes it harder for the veterinarians to reach these areas. All these increase the cost of veterinary services in Uganda and therefore farmers and herdsman are forced to treat their animals. This is evident in the results of the study where many farmers and herdsman are treating animals (Figure 2).

From this study, low levels of antibiotic residues of gentamycin, amoxicillin and oxytetracycline were detected in both the individual and the bulked milk samples that were collected in Kiboga district; when compared with FAO/WHO MRLs. These antibiotic residues were found in both the milk that was collected from the individual farms and the four milk collection centres, meaning that there is uncontrolled administration of antibiotics in these herds. This situation is similar to what was observed in other countries like Croatia, Ghana and Kenya among pastoralists where drug residues were found to occur in milk (Khaskheli *et al.*, 2008, Kang'ethe *et al.*, 2005 and Nisha *et al.*, 2008). In Uganda, Sasanya *et al* (2013) reported that 13% of milk samples contained antibiotic residues beyond the MRL. Among the milk samples from the bulk milk centers, oxytetracycline had the highest mean concentration followed by amoxicillin and gentamycin. This could be attributed to the ease of use of, oxytetracycline intramammary and its injectable solutions on the market that were readily available to these farmers. Farmers also believe that Oxytetracycline can treat nearly all forms of livestock diseases.

Despite the samples being positive for oxytetracycline, gentamycin and amoxicillin the detected concentrations were below the recommended maximum residual limits set for drug residues in milk and milk products by WHO. This is in conformity with a study by Bilandžić *et al.*, (2011) in Croatia where all the results obtained did not exceed the MRL. However, the observance of a slightly higher mean concentrations of antibiotic residues from individual farm milk samples for gentamycin (40 μ g/kg) and oxytetracycline (0.52 μ g/kg) could be attributed to the length of their recommended withdraw periods which is 5days for gentamycin and 7days for long acting oxytetracycline. These mean concentrations were higher compared to that of amoxicillin (0.134 μ g/kg) which has a recommended withdraw period of 2.5days (60 hours) (Table 3).

Furthermore, these high concentrations could be linked to the reported treatment with gentamycin and oxytetracycline mastitis tubes for subclinical mastitis in that area. This is comparable to findings in a previous survey in Uganda that established the presence of antibiotic residues in milk with 13% of the samples positive (Sasanya, *et al* 2013)

However in this study, a higher proportion of samples were positive with antibiotic residues as compared to the previous survey by Sasanya *et al.*, (2013). This difference could also point to the methods of detection that were used in the two studies. While the later study used the Charm Farm[®] test (field kits), this study used HPLC which is a more sensitive test. While the method of detection could be a likely explanation for the higher concentrations observed, there is also a high possibility that the increase is due to the growing trend of increase in the occurrence of antibiotic residues in milk. This is showed by the fact that majority of the farms sampled had antibiotic residues in milk.

Similar findings have been observed elsewhere in the world such as in Kenya where 16% of marketed milk had antimicrobial residues (Kang'ethe *et al.*, 2005) and in Ghana, 35.5% of milk samples were contaminated with one or more types of drug residues (Aning *et al.*, 2007). However the results of this study differed from the results of a study carried out by Bilandzic *et al.*, (2011) in Croatia where none of 119 raw milk samples collected had residues above the MRL that are set by European Union and Croatian legislation. This situation is because of the strict adherence to the drug withdraw periods and following the legislation in Croatia (Bilanzik *et al.* 2011). In Uganda however, there is no legislation on the occurrence of drug residues in milk (Sasanya *et al.* 2013)

Among the factors that were established to affect the occurrence of drug residues by this study were; observance of the drug withdrawal period which was significant with ($P=0.005$) (table 5) indicating that farmers milk animals that are undergoing treatment. It is also strongly supported by the results where 62% of farmers do not observe recommended withdraw periods. This relates to studies by Kaneene *et al.*, (1999) which indicated the lack of adherence to drug withdraw periods as one of the factors influencing occurrence of drug residues in milk.

