

**ASSESSMENT OF ANTIBIOTIC RESIDUES IN RAW COWS' MILK
PRODUCED BY SMALL SCALE DAIRY FARMS IN BAGAMOYO
DISTRICT, TANZANIA**

RIDHIWANI, RAMADHANI

**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN
PUBLIC HEALTH AND FOOD SAFETY OF SOKOINE UNIVERSITY OF
AGRICULTURE. MOROGORO, TANZANIA.**

2015

ABSTRACT

A cross-sectional study was conducted in Bagamoyo District, Tanzania between October, 2013 and March, 2014 to assess the antibiotic residues in raw cows' milk produced by small-scale dairy farms. One hundred and ten small-scale dairy farmers were interviewed at household level on the handling and use of veterinary drugs, access to and availability of veterinary services, livestock diseases frequency and means of treatment, most commonly used antibiotic, awareness of drug residues in raw milk and health risks associated with consumption of raw milk containing antibiotic residues. In addition 110 raw milk samples were collected from three available milk collection centers for laboratory analysis of antibiotic residues. Laboratory assessment included, screening qualitative test using microbial inhibition test, followed by quantification of oxytetracycline (OTC) residues levels for positively detected samples by using HPLC Technique. Prevalence of OTC residues was 10% and all positive detected samples had OTC residues above MRL levels by FAO/WHO-CAC 2-2012. The mean value level of OTC was $766.3 \pm \mu\text{g/l}$. About 83.6% of respondents call livestock officers in the event of a sick cow and 56.4% of cases OTC were prescribed. On health risks associated with consumption of raw milk with antibiotics, 91.8% of respondents were aware. Screening for residues at the milk collection centers is recommended and further investigation of the milk production practices among small-scale dairy farmers is required. This will provide a basis for designing appropriate and effective small scale milk production practices which will reduce milk contamination and help to protect the health of consumers in Tanzania.

DECLARATION

I, RIDHIWANI RAMADHANI, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other Institution.

RIDHIWANI RAMADHANI
(MSc. Student)

Date

The above declaration is confirmed by:

Prof. KAZWALA, R.R
(1st Supervisor)

Date

Prof. MUHAIRWA, A.P
(2nd Supervisor)

Date

COPYRIGHT

No part of this dissertation may be reproduced, stored in any retrievable system or transmitted in any form or by any means without prior written permission of the author or Sokoine University of Agriculture in that behalf

ACKNOWLEDGEMENTS

I highly thank the almighty God who helped me in every step of my studies at SUA. This dissertation is a result of contributions of many people whom I can't mention all by names. I am very much grateful to the Tanzania Bureau of Standards (TBS) for sponsoring my studies. I would also like to extend my special thanks to my supervisors Prof. R.R Kazwala and Prof. A.P. Muhairwa for their tireless guidance during the entire course of preparation of this dissertation. I real wish to express my sincere thanks for their professional guidance, readiness, commitment and helpful discussions.

My heartfelt appreciation should further go to the academic staff members of the Faculty of Veterinary Medicine for providing me with opportunity and facilities to conduct my research laboratory work. I would like also to express my entire gratitude to Tanzania Food and Drugs Authority (TFDA) and particularly, Director of Laboratory Services Mrs Charys Ugulum for permission to conduct part of my laboratory work in TFDA. I am very grateful to the Ward Extension Officers Mr. Dickson Chahe and Mr. Yahaya Mono in the study area for their assistance they provided during the field work. I also acknowledge the small-scale dairy farmers who participated in the study and provided relevant information regarding their animals and the use of antibiotics. I humbly appreciate the support from my wife Mrs. Halima Matange for her encouragement which enabled smooth accomplishment of both course work and research part of my study.

DEDICATION

This work is humbly dedicated to my late Father Ramadhani Matange, my late Mother Mwanaid Seif, and my wife Halima R. Matange, and our children Faraja and Jaffar for their patience during the period of my study.

TABLE OF CONTENTS

ABSTRACT	i
DECLARATION	ii
COPYRIGHT	iii
ACKNOWLEDGEMENTS	iv
DEDICATION	v
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF APPENDICES	xii
LIST OF ABBREVIATIONS AND SYMBOLS	xiii
CHAPTER ONE	1
I.0 INTRODUCTION	1
1.1 Background information.....	1
1.2 Problem Statement and Study Justification.....	5
1.3 Research Objectives	5
1.3.1 The Overall Objective.....	5
1.3.2 Specific Objectives	6
CHAPTER TWO	7
2.0 LITERATURE REVIEW	7
2.1 General Overview.....	7

2.2 Livestock and dairy production in Tanzania	8
2.3 Uses of antibiotics in animals production	10
2.4 Occurrence of antibiotics residues in animal products	10
2.5 Commonly used antimicrobial agents in dairy Cattle	12
2.6 Uses of antimicrobial drugs in Tanzania	13
2.7 Potential health effects of antibiotic residues to human being	15
2.8 Detection of antibiotic residues in raw cow milk	16
2.8.1 Screening method of antibiotic residues in raw milk	16
2.8.2 Agar well diffusion microbial inhibition assay.....	17
2.8.3 High performance liquid chromatography technique	18
CHAPTER THREE	21
3.0 MATERIALS AND METHODS	21
3.1 Study area	21
3.2 Study design	23
3.3 Study population.....	23
3.4 Sample size determination.....	24
3.5 Ethical consideration	24
3.6.1 Sampling for small-scale dairy farmers	25
3.6.2 Pretesting of data collection tools.....	25
3.6.3 Sociological data collection	25
3.6.4 Raw milk sampling for laboratory analysis	26

3.7 Laboratory analysis of antibiotic residues.....	27
3.7.1 Microbiological inhibition screening test for drug residues	27
3.7.2 Quantification of oxytetracycline residues in milk by HPLC technique	29
3.8 Knowledge, attitudes and handling practices in usage of veterinary drugs	40
CHAPTER FOUR.....	41
4.0 RESULTS.....	41
4.1 Awareness of small-scale dairy farmers on veterinary drugs usage and factors leading to presence of antibiotic residues in raw milk in Bagamoyo District.	41
4.1.1 Demographic characteristics of the study population.....	41
4.1.2 Distribution of the respondents in the study area	41
4.1.3 Type of farming system and animal health management	42
4.1.4 Commonly reported animal diseases affecting dairy animal.....	42
4.1.5 Animal diseases, veterinary drugs and frequency of use.....	42
4.1.6 Problem associated with the use of veterinary drugs to dairy animal	43
4.1.7 Awareness of the respondents on health risks associated with consumption of raw milk with antibiotic residues.	43
4.2 Qualitative screening test for antibiotic residues	44
4.3 Quantitative analysis of OTC in raw milk samples.....	44
CHAPTER FIVE.....	47
5.0 DISCUSSION	47

5.1 Farming system and related practices	48
5.2 Awareness of small-scale dairy farmers on antibiotic residues in raw milk	49
5.3 Oxytetracycline residues in raw milk	52
CHAPTER SIX	55
6.0 CONCLUSIONS AND RECOMMENDATIONS.....	55
6.1 Conclusions	55
6.2 Recommendations	56
REFERENCES.....	57
APPENDICES	68

LIST OF TABLES

Table 1: Maximum Residues Limits for Antimicrobial Veterinary Drugs in Milk.	13
Table 2: OTC Standards calibration curve.....	35
Table 3: Recovery and precision of OTC determined in spiked UHT milk sample (n=4).....	39
Table 4: Determined OTC levels in raw milk samples in Bagamoyo District.....	45
Table 5: Residues and levels of oxytetracycline residue in raw cow's milk	46

LIST OF FIGURES

Figure 1: A map of Bagamoyo District showing study wards and sample collection points.	22
Figure 2. Collected Raw Milk Samples	27
Figure 3: Calibration curve with five concentration points of OTC standards.	36
Figure 4: Antibiotic Inhibition Test plate with zones of inhibition.	44

LIST OF APPENDICES

Appendix 1: Semi-Structured Questionnaire for Small-Scale Dairy Farmers: 68

Appendix 2: Chromatogram of OTC standard solution of Blank Spiked. 73

Appendix 3: Chromatogram of known control milk sample with OTC Residues 74

Appendix 4: Chromatogram of positive OTC Spiked at a concentration of 1000 μ g/l 75

Appendix 5: Chromatogram of OTC Standard solution at a concentration of 200 μ g/l ... 76

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Descriptive meaning
AOAC	Association of Official Analytical Chemists
HPLC-UV	High Performance Liquid Chromatography with UV Detector
CRS	Certified Reference Standards
SPE	Solid Phase Extraction
PDA	Photodiode Array Detector
µg/ml	microgram per millitre
CAC	Codex Alimentarius Commission
CBPP	Contagious bovine pleura-pneumonia
ECF	East Coast Fever
et al	and others
FAO	Food and Agriculture Organization of the United Nations
WHO	World Health Organization of the United Nations
FMD	Foot and Mouth Disease
LOD	Limit of Detection
LOQ	Limit of Quantification
R ²	Correlation Coefficient
+Ve	Positive detected
EDTA	Ethylene diamine tetra acetic acid disodium salt

LC	Liquid Chromatography
IDF	International Dairy Federation
ISO	International Organization for Standardization
MRL	Maximum residual limit
°C	Degrees Celsius
SUA	Sokoine University of Agriculture
TFDA	Tanzania Food and Drugs Authority
TBS	Tanzania Bureau of Standards
UHT	Ultra Heat Treatment

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Antibiotics are vital drugs considered as the ultimate strategy to treat animal infections. Their effectiveness is however, threatened by extensive and inappropriate use of these, not only in cattle but also in human medicine. In veterinary practice, antibiotics are utilized at therapeutic levels primarily to treat diseases and to prevent infections (Aning *et al.*, 2007). They are also used at sub-therapeutic levels to increase feed efficiency, promote growth and prevent diseases (Mosalagae *et al.*, 2011). The frequent use of antibiotics may result in drug residues that can be found at different concentration levels in products of animal origin, such as milk or meat. Presence of drugs or antibiotics residues in food above the maximum level recognized world-wide by various public authorities is illegal (Aning *et al.*, 2007).