Keeping of records on the farm is also another factor that significantly affected the occurrence of drug residues in milk with ($P=0.031$). This could be explained by the fact that if there are no records on the farm, the farmer is not likely to know which animals are sick, which animals are undergoing treatment, when they started the treatment, when they are expected to finish the treatment, which drugs are being used and the recommended withdrawal times for those drugs. If all this is not in place the farmer will just milk all animals regardless of whether they are undergoing treatment or not. These two factors above concur with the results by Kaneene *et al.*,

(1987) where observance of recommended drug withdrawal periods and keeping of records influenced the occurrence of drug residues among dairy farmers in Michigan. It also relates to a study in Kenya by Shitandi & Sternesjo2004, which identified poor or no treatment records as a factor influencing the occurrence of drug residues in milk

The number of milking animals was also found to be influencing the occurrence of antibiotic residues in milk with ($P=0.013$) as showed in (Table 5). The more the milking animals the higher the chances of detection of antibiotic residues in milk. Some of the animals there could be undergoing treatment. It could also be explained by the fact that the strictness and keenness in terms of drug management reduces as the number of animals increases. This creates the likelihood of the occurrence of antibiotic residues in milk.

The type of grazing system was found to be another factor that influenced the occurrence of antibiotic residues in milk with ($P=0.033$) as showed in (Table 5). It was observed that antibiotic residues occurred more in the ranching farmers followed by the paddocking then the zero grazing farmers. This could also be explained that as the number of animals increases on the farm, there is more intensification of practices in terms of feeding and treatment. There is also more exposure to disease causing organisms in an intensified system than a zero grazing system which calls for the use of more drugs on the farm. An intensive system also tends to employ more people owing to the demands of the system of rearing animals.

An intensive system requires more workers handling drugs and these are subject to error from time to time while in a zero grazing unit it could only be one person administering the drugs this reduces the risk of improper handling of drugs and thus reduces their occurrence in milk. Results from this study relate with a mail survey of Michigan farmers where it was found that most residues were due to: insufficient knowledge about drug withdrawal periods, errors due to hired help, insufficient records of treatment, and identification of treated animals (Mellenburger, 1998).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Residues of amoxicillin, tetracycline and gentamycin were present in raw cow milk from Kiboga district but all levels were below the FAO/WHO MRL levels. Higher concentrations of antibiotic residues were observed in antibiotics with longer recommended withdrawal periods. There is low level of knowledge and a poor perception about the occurrence and effect of antibiotic residues milk among livestock farmers in the district.

6.2 Recommendations

- More studies should be carried out to establish the occurrences of antibiotics in milk especially the classes of antibiotics that were not covered by this study for example macrolides, quinolones and sulphonamides as well as other factors that may influence the occurrence of antibiotics in milk like seasonality.
- Continuous sensitization of farmers, farm workers and the general public by veterinarians on the causes and implications of antibiotic residues in milk.
- The government should enforce regulations regarding the use of antibiotics in animals especially acquisition, administration and following of the recommended withdrawal periods more so with antibiotics that have longer recommended withdrawal periods
- Rapid screening kits should be availed at milk collection centers to test for drug residues in milk and incentives such as higher pricing offered to farmers whose milk meets the required standards.

REFERENCES

- Agriterra (2012). Identification of livestock investment opportunities in Uganda, 126. *Retrieved from www.agriterra.org*
- Aning, K. G., Donkor, E. S., Omore, A., Nurah, G. K., Osafo, E. L. K., & Staal, S. (2007). Risk of Exposure to Marketed Milk with Antimicrobial Drug Residues in Ghana. *The Open Food Science Journal*. 1, vol. 1, no. 1. pp. 1–5.
- Bilandžić, N., Kolanović, B. S., Varenina, I., & Jurković, Z. (2011). Concentrations of veterinary drug residues in milk from individual farms in Croatia. *Scientific note - Znanstvena bilješka, Mljekarstvo* 61 (3), pp. 260-267.
- Bingi, S., & Tondel, F. (2015). Recent developments in the dairy sector in Eastern Africa, *Towards a regional policy framework for value chain development, briefing note no. (78)*.
- Bubuku.J.N (2013). Awareness of health risks as a result of consumption of raw milk in Arusha city and Meru district,Tanzania. M. Sc Thesis, Sokoine University of Agriculture, Morogoro,Tanzania.
- Codex Alimentarius Commission(CAC), (1993), *Report of the thirtieth session of the codex committee on pesticide residues*, pp 22–26.
- Cinquina, A. L., Longo, F., Anastasi, G., Giannetti, L., & Cozzani, R. (2003). Validation of a high-performance liquid chromatography method for the determination of oxytetracycline, tetracycline, chlortetracycline and doxycycline in bovine milk and muscle. *Journal of Chromatography A*, 987(1-2), pp 227–233.
- Dairy Couincil. (2007). The importance of milk and dairy products as part of a healthy balanced diet 1st Edition, Londin, United Kingdom,pp 5-15.