Milk production in Tanzania is carried out under two major production systems; namely the traditional and the commercial dairy production systems. Seventy percent of the milk produced in the country comes from the traditional sector (indigenous cattle) kept in rural areas, and the remaining come from improved cattle mainly kept by smallholder producers (Njombe *et al.*, 2011). Smallholder dairy producers' supply about 90% of the milk consumed in the Dar es Salaam city and remarkably 74% of all milk is marketed raw through informal channels (Kivaria *et al.*, 2006). However, both traditional and improved dairy systems are constrained by diseases as a consequence to poor quality feeds, inadequate veterinary services and technical support services

(Shitandi, 2004; Kurwijila *et al.*, 2006). Ensuring that consumers' health is adequately protected from hazards associated with the use of food is one of the major challenges in developing countries including Tanzania (Kurwijila *et al.*, 2006; Kurwijila *et al.*, 2009).

To ensure food safety, control measures must be taken along the food value chain from primary production to the table (Kurwijila *et al.*, 2009). Today, antimicrobial drugs are used to control, prevent, and treat infections and to enhance animal growth and feed efficiency (Mitchell *et al.*, 1998). Currently, approximately 80% of all food-producing animals receive medication for part or most of their lives (Mitchell *et al.*, 1998). The most commonly used antimicrobials in food producing animals are the β -lactams, tetracyclines, aminoglycosides, lincosamides, macrolides, pleuromutilins, and sulfonamides (Lee *et al.*, 2000; Grane, 2000).

High incidence of tropical tick borne diseases coupled with other infectious diseases conditions (Mosalaga *et al.*, 2011) necessitates use of antimicrobial agents in dairy cattle in Tanzania. In situation where proper withdrawal period is not observed or not precisely known by cattle keepers (Katakweba *et al.*, 2012) drug residues in milk is likely to occur. Residues of drugs in milk and meat is a potential health risks because of failure to observe the mandatory withdrawal time/periods, illegal or extra-label use of veterinary drugs and incorrect dosage levels application (Kurwijila *et al.*, 2006). Effects of antibiotic residues include selection of antibiotic -resistant bacteria which could later be transferred from animals to humans, through contaminated milk products (Syt, 2011).

In addition to residual effect of drugs to human consumers, antibiotic residues in milk affect industrial processing of milk to other products. A fermentation step is frequently employed for the manufacture of food products, such as cheese and yoghurt, from milk. Milk contaminated with antibiotic residues, and subsequently employed in the manufacture of cheese or yoghurt, can result in the inhibition of culture development, due to elimination or reduction of the micro-organism necessary to allow fermentation (Shitandi and Sternesjo, 2004; Kurwijila *et al.*, 2006). Consequently as a result, milk and milk products contaminated with antibiotics beyond a recommended residue levels, are considered unfit for human consumption (WHO/FAO-CAC, 2012; Hillerton *et al.*, 1999).

Good quality milk has normal chemical composition, completely free from harmful bacteria and harmful toxic substances, free from sediments and extraneous substances, which have lower degree of titratable acidity, of good flavour, adequate in preserving quality, and low in bacterial counts (Hossain *et al.*, 2011). High-quality milk apart from containing low bacterial count also contains a low number of somatic cells and is free of human pathogens and antibiotic residues (Oliver *et al.*, 2009). Testing for microbiological quality, chemical and antibiotic residues are not practiced at small-scale dairy or milk collection centers in Tanzania and the test done for raw milk wholesomeness at the collection centers is only lactometry test of specific gravity Msangi, (2006). To protect the public against possible health risks caused by drug residues hazards, regulations regarding veterinary use of drugs including withholding periods after antibiotics therapy and tolerance levels have been formulated (WHO/FAO-CAC, 2012) and are strictly adhered in developed countries (Lee *et al.*,

2001; Donoghue, 2003). However, such regulations are not usually adhered to in countries where routine monitoring of veterinary drug residues in food is not done (Shitandi and Sternesjo, 2001).

In Tanzania, about 80-90% of households in urban centres purchase raw milk from street vendors or via home delivery with little control of microbial and antibiotic residues contamination (Msangi, 2006; Kurwijila *et al.*, 2009). Milk contamination can be reduced through effective good hygienic practices from farm level to the final consumer (WHO/FAO-CAC, 2004). Maximum Residue Limits (MRLs) are the levels of drug legally permitted and recognized as acceptable in a food, resulting from the correct use of a veterinary drug, which should occur in food. MRLs are based on the type and amount of residue considered to be without any toxicological hazard for human health as expressed by the acceptable daily intake and an additional safety factor. MRLs give an indication of food safety and provide trading standards (FAO/WHO-CAC, 2012).

According to Msangi, (2006) raw milk quality and safety assessment is neither done after milk production at the households nor is it done before selling to consumers. Further studies by (Kurwijila *et al.*, 2006; Karimuribo *et al.*, 2005) showed that antimicrobial residues levels such as oxytetracycline, sulphadimidine and penicillin in raw milk from the small-scale dairy farms sector are not well known. Nevertheless, limited studies (Swai and Schoonman, 2011; Kivaria *et al.*, 2006; and Kurwijila *et al.*, 2006) have been conducted on milk quality and safety in terms of levels of milk contamination, microbiological quality, antimicrobial residuals

and milk adulterations. However, all previous studies employed qualitative assays which indicate presence of antibiotics but could not establish whether the residue exceed the MRL. The present study was designed to assess the antibiotic residues in raw milk by providing quantitative information on residues of the most commonly used antibiotics and the factors that contribute to their occurrence in Bagamoyo District, Tanzania.

1.2 Problem Statement and Study Justification

Understanding of the quality and safety of milk sold to the market has ultimate benefits to the consumers of milk and other dairy products. In addition equal benefits are obtained by the producers of milk (farmers) and dairy industry are assured of good milk that can be safely processed to other products. It was therefore envisaged that this study will add information to the chemical safety and quality of milk in Tanzania and provide baseline data for further investigation on milk safety. The obtained information would also assist milk producers, Regulatory Authorities and consumers to contribute to the formulation of control strategies on the use of antibiotics as veterinary drugs in treating and preventing various cattle diseases.

1.3 Research Objectives

1.3.1 The Overall Objective

To assess veterinary drugs usage and determine antibiotic residue levels in raw cows' milk produced by small-scale dairy farmers in Bagamoyo District, Tanzania.

1.3.2 Specific Objectives

- i) To determine the most commonly used antibiotics to treat cattle diseases in the study area.
- ii) Qualitative screening of antibiotic residues in raw cows' milk produced by small-scale dairy farmers in the study area.
- iii) Quantitative determination of antibiotic residue levels in raw milk for the most commonly used antibiotics to treat diseases by small-scale dairy farmers in the study area
- iv) To assess knowledge, attitudes and practices of small-scale dairy farmers with respect to use and factors contributing to the presence of antibiotic residues in raw cows' milk.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General Overview

Livestock production in Tanzania largely depends on the traditional sector which is faced by several challenges such as poor animal genetic potential, poor management and diseases (Mellau *et al.*, 2010). It was reported that, apart from the high prevalence of animal diseases, their control in livestock remains problematic because of inadequacy of veterinary services particularly in rural areas. The factor contributing to unreliable veterinary services delivery in rural areas of Tanzania includes shortage of veterinary staff, poor transport facilities, limited diagnostic facilities and drugs (Mellau *et al.*, 2010; Komba *et al.*, 2012). Sick dairy animals are often treated with antibiotics; failure to follow required withdrawal time/periods after these treatments is the most commonly cited reason for drug residues in marketed milk (Zwald *et al.*, 2004). Dairy markets in most sub-Saharan African countries are mostly supplied by small-scale farm enterprises (de Leeuw *et al.*, 1999). Ensuring that dairy products are free from veterinary chemical residues is one of the challenges in developing countries (Kurwijila *et al.*, 2006).

While antibiotics are required to treat infections in individual animals, they are also used prophylactically to prevent the occurrence and spread of infections in intensive production systems such as cattle, pigs and poultry (Grane, 2000; Kurwijila *et al.*, 2006). The presence of antibiotic residues in animal products such as milk, meat and eggs can present hazards for the public health, industry and environment (Kurwijila *et al.*, 2006; Nonga *et al.*, 2009; and Katakweba *et al.*, 2012). The undesirable effects

can be classified as toxicological, selection of resistant strains of bacteria and allergic reactions. A major public health concern is the increasing occurrence of strains of bacteria resistant to antibiotics (Kurwijila *et al.*, 2006; Katakweba *et al.*, 2012) which would mean antibiotics are losing their effectiveness to treat some common infections. This phenomenon has resulted in the removal or restriction on the use of certain antibiotics and prolonged exposure to antibiotics as a result of consuming residues contained food is contributing to antibiotics resistance (Shitandi, 2004; Nonga *et al.*, 2009; Katakweba *et al.*, 2012).

The presence of antibiotic residues in food is of major industrial importance as fermentation step is frequently employed for the manufacture of food products such as cheese and yoghurt from milk. Inhibition of culture development can occur due to milk containing antibiotic residues which subsequently lead to elimination or reduction of the micro-organism necessary to allow fermentation (Shitandi and Sternesjo, 2004)

2.2 Livestock and dairy production in Tanzania

Tanzania's livestock production is mainly smallholder based and the country has the third largest livestock population in Africa after Sudan and Ethiopia (Njombe *et al.*, 2011). Out of the total 88.6 million hectares of land resource, 60 million hectares are rangelands suitable for livestock grazing, able to carry up to 20 million Livestock Units. However, due to tsetse infestation and other constraints, only 40% of the rangelands is utilised for grazing 18.5 million cattle; 13.1 million goats and 3.6 million sheep (Njombe *et al.*, 2011). More than 90% of the livestock in the country are of indigenous types and are kept in the traditional sector, having a characteristically

low productivity yet well adapted to the existing harsh environment including resistance to diseases (Njombe *et al.*, 2009).

Smallholder dairy farming has a significant role in the alleviation of poverty and reduction of malnutrition in Tanzania. Dairy animals provide regular household income, employment and nutritious food. However, the dairy farming suffers from constraints that limit realization of this potential mainly due to the factors such as diseases, poor management practices and sub-optimal feeding regimes (Mdegela *et al.*, 2009).

In Tanzania milk production is mainly from cattle, of the 18.8 million cattle found in the country about 560,000 are dairy cattle which consist of Friesian, Jersey, Ayrshire breeds and their crosses to the East African Zebu (Njombe *et al.*, 2011). The rest are indigenous cattle raised as dual purpose animals that are for milk and meat production. About 70% of the annually produced milk comes from traditional sector (indigenous cows), whereas the commercial sector (dairy cows) produce about 30%. Currently, only a small proportion (10%) of marketable surplus of milk produced annually is filtering into the urban markets and processing plants. Remoteness and poor infrastructure constitute the largest bottlenecks to collection and marketing of milk. Thus the milk produced is mostly consumed locally and quite often a significant amount is left for the calves. However, some producers who depend on milk for their cash income are willing to exploit the available opportunity in marketing their produce, for example, Maasai herders who transport milk by bicycles over long distances, for sale at collection centres located along the Dar es Salaam – Chalinze –

Segeera and Dar es Salaam – Chalinze – Morogoro – Dodoma highways (Njombe *et al.*, 2011).

2.3 Uses of antibiotics in animals production

In veterinary practice antibiotics are used largely for four purposes, for therapeutic use to control bacterial infection (treat sick animals), prophylactic use to prevent infection. The prophylaxis application of antibiotic can be to both individual animal and to groups of animals; the application of antibiotic to groups of animal at the time when only single animal of the group present symptoms of the disease, but it is expected that most of the group will become affected, is referred to as metaphylaxis with such treatment regimes, the antibiotics are commonly applied via feed or water; and also antibiotics are used in animals production as growth promoters to improve feed utilization and production (Katakweba *et al.*, 2012; Kurwijila *et al.*, 2006; Grane, 2000). The frequent use of antibiotics may result in veterinary drugs residues in the products of animal origin such as milk or meat (Kurwijila *et al.*, 2006; Mmbando, 2004)

2.4 Occurrence of antibiotics residues in animal products

Antibiotics residues are remnants of antibiotics or their active metabolites that are present within tissues or products e.g. meat, milk and eggs from treated animals (WHO/FAO-CAC 2-2012). A number of factors may be responsible for persistence of drugs residues in food of animal origin. The main reason reported is failure to observe withdrawal times (Shitandi, 2004). Following antibiotic administration, withdrawal times are specified after which time the animal or animal products are fit for human

consumption (Katakweba *et al.*, 2012; Kurwijila *et al.*, 2006 and Shitandi *et al.*, 2004). Levels of the drug and their metabolites may persist at unacceptable levels and consumers can be exposed to them (WHO/FAO-CAC 2- 2012). The presence of residues may result from failure to observe the mandatory withdrawal periods, illegal or extra-label use of drugs and incorrect dosage, non-existence of restrictive legislation or their inadequate enforcement, poor records of treatment failures, lack of advice of withdrawal period especially in the developing countries (Ivona and Mate, 2002).

Residues of antibiotics in milk most often originate from poor livestock diseases management, poor milkhandling and unhygienic condition at farm-level, but do not rule out market-level practices which introduce antimicrobials to milk (Kurwijila *et al.*, 2009; Shitandi, 2004). However, unconfirmed reports suggest that some unscrupulous milk market agents may add antibiotics, among other chemicals, to lengthen the shelf life of milk (Kurwijila *et al.*, 2006). The presence of antibiotics drug residues in milk above allowable limits (Table 1) is a serious food safety risk because it may lead to consumers allergies (Lee *et al.*, 2000; Oslonet *al.*, 1975 cited by Kurwijila *et al.*, 2006) or to selection of antibiotic resistant bacteria populations (Nijsten *et al.*, 1996). Veterinary drug residues also alter the processing qualities of raw milk by inhibiting starter cultures used in the preparation of cheese and other fermented dairy products (Katlaet *al.*, 2001; Broome *et al.*, 2002; Shitandi, 2004).

The public health risk to antibiotics residues seems particularly high in developing countries, where there may be no local legislations regulating maximum tolerance

limits for marketed products and recommended withdrawal times are violated (Shitandi, 2004; Kurwijila *et al.*, 2006). Furthermore there are no national programmes for routine monitoring of residues in food products despite large-scale use of various antibiotics (Grane, 2000; Shitandi, 2004).

2.5 Commonly used antimicrobial agents in dairy Cattle

Antimicrobial agents are found in different groups which are available for treatment of infected livestock. The most common groups includes tetracyclines, beta-lactams, sulphonamides, aminoglycosides, macrolides, and chloramphenicol (Omore *et al.*, 2002; Movassagh and Karami, 2010; Pecou and Diserens, 2011). These antibiotics may be used alone or at times in combination when treating dairy cattle. Several studies have been done on commonly used antibiotics in livestock in different countries. In Tanzania studies conducted by (Kurwijila *et al.*, (2006); Mmbando, (2004); Nonga *et al.*, (2009); Midenge, (2011)) revealed that, tetracycline particularly oxytetracycline, β -Lactam (penicillin G and sulphonamide are commonly used antibiotic veterinary drugs. These studies also showed that there is rampant and indiscriminate use of antibiotics among the livestock keepers in Tanzania.

Table 1: Maximum Residues Limits for Antimicrobial Veterinary Drugs in Milk.

Antimicrobials	MRL (µg/l)
Tetracycline	100
Chlortetracycline	100
Oxytetracycline	100
Doxycycline	100
Benzyl Penicillin /procaine	4
Ampicillin	4
Amoxycillin	4
Cloxacillin	30
Dicloxacillin	30
Oxacillin	30
Streptomycin	200
Erythromycin	4
Gentamycin	200
Tylosin	100
Lincomycin	150
Monensin	2
Sarafloxacin	100
Spectinomycin	200
Sulfamethazine	25
Sulfadimethoxine	25
Sulfamerazine	25
Sulfathiazole	25
Sulfamethoxazole	100
Sulfanilamide	100
Sulfadiazine	100

Source:FAO/WHO-Codex Alimentarius Commission: Maximum Residues Limits

(MRL) for Veterinary Drugs in Foods- CAC/MRL 2-2012 Standard.

2.6 Uses of antimicrobial drugs in Tanzania

In Tanzania, the use of supplementary feeding involving the use of feed additives, growth promoter in dairy animal is virtually non-existent (Mmbando, 2004).

However, a lot of veterinary drugs such as OTC, penicillin and sulphadimidine are

used abusively to treat and protect cattle against various diseases (Katakweba *et al.*, 2012). From this perspective the most likely source of veterinary drug residues especially OTC will be from the treatment (therapeutic) and prophylaxis of diseases rather than from feed additives (Kurwijila *et al.*, 2006).

Oxytetracycline residues are likely to originate from the treatment of various diseases such as east coast fever (ECF), anaplasmosis, foot and mouth diseases (FMD), contagious bovine pleuro-pneumonia (CBPP) and heart water (Midenge, 2011). Much of the treatment is done by the livestock keeper themselves with questionable adherence to the right doses and even when the treatment done by the veterinary doctor or livestock officer, withdrawal period is rarely observed (Shitandi and Sternesjo, 2001; Kurwijila *et al.*, 2006).

Several studies have been conducted in Tanzania to assess the extent of use and occurrence of veterinary drugs in food of animal origin (Mmbando, 2004; Kurwijila *et al.*, 2006; Nonga *et al.*, 2009; Katakweba *et al.*, 2012) in the study that involved smallholder livestock keepers reported that, the most commonly used antibiotics were oxytetracycline, sulphadimidine, penicillin-streptomycin and gentamycin. Antimicrobial residues in milk in Tanzania were reported by (Karimuribo *et al.*, 2005; Kurwijila *et al.*, 2006 and in cattle meat (Mmbando, 2004). The study conducted in Morogoro Municipality reported high level of antimicrobial residues in eggs and chicken meat from commercially raised poultry by small scale farmer (Nonga *et al.*, (2009a,b). The reason implicated to this high level occurrence of antimicrobial residues was the use of antimicrobial drugs as prophylaxis and treatment of common

chicken diseases accompanied with failure to observe withdrawal period. It was further reported that, although some farmers particularly in poultry keepers were aware of good agricultural practices such as implementation of withdrawal period, but they were more concerned on the income (money) than the health of the public (Nonga *et al.*, 2009). All these studies revealed gross lack of awareness on health threats associated with antibiotics resistance among the farming community thus posing serious public health threats in Tanzania.