- DeVincent SJ, V. C. (2006). Introduction to animal antimicrobial use data collection in the United States: methodological options. *Journal of Preventive Veterinary Medicine* ,73 pp,105-109.
- Dieye. PN, Duteurtre.G, JR Cuzon.JR, D(2004). Livestock, Liberalisation and Trade Negotiations in West Africa .*Communication(full paper) to the 1st Inaugural Symposium of the African Association Of Agricultural Economics (AAAE), Nairobi Kenya*,pp 22-44
- Douglas R. Call, Louise Matthews, Murugan Subbiah, and J. L. (2013). Do antibiotic residues in soils play a role in amplification and transmission of antibiotic resistant bacteria in cattle populations? Published online 2013 Jul 11. doi: 10.3389/fmicb.2013.00193
- Geoff B. & Are N. (2008). North Carolina's Dairy Industry Stabilization and Growth Program Strategic Plan, *Dairy Advantage* pp1-28.
- Food and Agriculture Organisation (FAO). (2011). Dairy Development Institutions in East Africa, Rome, Italy pp, 36–39.
- Haug, A., Høstmark, A. T., & Harstad, O. M. (2007). Bovine milk in human nutrition Open access article <http://www.lipidworld.com/content/6/1/25>.
- International Dairy Federation (IDF) , (2013). The Economic Importance of Dairying
- Kaneene, J. B., & Ahl, A. S. (1987). Drug Residues in Industry□: *Journal of Dairy Science*, 70 2176–2180.
- Kang'ethe, E. K., Aboge, G. O., Arimi, S. M., Kanja, L. W., Omore, A. O., & McDermott, J. J. (2005). Investigation of the risk of consuming marketed milk with antimicrobial residues in Kenya. *Food Control*, 16(4), 349–355.
- Katakweba, A. A. S., Mtambo, M. M. A., Olsen, J. E. and Muhairwa, A. P. (2012). Awareness of human health risks associated with the use of antibiotics among livestock keepers and

- factors that contribute to selection of antibiotic resistance bacteria within livestock in Tanzania. *Livestock Research for Rural Development* Volume 24, Article #170. [<http://www.lrrd.org/lrrd24/10/kata24170.htm>] .
- Kebede, G., Zenebe, T., Disassa, H., & Tolosa, T. (2014). Review on Detection of Antimicrobial Residues in Raw Bulk Milk in Dairy Farms, 6(4), 87–97.
- Khaskheli, M., Malik, R. S., Arain, M. A., Soomro, A. H., & Arain, H. H. (2008). Detection of β -Lactam Antibiotic Residues in Market Milk. *Pakistan Journal of Nutrition*, 7(5), 682–685.
- Kurittu, Jussi; Lönnberg, Stefan; Virta, Marko; Karp, M. (2000). Qualitative Detection of Tetracycline Residues in Milk with a Luminescence Luminescence-Based Microbial Method *Journal of Food Protection* 7(5) pp. 953-957
- Lint, W. D., & Krachtcentrale, M. (2013). Dairy Nation Holland. Holland, Netherlands pp.55.
- MAAIF. (2008). The National Livestock Census Report.
- Marshall, B. M., & Levy, S. B. (2011). Food animals and antimicrobials: Impacts on human health. *Journal of Clinical Microbiology*, 24(4), 718–733.
- McEwen, S.A. (2006). Antibiotic use in animal agriculture: what have we learned and where are we going? *Journal of Animal Biotechnology*, 17:239-250.
- Mellenburger, R.W. (1998). Milk antibiotic violations, page 11-14, Michigan Dairy Review, Vol. 3 (1), Michigan State University, East Lansing
- Midenge, B. Y. (2011). Awareness on recommended veterinary drugs withdrawal period among small scale dairy cattle farmers in Kinondoni Municipality. M.Sc Thesis, Muhimbili University of Health and Allied Sciences, Dar es salaam, Tanzania.
- Munguba, E.O. (2001). Management and economics of dairy cows mastitis in the urban and periurban area of Addis Ababa. M.Sc Thesis, Addis Abba University, Ethiopia