2.7 Potential health effects of antibiotic residues to human being

The antibiotic residues when taken above the maximum residue limit (MRL) can result in potential health effects to the human being (Goffova *et al.*, 2012). The effects include the occurrence of resistant strains of bacteria in humans, toxicity effects of the drug, allergic reactions (hypersensitivity reactions) in sensitized persons and inhibition of starter cultures in production of cultured milk products such as yogurt and also the manufacture of cheese (Movassagh and Karami, 2010; Kaya and Filazi, 2010). Other effects includes intestinal dysbiosis (Goffová *et al.*, 2012). Also some drugs or their metabolites possess carcinogenic potential e.g. meat preserved with sodium nitrate and contains sulphamethazine residues, may develop a triazine complex that has a considerable carcinogenic potential (Goffová *et al.*, 2012). Prolonged ingestion of tetracycline in food has detrimental effects on teeth and bones in growing children. Some reports have also associated drug residues to destroy useful microflora of gastrointestinal tract, especially in children leading to enteritis problems (Goffová *et al.*, 2012).

2.8 Detection of antibiotic residues in raw cow milk

There are several methods for analysis of antibiotics in various biological and pharmaceutical matrices and these consist of screening methods and chromatographic techniques for detection of qualitative and quantitative levels of antibiotic residues (Alkan, 2007). The screening is performed by microbiological, enzymatic and immunological methods and they are based on the susceptibility of bacteria to different antibiotics (Syt, 2011).

There are also various chemical, microbiological and immunological assays used to detect antibiotic residues in milk. The chemical methods include High Performance Liquid Chromatography (HPLC), Gas-liquid chromatography, Radioimmunoassay, Thin-Layer Chromatography (TLC) and Electrophoresis (Ramirez *et al.*, 2003).

Microbiological assays for detection of antibiotic residues utilizes bacteria such as *Bacillus stearothermophilus* and *Bacillus subtilis* because of their high sensitivity to detect a wide range of antibiotics commonly used in animal disorders (Syt, 2011). The microbiological assay is easy, fast, simple and cheapest method (Muriuki *et al.*, 2001; Abbasi *et al.*, 2011) however the method can detect only qualitative and/or semi-quantitative measurement of residues (Abbasi *et al.*, 2011). The bioassay techniques are less specific and sometimes produce false positive results (Abbasi *et al.*, 2011; Syt, 2011).

2.8.1 Screening method of antibiotic residues in raw milk

Efficient control of residues requires good screening tests, which must be less expensive, less time consuming than the more specific quantitative or confirmatory

method, easy to perform, allow simultaneous analysis of large numbers of samples and give rapid results (Syt, 2011). They may detect the presence of an antibiotic residue or a class of antibiotics and usually allow high sample throughput (Alkan, 2007; Syt, 2011). Screening methods have the capability for a high samples throughput and are used to sift large numbers of samples for suspect or potential non-compliant results. They are specifically designed to avoid false compliant results (Okerman *et al.*, 2004), thus, the number of so-called false-negative results of a screening test should be as low as possible, while a few false-positive results can be accepted as long as all positive results of the screening test are confirmed with chromatographic method.

2.8.2 Agar well diffusion microbial inhibition assay

This test involves preparation of Muller Hinton Agar in sterile glass plates, thereafter uniform streaking of *B. subtilis*, followed by creating wells/holes on the media using sterile boring glass rods. Then 10µl of sample pipetted in the wells and the plates incubated at temperature of 37⁰C for 18-24hours. After incubation the cultures examined for bacteria growth inhibition zone in case of antibiotic positive results. The diameters of the inhibition zones measured with callipers. Screening of milk and other animal food samples for the presence of antibiotic residues is usually performed with the help of microbial inhibition assays. Their sensitivity to different drugs depends on the indicator microorganism used and the concept of the test. Microbiological assays for the detection of antibiotic residues utilize the genus *Bacillus*, because of its high sensitivity to the majority of antibiotics (Jevinova *et al.*, 2003).

2.8.3 High performance liquid chromatography technique

High performance liquid chromatography (HPLC) is a chromatographic technique used to quantify the levels of antibiotics residues in different biological, pharmaceutical and food matrices. It has got different detection modes such as spectrometry, fluorescence, mass spectrometry, particle beam (PB), fast atom bombardment (FAB), atmospheric pressure chemical ionization (APCI) and Electrospray ionization (ESI) (Pena *et al.*, 2007).

Antibiotics residues such as penicillin G and oxytetracycline can be successfully detected and quantified in various biological matrices using HPLC in the reverse phase mode, with different detection modes, such as spectrometry, fluorescence and mass spectrometry (Muriuki *et al.*, 2001; Abbasi *et al.*, 2009; Olatoye and Ehinmowo, 2010; Bedada *et al.*, 2012). HPLC usage is increasingly being used in the field of residue analysis. The variety of mobile phases, the extensive library of column packing and the variation in modes of operations are the reasons for this method to be in demand.

In residue analysis of edible animal products, the samples often have much higher concentrations of endogenous interfering components but a very low content of residues. It is necessary to assess variety of producers for isolations, derivatization and quantitation of the compound of interest since the nature and concentration of these components can vary widely (Nollet, 1992). Sample deproteinization is the first step in animal originated food residue analysis. Mineral or organic acids like hydrochloric or trichloroacetic acid and/or water miscible organic solvents such as

acetonitrile, acetone or methanol, which precipitate the proteins and allow their removal by centrifugation, are used frequently. Sample deproteinization helps releasing protein-bound residues besides protecting the HPLC column from irreversible contamination (Pena *et al.*, 2007)

In most conditions analyte extraction into a solvent is the second step where extraction efficiency is determined by the polarity of the extracting solvent, the pH of the sample/solvent system and the sample-to-solvent volume ratio. Extract clean-up process is usually involved as the third step in sample preparation. The easiest procedure is a simple liquid-liquid partitioning between two immiscible solvents, where the analyte is selectively partitioned in one of the two phases (Nollet, 1992).

HPLC analysis of antimicrobial residues is mainly performed in either reverse phase mode or in the ion exchange mode. The efficiencies in the ion exchange mode are determined to be lower than those obtained by normal- or reverse-phase HPLC. Usually excessive tailing due to the in-homogeneity of the absorbent surface is obtained. Many parameters can influence both the resolution of the compounds and column efficiency in reverse-phase HPLC. In order to obtain best results a combination of the appropriate stationary/mobile phase system and the mode of elution (isocratic or gradient) must be determined. Alkyl-bonded (C8, C18) stationary phases are used with mobile phases such as methanol or acetonitrile. The content of the organic modifier in the mobile phase is a function of both the polarity of the analyte and the type of column packing (Nollet, 1992).

For residue analysis fluorescence detection has been proved to be a valuable tool where interferences from food components must be reduced or eliminated.

Fluorescent derivatives of many non-fluorescing solutes emerging from the chromatographic column can be prepared using specific fluorescence-labeling reactions. Comparing retention times is the key for identification of eluted compounds with reference compounds processed in an identical manner. Sometimes retention times are not enough by itself since a retention time can be observed for more than one compound or several components can be eluted at same retention time and chromatograph may show only one peak. Repeating the sample analysis on a different packing material can contribute to more satisfying results (Nollet, 1992).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

Bagamoyo district is located in Pwani Region, having an altitude with average elevation of 184 metres (604 ft) above the sea level, Longitude 38.5° East and Latitude 6.3° South. Bagamoyo District is one of the six districts of the Pwani Region of Tanzania. It is bordered to the North by the Tanga Region, to the west by the Morogoro Region, to the East by the Indian Ocean and to the South by the Kibaha District, it is estimated to have a population of 311,740 (Tanzania National Census, 2012). Rainfall pattern is characterized by two rainfall seasons, typical for the tropics: a long rainy season (March to May) and a short rainy season (October to December). Average annual rainfall ranges from 800mm to 1000mm and the average temperature is 28°C. Bagamoyo District was proposed as a study site, because it has accessible raw milk collection centers namely Msata, Chalinze and Lugoba (Figure 1). These collection centers are the filters through, whereby raw milk produced by small scale producers is supplied to Dar es Salaam city.

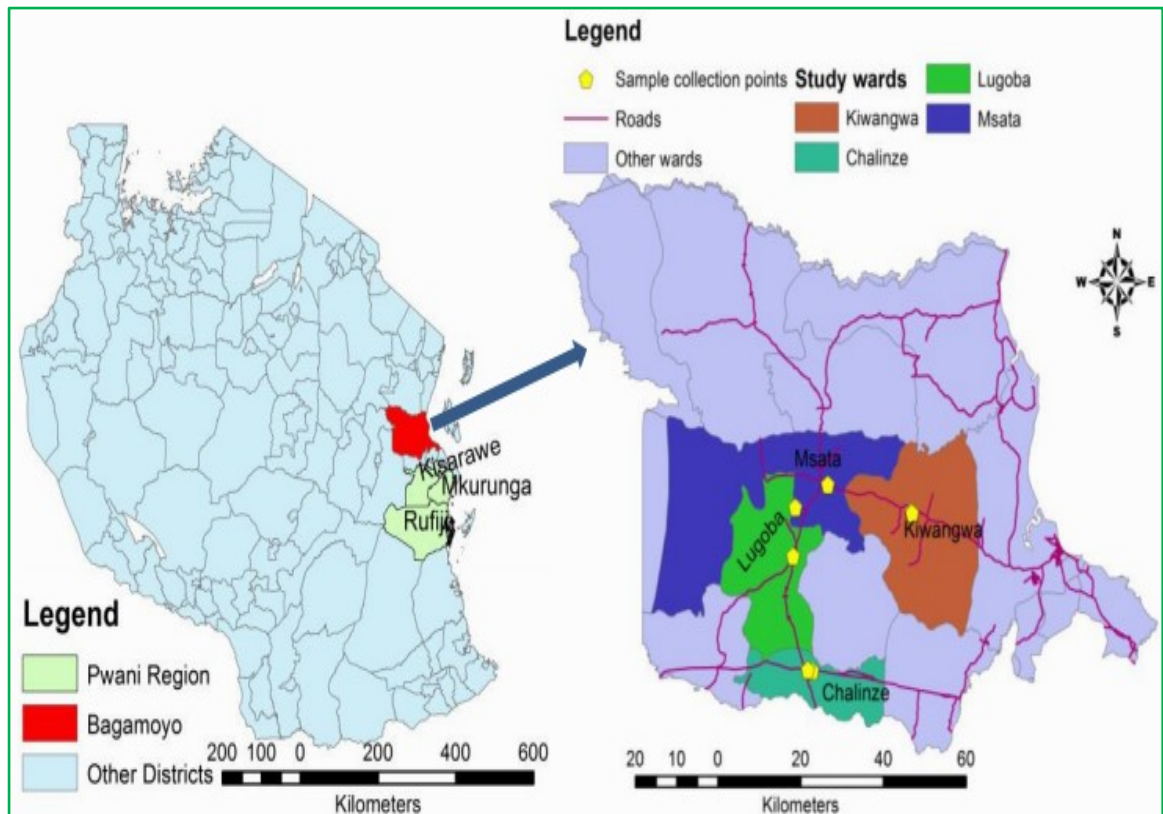


Figure 1: A map of Bagamoyo District showing study wards and sample collection points.

Bagamoyo District consists of 22 wards, however Msata, Kiwangwa, Fukayosi, Lugoba, Msoga and Chalinze (Bwilingu and Pera) wards were purposively selected because of large number of small-scale dairy farmers and presence of established raw milk collection centres. According to wards' Livestock Office records, Msata ward have 44 small-scale dairy farmers with 257 dairy cattle, Lugoba ward has 20 small-scale dairy farmers with 300 dairy cattle and Chalinze ward has 74 small-scale farmers with 566 dairy cattle. Each small-scale dairy farmer has cattle numbers ranging from one to five. The milk collected from those centres is supplied to different locations of Dar es Salaam city.

3.2 Study design

A cross-sectional study design was employed which allowed samples to be collected from small-scale dairy farms with respect to available milk collection centres in Bagamoyo district, namely Msata, Lugoba and Chalinze. Msata collection centre involved dairy farmers from Msata, Kiwangwa and Fukayosi Wards, while Lugoba collection centre drew farmers from Lugoba and Msoga Wards and Chalinze collection centre were involved dairy farmers from Bwilingu and Pera wards. Semi-structured questionnaire was also developed and used for data collection in the study area. In order to obtain the true representative samples a survey was conducted to identify individual farms that are producing and selling raw milk to the collection centres.

3.3 Study population

The population was considered to be heterogeneous with various gender and age groups. From the population, a representative randomly selected number of small-scale dairy farmers in Msata, Lugoba and Chalinze were considered in the study. The inclusion criteria were; small-scale dairy farmers both women and men who keep from one to five dairy cattle, or willingness to participate in the study and able to give information and accessibility of the place during data collection. The exclusion criteria included; unwillingness to participate in the study, unable to give information asked and living in inaccessible areas. Also those who had no time for interview were excluded.

The collection centres formed a cluster of the farmers who sell raw milk to a particular centre. Small-scale dairy farmers were visited once and the semi-structured questionnaires were administered to randomly selected study participants from whom milk samples were collected for laboratory analysis. Farmers from which samples were drawn were identified by numbers 1 to 110 while the milk collection centres were identified by letters A, B and C representing Msata, Lugoba and Chalinze respectively.

3.4 Sample size determination

Sample size required for the study were determined according to Thrusfield (2005) at 95% CI, 5% precision and with expected occurrence of antimicrobial residues prevalence of 7% in Dar Es Salaam Tanzania (Kivaria *et al.*, 2006). The formula for unknown population was as follows;

$$n = \frac{Z_{\alpha/2}^2 pq}{d^2}$$

Where n = Herd and estimated sample size

Z = t value for an expected confidence level (1.96)

p = Antimicrobial prevalence of 7%

d = selected accepted error or precision (as 0.05)

The chosen 110 small-scale farmers were 41 from Msata, 19 from Lugoba and 50 from Chalinze whom each provided raw milk samples from their cattle for analysis.

3.5 Ethical consideration

Research permit was provided by the Vice Chancellor Sokoine University of Agriculture and verbal consent was obtained from each of the head of household prior to commencement of interviews and sampling. Participation in the study was on

voluntary basis. All the information collected from the participants and the laboratory results obtained after raw milk samples analysis were kept under the custody of the researcher as confidential.

3.6 Sample collection

3.6.1 Sampling for small-scale dairy farmers

Selection of small-scale dairy farmers depended on the concentration of farmers in a particular collection centre and based on the respondent's willingness to participate in the study. Researcher visited small-scale dairy farmers, using good communication skills and assurance of confidentiality; 110 participants were immediately enrolled for questionnaire and sample collection. Simultaneously, 110 samples of raw milk were collected for laboratory analysis for qualitative and quantitative determination of antibiotic residues.

3.6.2 Pretesting of data collection tools

Pretesting of questionnaires was done to test the clarity, sequence of questions and estimate the duration of each questionnaire. A total of five respondents were interviewed and findings were used to improve the questionnaires. The revised version of the questionnaire was then translated into "Kiswahili", the national language understood by majority of Tanzanians.

3.6.3 Sociological data collection

Sociological data were collected from respondents (small-scale dairy farmers) during household visits by interviews using semi-structured questionnaires with both closed

and open ended questions (Appendix 1), The information gathered included general health status of cattle, common diseases in the area, diseases management and control, mostly common used antibiotic to treat cattle diseases, treatment practices in use of antibiotic drugs on their farms specifically; documentation of antibiotic administered/usage, how small-scale farms keep treatment protocols, compliance to withdrawal periods, illegal or extra-label use of veterinary drugs and incorrect dosage levels. Furthermore the questionnaires were used to assess producers awareness on the risk factors for antibiotic residues through; knowledge, attitudes and practices of dairy farmers on the use of antibiotics in cattle production.

3.6.4 Raw milk sampling for laboratory analysis

Raw milk sampling was done after questionnaire interviews was completed. Samples from each individual farm were collected directly from the milking containers using 100ml clean sterile plastic sample bottles (figure 2). All collected samples were immediately cooled to 4°C in the cool box container with ice cubes and transported to the laboratory in the Department of Veterinary Medicine and Public Health, Faculty of Veterinary Medicine, Sokoine University of Agriculture (SUA) for qualitative analysis of antibiotic residues. The samples were stored in freezer at -20°C before analysis.



Figure 2. Collected Raw Milk Samples

3.7 Laboratory analysis of antibiotic residues

3.7.1 Microbiological inhibition screening test for drug residues

For test and interpretation of the results this study followed the general guidelines of National Committee for Clinical Laboratory Standards (NCCLS, 2002). In microbiological inhibition test for drug residues agar well diffusion method was employed as previously described by Association of Official Analytical Chemists (AOAC, 2000) and (Syt, 2011). Briefly, Muller Hinton agar (MHA) (Laboratorios Conda S.A C/La Forja 9.28850 Torrejon de Ardoz, Madrid Spain) was prepared, autoclaved and then left to cool to 48⁰ before being poured in a sterile glass Plate for cooling and solidification, Subsequently 0.1 ml of already prepared *B. subtilis* (ATCC 6633) bacterial suspension was added to each plate. Then *B. subtilis* was uniformly streaked by using sterile cotton swabs, then four wells or holes with diameter of 10mm were punched into the agar layer using sterile boring glass rod on each plate. The wells were at the distance of at least 30mm from each other. A 10 μ l of the test raw milk sample was pipetted in the holes/wells and after a pre-diffusion period of about 1 hour at room temperature, then plates were incubated at 37⁰C for 24 hours.

These tests were performed in duplicate and both positive and negative controls wells/holes were included in each plate. Positive control was 10µl of 5% oxytetracycline (Alfavet Animal Health care-UK) and Pen&strep (Norbrook Laboratories Ltd- UK) mixed with 1ml of drug residues free UHT milk (Brookside Dairy Limited - Kenya), while the negative control was 10µl drug residues free UHT milk (Brookside Dairy Limited - Kenya). After 24 hours of incubation the cultures were examined for bacteria growth, the diameters of inhibition zones were measured with slipping callipers.

The diameter of the zones of inhibition from the end of hole/well margin to beginning of bacterial growth was measured using calliper and an inhibition zone of ≥ 2 mm was considered as positive and diameter of inhibition zone measured less than 2mm was considered negative. These diameters were measured minus the diameter of the punch well which was 10mm (Omija *et al.*, 1994; Ailal *et al.*, 2009; Nonga *et al.*, 2009).

The results of present study revealed high susceptibility of *B. subtilis* for most antibiotics which frequently used in veterinary treatment, and this agreed with what mentioned by (Al-Mohana *et al.*, 2010) who preferred using of *B. subtilis* because it is sensitive for most antibiotics as compared with other bacteria as well as this bacteria are non- pathogenic, grow rapidly, easy to preserve and cost effective. All these factors explain why present study selected these bacteria for detection of antibiotics residues in milk. These findings pointed to efficiency of these bacteria for detection of antibiotics residues in milk even under low concentration in the product. However, because antimicrobial inhibition assay using *B. subtilis* cannot give any information about the type of antibiotic and the amount that is found in the milk, hence it need to

be confirmed by another method usually High performance Liquid chromatography (HPLC) as per this current study.