- Nampoothiri, V. M., & Dominique, G. (2014). Drug residues in milk, Open access <http://www.doublehelixresearch.com/RNFU> ©Double 14, 158–164.
- Nisha, A. R. (2008). Antibiotic Residues - A Global Health Hazard, *Veterinary World*, Vol.1(12) pp 375–377.
- Nonga, H. E., Mariki, M., Karimuribo, E. D. and Mdengela, R. H. (2009). Assessment of antimicrobial usage and antimicrobial residues in broiler chicken in Morogoro municipality, Tanzania. *Pakistan Journal of Nutrition* 8: 203 - 207.
- Olufemi Isaac Olatoye, Oluwayemisi Folashade Daniel,² and Sunday Ayobami Ishola (2015) Screening of antibiotics and chemical analysis of penicillin residue in fresh milk and traditional dairy products in Oyo state, *Nigeria Vet World*. 2016 Sep; 9(9): 948–954.
- Ombui, J. N. (1994). Antibiotic residues in milk received by dairy cooperatives societies in Kiambu district, Kenya. *East African Medical Journal* Vol 71: pp 628 - 630.
- Omoro, A., Lore, T., Staal, S., & Kutwa, J. (2005). Addressing the public health and quality concerns towards marketed milk in Kenya, *SDP Research and Development Report 3* Smallholder Dairy (R& D) Project.
- Rastogi, N. K., Raghavarao, K. S. M. S., & Niranjana, K. (2016). Opportunities and Challenges in Uganda's dairy industry, *East Africa agribusiness article Monday 25th April, 2016* East Africa agribusiness.
- Redding, L. E., Cubas-Delgado, F., Sammel, M. D., Smith, G., Galligan, D. T., Levy, M. Z., & Hennessy, S. (2014). Understanding the use of antibiotics on small dairy farms in rural Peru. *Journal of Preventive Veterinary Medicine*, 113(1), 88–95
- Ruegg, P. L., Science, D., & Wi, M. (2013). Antimicrobial residues and resistance: understanding and managing drug usage on dairy farms, *Proceedings of the Central*

Veterinary Conference, Kansas City, MO, August 2013

- Ruegg P. L (2005). Relationship between bulk tank milk somatic cell count and antibiotic residues, *a report to the University of Wisconsin Madison, Wisconsin.*
- Sasanya J.J, Ejobi F, Enyana J, O. D. and S. G. (2013). Public Health Perspectives of Penicillin G Residues in cow Milk and Edible Bovine Tissues collected from Mbarara and Masaka Districts, Uganda. *African Journal of Animal and Biomedical Sciences*, 53(2), 1689–1699.
- Sawant, A. A., Sordillo, L. M., & Jayarao, B. M. (2005). A Survey on Antibiotic Usage in Dairy Herds in Pennsylvania. *Journal of Dairy Science*, 88(8), 2991–2999.
- Shitandi, A. 2004. Risk factors and control strategies for antibiotic residues in milk at farm level in Kenya. Doctoral dissertation. ISSN 1401-6249. ISBN 91-576-6477-304). ,
- Shitandi, A., & Sternesjö, A. (2004). Factors contributing to the occurrence of antimicrobial drug residues in Kenyan milk. *Journal of Food Protection*. 2 pp. 228-419, pp. 399-402(4)
- Syit, D. A. (2009). Detection and determination of oxytetracycline and penicillin g antibiotic residue levels in bovine bulk milk from Debrezeit and Nazareth dairy farms. *Proceedings of the 1st International Technology, Education and Environment Conference (c) African Society for Scientific Research (ASSR)*, 11(3), 1153–1156. doi:10.1016/S0031-9422(00)88470-8
- Takele Beyene (2016) Veterinary Drug Residues in Food-animal Products: Its Risk Factors and Potential Effects on Public Health, *Journal of Veterinary Science & Technology* 9(9): 948–954.
- TechnoServe Uganda (2008) The Dairy Value Chain in Uganda. A report for Easr Africa Dairy Development program
- Thorsten Christian, Rudolf.J.Schnieder, Harald A.Farber, Dirk Skutlarek, H. E. G. (2004). Determination of Antibiotic Residues in Manure, Soil and surface Waters. *Doctoral*

dissertation, Institute of Hygiene and Public Health, University of Bonn Sigmund-Freud -

Str.25,53105 Bonn, Germany.