3.7.2 Quantification of oxytetracycline residues in milk by HPLC technique

The positive detected milk samples were confirmed and quantified by using HPLC-uv and the test was done according to (AOAC 995.04). Procedure methods and as described by (Ghinidi *et al.*, 2003) with some modifications at the Tanzania Food and Drug Authority (TFDA) Laboratory Dar es salaam, Tanzania.

i) Testing principle

Partially thawed raw milk samples were extracted with McIlvaine Buffer/EDTA solution. After centrifugation, the extracts containing the oxytetracyclines were cleaned-up by passing through C18 SPE Cartridges. Oxytetracyclines were eluted from the cartridge with methanolic oxalic acid, evaporated and reconstituted with aqueous methanol and analyzed by reverse phase High Performance Liquid Chromatography (HPLC-UV)

ii) Equipment and Apparatus

Analysis was carried out on a HPLC Model LC-20AT Prominence Liquid Chromatography series, serial No.C213346 015664, SHIMADZU Corporation, Japan.), equipped with constant flow pump, degasser (DGU 20A₅ Serial L-20244 07326), column oven (CTO-10A VP Serial C 210447 06967), auto sampler (OPTIMAS- Spark) and computer software. Separations were carried out by Zorbax Eclipse XDB-Analytical C18 (150x 4.6 mm, 5µm) Column Agilent Technologies-

USA and analyte were detected by PDA-Detector with variable wavelength UV detector (SPD-20A), CBM- 20A Prominence serial No L 202347 09807 SHIMADZU Corporation Japan. Other equipments used were;

- a) Centrifugation during sample preparation and extraction was performed with a ROTOFIX 32A ®(ZENTAFUGEN)-Centrifuge made from Germany.
- b) Sonicator & Degasser used to degas mobile phase- Branson ® 3210E-DTH, UK
- c) Nitrogen drier used to dry sample during extraction process-Thermo Scientific heating module ®No. 8824
- d) PH Meter measuring ± 0.05 unit,- JENWAY ® 3540 model-UK
- e) Analytical balance-Readability 0.0001g – OHAUS ® corporation-USA
- f) Mechanical shaker/Vortex mixer- SFI, Sturt Scientific- UK
- g) Clean-up Solid phase extraction (SPE) Cartridges-VARIAN Bond Elut SAX made from USA
- h) Disposable syringe filters-(0.45 μ m)-lot no. SF2350812, XIVA-SICHEM BIOTECH- Delhi India
- i) Sterile membrane filter- 0.45 μ m pore size, Cat no. 7141114 supplied from Japan

iii) Chemicals and reagents

- a) Methanol-HPLC grade, acetonitrile- HPLC grade and Water- HPLC grade were supplied from Fishers Scientific Limited- UK.

- b) Disodium hydrogen phosphate dihydrate, oxalic acid-dihydrate, citric acid-monohydrate, Sodium phosphate-dibasic anhydrous, Ammonium hydroxidewere purchased from Fishers Scientific Limited, UK.
- c) Ammonium acetate (Analytical grade) was supplied by Scharlau chemie S.A,
- d) EDTA, trifluoroacetic acid was purchased from Ps Park Scientific Limited-UK.
- e) Distilled and deionized water was obtained from TFDA Laboratory and
- f) Oxytetracycline CRS was supplied from council of Europe (batch/lot no 4 a)

iv) Extraction solvents

- a) McIlvaine buffer(pH 4.0 ± 0.05)

The amount of 21.0 g of citric acid was taken and dissolved in 1 liter distilled water in a volumetric flask. In another flask, 28.4 g of (Na_2HPO_4) anhydrous disodium hydrogen phosphate was dissolved in 1 liter distilled water. McIlvaine buffer was then made by combining 1 liter citric acid and 625 ml of disodium hydrogen phosphate in a 2 liter volumetric flask. The pH was adjusted using 0.1M hydrochloric acid.

- b) McIlvaine buffer- EDTA solution (McIlvaine Buffer/0.1M EDTA)

On weekly basis McIlvane buffer was prepared as described by Ghinidiet *al.*, (2003).To 1.625 liter McIlvaine buffer, 60.5 g of disodium ethylenediamine-tetracetate (EDTA dehydrate) was added and mixed until dissolved completely.

v) Elution solvent

Every day fresh methanolic oxalic acid solution was made by dissolving 1.26 g oxalic acid dihydrate in HPLC grade methanol in 1 liter volumetric flask and mixed until dissolved.

vi) Liquid chromatography (LC) mobile phase

Ammonium acetate- Acetonitrile- Solution in a ratio of 77:23 was used (Ghinidi *et al.*, 2003 and USA: CLG-TET2.04, 2011). The 0.05M Ammonium acetate solution was prepared by weighing 3.85g of ammonium acetate and dissolved into a 1Litre distilled water and adjust PH of solution to 3.0 ± 0.5 with trifluoroacetic acid. The solution was also filtered through 0.45 μ m pore size membrane filter and degassed in the ultrasonic/degasser.

vii) Preparation of oxytetracycline standard**a) Stock standard- (1000 μ g/ml)**

Stock standard was prepared by adding 108 ± 0.1 mg of oxytetracycline reference standard to methanol in 100ml volumetric flask at room temperature.

b) Working standard- (25 μ g/ml)

Working standard was prepared by first, preparing 100 μ g/ml and then pipetting 2.5ml of the prepared solution into 10ml volumetric flask. Subsequently diluting with methanol to the volume and mixed thoroughly and stored in the refrigerator

c) Oxytetracycline chromatographic standard solutions

The oxytetracycline chromatographic standard solutions- (12.5µg/l, 25µg/l, 50µg/l, 100µg/l and 200µg/l) were prepared from the oxytetracycline working standard (1000µg/l) which was diluted accordingly to make the desired concentration.

3.7.2.1 Sample preparation and extraction

i) Sample extraction

Homogenised, previously frozen raw milk samples (5.0 ±0.1ml test milk), showing no signs of souring or curdling was pipetted into 50ml polypropylene centrifuge tube and centrifuged for 15minutes at 250rpm at approximately 10⁰Ctemperature to separate cream.The contents were then mixed with 20 ml McIlvaine/EDTA solution and shaken for 10 minutes on flat bed shaker or vortex mixer at high speed.

The content of the tubes were then centrifuged for 20 minutes at a speed of 4000 rpm at approximately 15⁰C. The supernatant was filtered through GF/B filter paper after moistening with McIlvaine buffer-EDTA solution.

ii) Sample clean up by solid phase extraction(SPE)

Sold phase extraction (SPE) cartridges were attached to an SPE vacuum manifold connected to 75ml reservoir to each cartridge. The Bond Elut-SAX C18 cartridges were first conditioned with 20ml methanol followed by 20ml of HPLC- grade water, at approximately 1.5- 2.5ml/minute, vacuum was applied gently and eluate were discarded.The final extract supernatant of test solution was then applied into C18 cartridge followed by drained cleaned and rinsed the cartridge with 20ml HPLC-

grade water and eluate were discarded. The cartridges were dried when water rinse was completed and continued to draw air through cartridge for approximately 2 minutes.

iii) Elution step to remove residues from the cartridge

Oxytetracycline residues test solution from the cartridges were eluted with 5.0ml methanolic oxalic acid and filtered through 0.45µm syringe filtration cartridges into LC autosampler vials and loaded into autosampler. Finally, 20µl of the solution was injected into the HPLC-UV system. Samples were analyzed at 365nm wave length.

3.7.2.2 HPLC analysis for OTC

Using 0.45µm pore size micro syringe filter the test solution and the standards were filtered into the LC auto sampler vials and loaded into auto sampler. The analysis and quantification of the OTC residues in the extract was done using a high-performance liquid chromatography (LC-20AT Model Shimadzu Prominence series -Japan) equipped with CBM-20A auto injector with sample cooler CTO-10AS VP and DGU-20A₅ on-line vacuum degassing solvent delivery unit, a constant flow pump and a variation wavelength UV detector set at 365 nm. The separation was done on Zorbax Eclipse XDB-Analytical C18 (150x 4.6 mm, 5µm) Column with Ammonium acetate-Acetonitrile solution, (77:23) as the mobile phase by gradient mode, the mobile phase flow-rate of 1.2 ml/min at room temperature and the sensitivity range was 0.08 ppm. The HPLC analysis was performed for 5 minutes in each sample.

To get the concentration of a given sample, reference standards of known concentrations were used to determine the concentration of the sample extract. For the

determination of oxytetracycline residues, the OTC standard solutions at different concentrations of 12.5, 25, 50, 100 and 200 µg/l was injected in ascending order (table 2). The extract from each sample was injected in duplicate to obtain average peak height of positive samples. The samples were considered to be positive for tetracycline if their retention time and peak corresponded to that of the reference standard. The retention time of the standard was at 2.7 minutes (figure 3).

i) Method development for HPLC analysis of raw milk samples

Prior to extraction of OTC in an unknown raw milk samples, the method was developed under the laboratory conditions suitable for analysis using HPLC. The developed method was later on used to determine OTC residues concentration of the raw milk sample in the present study.

Table 2: OTC Standards calibration curve

OTC Reference standards (ug/l)	Area of the chromatography
12.5	800.3
25.692	1473.3
47.465	2478
93.009	4853
198.561	10289

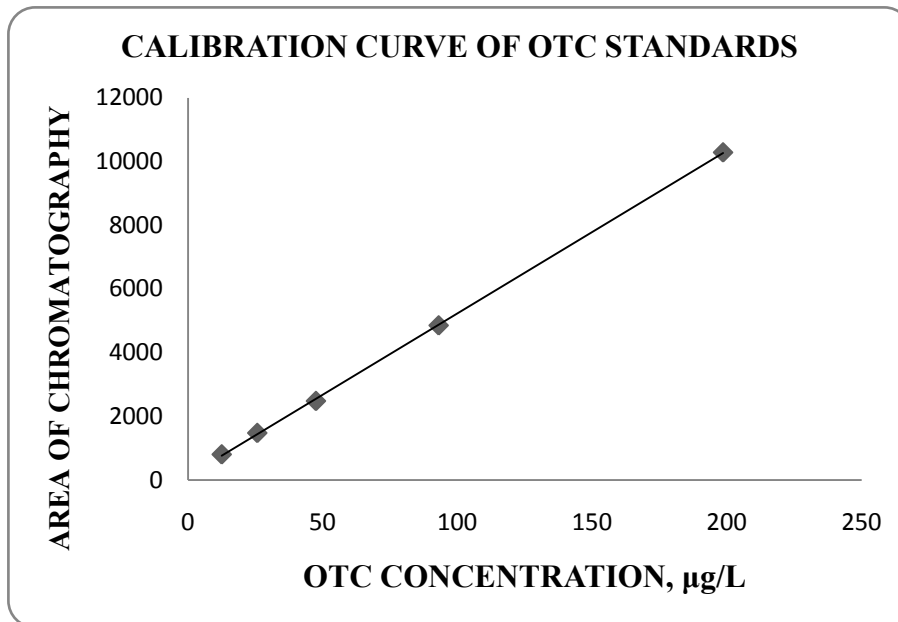


Figure 3: Calibration curve with five concentration points of OTC standards.

ii) Preparation of OTC standard solutions

One hundred and eight milligram of OTC standard solution was weighed and transferred into 100 ml amber volumetric flask, then was dissolved in methanol at room temperature. This OTC stock standard solution-1000µg/ml was protected from light by using amber volumetric flask and stored at -20°C for three months and it was used to prepare the working OTC standard by serial dilutions.

The OTC intermediate stock solution-100µg/ml was then prepared by diluting 10 ml of stock standard solution in 100 ml volumetric flask with methanol at room temperature and stored in a -20°C. It was prepared on a weekly basis. The intermediate stock solution was further diluted to get the OTC working standard solution 25µg/ml by taking 2.5 ml of OTC intermediate stock solution in 10 ml volumetric flask with methanol and mixed at room temperature. Working standard was used to prepare 1000µg/l, Then five (5) oxytetracycline chromatographic

standard solutions at 12.5, 25, 50, 100 and 200 µg/l concentrations were prepared into separate 10 ml volumetric flasks containing 5 ml methanolic oxalic acid solution and mixed. Then distilled water was added to bring the flask to volume.

Under gradient conditions, different standard concentrations of the OTC analytical standard solutions prepared were injected in ascending order and the results were plotted automatically on the integrator. The machine was set to run for 5 minutes and OTC was detected at 2.7 minutes as retention time with the peak height increasing with increase in concentrations.

iii) **The positive and negative control samples**

A known negative and positive samples were used as quality control. Negative controls were those known to be free from OTC contamination. Positive controls were those known to be free from OTC that have been fortified (spiked) with a known quantity of OTC (Table 3). In this study, Antibiotic free UHT milk (Brookside Dairy) was purchased from Dar es Salaam Shoprite super market. The negative control samples of duplicate UHT milk were treated the same way as the raw milk samples through undergone extraction, SPE clean-up and elution with methanolic oxalic acid. Duplicates UHT milk spiked with 1000µg/l of OTC standard solutions were used as the positive control samples.

Two hundred microliter (200µl) of the working solution of OTC standard (25µg/ml) was pipetted into 5ml volumetric flask and antibiotic free UHT milk was added to the volume and mixed thoroughly by shaking for 10seconds and left for 30 minutes before

start extraction procedure with McIlvaine buffer, solid phase extraction and elution from the cartridges. The control samples were run through the analytical column in a HPLC machine mobile phase conditioned with Ammonium acetate- Acetonitrile solution in a ratio of 77:23 respectively. The column temperature and flow rate were maintained as the case for the OTC analytical standard solutions. The retention time of the control samples was compared with those of the OTC analytical standard solution alone. During extraction blank sample eluted from SPE cartridge was included to check for the analytical column efficiency.

iv) LOD and LOQ of an analytical system

Limit of detection (LOD) and Limit of quantification (LOQ) depend on the noise and drift of the detection equipment. Absolute detector LOD can be determined by injecting a sample directly into detector. It is often expressed as minimum detectable level. However, the LOD depend on the oxygen content of the mobile phase, the injection system, peak broadening on the column and temperature differences among system component. Taking these factors into account, the LOD was defined as 3 times the noise level and LOQ was defined as 10 times the noise level and in this study, it was determined by the analysis of spiked free from antibiotic UHT milk (Brookside brand) sample with 10 μ g/l. The recoveries of the antibiotics were calculated at four different concentration levels of OTC Standards (50, 100, 500, 1000 μ g/l) and on average the recovery percentage was 83% (Table 3).

Table 3: Recovery and precision of OTC determined in spiked UHT milk sample (n=4).

OTC level (µg/l)	Spiked	Determined Time	Recovery level (µg/l).	Mean recovery level (µg/l)	% Recovery
50		1	28.269	27.379	54.8
		2	26.934		
		3	26.934		
100		1	66.024	68.821	68.8
		2	67.522		
		3	72.918		
500		1	532.166	558.249	111.6
		2	552.881		
		3	589.700		
1000		1	1007.021	978.6885	97.9
		2	950.356		
		3	-		

Mean %Recovery = 83.3 and therefore extraction efficiency was 83.3%

v) Calculations

The HPLC machine data output measured and recorded the peak area for OTC standard solutions and the test solutions. Using data from the OTC standard solutions, the best fit line of data was determined using the linear regression equation as follows; $y = mx + b$ whereby: y = Sample peak area; x = OTC concentration in µg/l; m = slope and b = intercept of y . Also the correlation coefficient (R^2) was determined from this equation and was found to be 0.99636. The quantification of OTC residues of the sample analyte was determined by using the peak area of the analyte and values from the regression slope and intercept of the OTC standard solutions. Each oxytetracycline standard curve should be linear. Concentration of oxytetracycline residues in original raw milk was calculated directly since the dilution factor of injected test solution was

1 (eluted final volume in SPE cartridge). Care was taken with integration and therefore baselines were checked for each chromatogram determined by automated data systems.

3.8 Knowledge, attitudes and handling practices in usage of veterinary drugs

A simple random sampling technique was employed in which the small-scale dairy farmers willing to participate in the study were interviewed at the household level. Semi-structured questionnaire was prepared and designed to capture information on diseases status, mostly common antibiotics used, frequency of using veterinary drugs, common type of farming system practiced, awareness of withdrawal period/time, means of access veterinary drugs, the reasons for using antibiotics, awareness on the effects of antibiotic residues in human health, storage practices and condition for drugs, raw milk consumption pattern, and training in regard to antibiotics usage.

3.9 Data analysis

Raw data from survey and laboratory analysis were entered and stored in MS-Excel spread sheet and analysed to describe samples with MRLs above the recommended values and therefore containing potentially harmful concentration of antibiotic residues. Proportions were computed using Epi-Info database (version 7 of 2012). Epi-Info database was also used to compute descriptive statistics for measure of central tendency and measure of variability. Continuous and proportions of categorical variables were computed and Chi-square used to compare for statistical significance at a critical probability of $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Awareness of small-scale dairy farmers on veterinary drugs usage and factors leading to presence of antibiotic residues in raw milk in Bagamoyo District.

4.1.1 Demographic characteristics of the study population.

Results showed that there were more males than females whereby, males were 71.8% (95% CI= 62.4-80, n=79) and 28.2 % (95% CI = 20.0-37.6, n=31) were females. Respondents were of different age, ranged from youth 16.4% (15- 4 years), adult 54.6% (36-55 years) and elder 29.1% (above 55 years). Their education level ranged from informal education to college education. The majority of the respondents had primary education (58.2%) followed by secondary education (27.3%), Informal education were (8.2%) and College education (6.4%).

4.1.2 Distribution of the respondents in the study area

A total of 110 small-scale dairy farmers were interviewed in the selected households from three (3) raw milk collection centres/wards in Bagamoyo district. The collection centres where the respondents were obtained include Msata, Lugoba and Chalinze. However, Msata collection centre involved respondents from Msata, Kiwangwa and Fukayosi wards, while Lugoba collection centre involved respondents from Lugoba and Msoga wards and Chalinze collection centre involved respondent from Bwilingu and Pera wards . Out of the 110 small-scale dairy farmers interviewed in the three collection centres of the Bagamoyo district, 45.5% (95% CI = 35.9-55.2, n=50) were from Chalinze, 17.3% (95% CI= 10.7- 25.7, n=19) were from Lugoba and 37.3% (95% CI= 28.3- 47.0, n= 41) were from Msata.

4.1.3 Type of farming system and animal health management

Most of the respondents practiced semi intensive grazing system which constituted 59.1 % (95% CI = 49.3-68.4, n= 65), followed by zero grazing system with 21.8%, n=24, extensive grazing system 14.6%, n=16 and the last were communal grazing system with 4.6%, n=5. On the other hand results indicated that, veterinary drugs for treating dairy animals were obtained from different markets and these were the veterinary pharmacy (49.1%, n=54), the livestock field officers (48.2%, n=53) and cattle market (2.7%, n=3).

4.1.4 Commonly reported animal diseases affecting dairy animal

The most commonly mentioned diseases were East Coast Fever (ECF) 95%CI, (58.2%, N=64), followed by Anaplasmosis (15.5%, n=17), Trypanosomiasis (11.8%, n=13), Mastitis (6.4%, n=7), Foot and Mouth diseases (FMD) (2.7%, n=3). On the other hand respondents reported Lumpy skin disease, Contagious Bovine Pleuropneumonia (CBPP) and worms with 1.8% each.

4.1.5 Animal diseases, veterinary drugs and frequency of use

The results showed that small-scale dairy farmers use OTC, pen & streptomycin, sulphonamides and gentamycin drugs to treat cattle diseases. In comparison OTC was the most frequently mentioned veterinary drug used in the area (56.4%, n= 62), followed by pen & streptomycin (penicillin G, 29.1%, n=32), sulphonamides (11.8%, n=13) and gentamycin (2.7%, n= 3). On the other hand, most of respondents treat their animal by calling the Veterinary officer (83.6%) and only 16.4% reported to treat sick animal themselves. Also respondents mentioned period when normally their cattle

received treatment as (91.8%) when animals are sick, 0.9% continue even after recovery and 7.3% they treat the animal even if not sick as preventive measures.