APPENDICES

Appendix 1: Questionnaire on knowledge and practices regarding occurrence of antibiotic residues in milk

MAKERERE UNIVERSITY

COLLEGE OF VETERINARY MEDICINE ANIMAL RESOURCES AND BIOSECURITY

SURVEY ON OCCURANCE OF DRUG RESIDUES IN MILK IN KIBOGA DISTRICT CENTRAL UGANDA

Reason for Farm Visit: To collect information regarding the occurrence of drug residues in milk in Kiboga district. The information obtained will be highly confidential and shall be shared with the farmers. It will also be used to develop useful guideline to improve milk quality in the district.

1. OWNERSHIP AND LOCATION OF FARM

1.1. Name of Farm/Farm owner-----

1.2. Location of farm:

District	Sub-County/Division	Parish	Village/ LCI

1.3. Farmer's age, sex and contacts

Age	Sex	Contact address	Telephone

1.4. Farmer education level

Tertiary	Secondary	Primary	None

2. KNOWLEDGE AND FACTORS INFLUENCING OCCURANCE

2.1. How many cows do you keep on the farm?

- a) 1-10
- b) 20-50
- c) 60-100
- d) More than 100

2.2. How many milking cows do you have?

- a) 1-10
- b) 20-50
- c) 60-100
- d) More than 100

2.3. Which type of grazing system do you use?

- a) Tethering
- b) Zero grazing
- c) Paddockking
- d) Ranching

2.4. Which are the commonest diseases encountered on the farm?

- a) TBDs
- b) Mastitis.
- c) Foot conditions
- d) Others specify

2.5. Who usually identifies and treats the diseased animals

- a) Farmers
- b) Herdsmen
- c) Veterinary doctor
- d) Others specify.....

2.6. Where do you buy drugs for treating animals?

- a) In the market
- b) Local drug shop
- c) From vendors
- d) Veterinary drug shop
- e) Others specify.....

2.7. Which are the commonest drugs used on the farm?

- a) Anti theirelials
- b) Anti biotics
- c) Anti protozoans
- d) Dewormers
- e) Acaricides

2.9. Do you keep records of all treated animals on the farm?

- a) Yes
- b) No

2.1.0. How are drugs handled and stored on the farm?

- a) In the cabin
- b) On the table
- c) Anywhere in the house

2.1.1. Do you usually read labels on the drug bottles?

- a) Yes
- b) No
- c) Sometimes
- d) Never

2.1.2. Do you usually give a combination of drugs to animals on the farm?

- a) Yes
- b) No
- c) Sometimes
- d) Always

2.1.3. Do you follow the recommended doses when treating animals?

- a) Yes
- b) No
- c) Sometimes
- d) Never

2.1.4 Which is the commonest route of drug administration used?

- a) Intra muscular
- b) Intra venous
- c) Oral
- d) Intra mammary
- e) Others specify.....

2.1.5 Do you milk treated animals separately?

- a) Yes
- b) No
- c) Some times
- d) Never

2.1.6. Do you discard milk for some days following treatment of animals?

- a) Yes
- b) No
- c) Sometimes
- d) Never

2.1.7 Where do you sell most of your milk?

- a) On the farm
- b) Vendored to clients
- c) Taken to the collecting centre
- d) All consumed at home

2.1.8. How is the milk transported for marketing?

- a) By vehicle
- b) By motorcycle
- c) By bicycle
- d) On foot

3. PERCEPTION

3.1. Do you think that the drugs administered to animals can end up in the milk?

- a) Yes
- b) No
- c) May be
- d) I don't know

3.2. Do you think it's okay for humans to consume milk with antibiotic residues?

- a) Yes
- b) No
- c) I don't know
- d) I don't care

3. What are some of the side effects that can be caused by consuming milk with antibiotic residues?

- a) Stomach pain
- b) Vomiting
- c) Scratching
- d) Damage to body organs
- e) I don't know