4.1.6 Problem associated with the use of veterinary drugs to dairy animal

In response to a question on the availability and accessibility of livestock services on a timely basis, 80.9% (95% CI = 72.3-87.8, n=89) of respondents reported presence of these services. 66.4% (95% CI = 56.7-75.1, n=74) reported to have no training on handling veterinary drugs, administration of veterinary drugs and handling of sick animal apart from the instructions given by the seller of the drugs at the time of purchase. Only 32.7% of respondents reported to receive the training on how to use veterinary drugs. On other hand respondents reported to obtain veterinary drugs from pharmacy (49.1%), n=54; from the livestock officers (48.2%), n=53 and only 2.7% reported to obtain from the livestock market, n=3. Respondents were also asked whether they were aware of drugs withdrawal period, majority 96.4% were knowledgeable and 91.8% complied by not selling milk from treated lactating cows.

4.1.7 Awareness of the respondents on health risks associated with consumption of raw milk with antibiotic residues.

On assessing the respondent awareness on the effects of consumption of raw milk contaminated with veterinary drugs residues, 91.8% of the respondents mentioned awareness of the effects of consumption raw milk contaminated with antibiotic residues while 2.7% of the respondents were not aware of danger and health effect of consuming raw milk contaminated with antibiotic residues. On the other hand 5.5% of the respondents said they did not know anything.

4.2 Qualitative screening test for antibiotic residues

The collected samples were 41 samples from Msata collection centre, 19 samples from Lugoba collection centre and 50 samples from Chalinze collection centre. The total of One hundred and ten (110) raw cows' milk samples were screened for antibiotic residues by microbiological inhibition assay using agar well diffusion method. The diameters of the zones of inhibition from the end of wells/holes margin to beginning of bacterial growth were measured and an inhibition zone of ≥ 2 mm was considered as positive and diameter of zone of inhibition measured less than 2mm were considered negative (Figure 9). Majority of collected raw milk samples from the small-scale dairy farmers, 99 samples (90.0%) were negatively detected for antibiotic residues i.e no visible transparent zone of inhibition around the wells in the nutrient (Muller Hinton) agar surface while 11 samples (10.0%) were positive detected for presence of antibiotic residues with mean zone of inhibition size 12.00 mm.

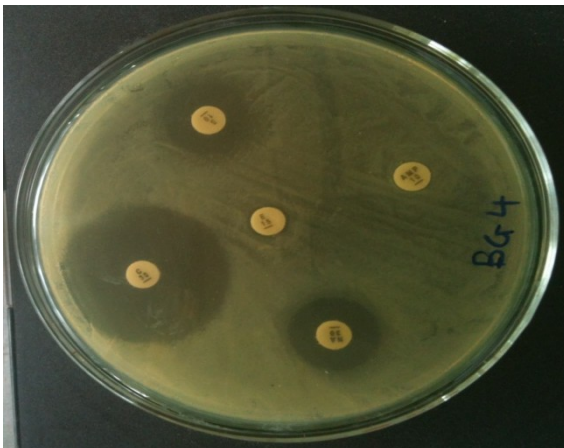


Figure 4: Antibiotic Inhibition Test plate with zones of inhibition.

4.3 Quantitative analysis of OTC in raw milk samples

Antibiotic positive detected raw milk samples were confirmed for mostly common used veterinary drug in the study area (OTC) residue levels through high performance

liquid chromatography (HPLC method) and the results are shown in the Table 4. The result revealed that all (11+ve) screened samples analyzed out of 110 raw milk samples had detectable oxytetracycline residues from which all 10% had OTC residues at violative levels above the WHO/FAO-CAC maximum residue limits (MRLs). According to Codex Alimentarius Commission standards (CAC) the maximum residues limits (MRLs) for OTC residues in milk is 100µg/l. In this current study the level of OTC residues obtained on average were, 553.985µg/l in Msata collection centre and 978.571µg/l in Chalinze collection centre, However Lugoba collection centre had no detectable level of OTC residues (Table 5). Hence therefore on average, in this study Bagamoyo district had OTC residue of 766.278µg/l which is almost eight times the recommended codex MRLs for milk.

Table 4: Determined OTC levels in raw milk samples in Bagamoyo District.

LOCATION	SAMPLE CODE	OTC RESIDUES (µg/l)
Chalinze	C3	395.334
Chalinze	C5	2841.11
Chalinze	C7	678.618
Chalinze	C8	482.963
Chalinze	C9	548.026
Chalinze	C10	925.375
Msata	A4	553.693
Msata	A5	597.745
Msata	A11	526.132
Msata	A12	719.871
Msata	A13	372.484

Out of total 110 raw milk samples OTC tested, 10.0% (n=11) samples were found to be positive for antibiotic residue, whereas 90.0% (n=99) samples were found to be negative for OTC antibiotic residue (Table 5). Out of 11 positive samples, oxytetracycline residues detected and quantified above the maximum residual limit

(MRL) of Codex Alimentarius Commission standard, where by all 11 positive detected samples had violative OTC residue levels (Table 4).

Table 5: Residues and levels of oxytetracycline residue in raw cow's milk

Collection centre	Number of sample	Sample with detectable levels %	Samples exceeding MRL %
Chalinze	50	12.0	12.0
Msata	41	12.2	12.2
Lugoba	19	0.0	0.0
Total	110	10.0	10.0

The total positive samples showed a significance variation ($P < 0.05$) in numbers of the positive samples for antibiotic residues in Chalinze collection centre as compared with other collection centres. On average Chalinze collection centre had 6 (12%) positive samples out of 50 samples collected in the area while Msata collection centre had 5 (12.2%) positive samples out of 41 samples collected in that area and Lugoba had no positive samples out of 19 collected samples. Furthermore on average, level of OTC residue in Chalinze was $978.571 \mu\text{g/l}$ while Msata had $553.985 \mu\text{g/l}$ and Lugoba had without contaminations.

CHAPTER FIVE

5.0 DISCUSSION

The overall objective of this study was to assess veterinary drugs usage and determine antibiotic residues levels in raw cows' milk produced by small-scale dairy farmers in Bagamoyo District, Tanzania. The results of the present study show that oxytetracycline is the most commonly used antibiotic in Bagamoyo District and detectable oxytetracycline residues are above the WHO/FAO-CAC Standard on veterinary residues in food" maximum residues Limits (MRLs). According to WHO/FAO-CAC Standard CAC/MRL 2-2012" the maximum residues limits (MRLs) for OTC residue in milk is 100µg/l (WHO/FAO-CAC Standard, 2012). The levels of OTC residues obtained were much higher as compared to maximum residue limit (MRL) for oxytetracycline residues in milk and their ranges were 372.5µg/l to 2841.1µg/l.

This study produced results which upholds the findings of a previous works in this field. The results match those of Olatoye and Ehnmowo, (2010) which indicated that oxytetracycline is one of the most commonly used antibiotics in livestock production. It is widely used in veterinary medicine mainly for the treatment of gastrointestinal, respiratory and skin bacterial infections, infectious diseases of locomotive organs and of genito-urinary tract as well as systemic infections and sepsis (Michalova *et al.*, 2004). Apart from being a broad spectrum antibiotic, it is also cheap (Olatoye and Basiru, 2013), readily available in veterinary shops and accessed easily by farmers without restrictions (Nonga *et al.*, 2009).

The present results further emphasises that tetracyclines are the most predominantly prescribed antibiotics in Africa and in all antibiotic-associated residues they represent 41% of cases, followed by β -lactams at 18% (Darwish *et al.*, 2013). Indeed the current findings compare well with Darwish *et al.*, 2013 who showed that 41% of OTC are prescribed and uses in bacterial infections diseases as well as non-bacterial pathogens of cattle. This could be due to it is known broad-spectrum characteristic with a bacteriostatic effect on the wide range of gram-negative and gram-positive bacteria (Jevinova *et al.*, 2002).

Consumption of milk contaminated with antibiotic residues pose serious health risk even if the milk will be heat treated before consumed. The findings by Navratilova *et al.* (2009) reported that, there is possibility of partial reduction of OTC residues but not total elimination following heat treatment of milk during milk processing. This means that even those majorities of the population in Bagamoyo District and elsewhere in Tanzania who are drinking boiled milk are at potential risk to OTC residues.

5.1 Farming system and related practices

Cattle in the study area are kept either in traditional herds or at small-scale backyard dairy herd and most of the small-scale dairy farmers practice semi-intensive grazing system (59.1%). During grazing animals are sometimes crossing traditional cattle herds which are grazed communally in the same area and therefore can predispose dairy animal to contact diseases. Since there is frequent occurrence of diseases which

needs uses of drugs, consequently compliance to withdrawal period could lead to loses especially when the milk production per cattle is low.

Milking at small-scale dairy farms is mostly done in a special crush and in some other cases in the cowshed often with concrete floor. Washing hands and cleaning the udder before milking is more common practice in small-scale dairy and normally this was done by using clean water. None of small-scale dairy farmers cooled milk on farms after milking at the house-hold level and therefore there was a possibility of adding antibiotic so as to lengthen shelf life of raw milk during transporting to the collection center (Kurwijila *et al.*, 2006). All small-scale dairy farmers used plastic utensils for milking and transporting milk to the collection center for selling. This practice could be the source of milk contamination at the small-scale dairy farms who sell their milk more often to milk collection centers or sometimes to their neighbors.

Quality check of the collected milk is mainly performed at the milk collection centers which are owned privately. The quality check consists of only physical verification and testing of specific gravity by using a lactometer and collection centers do not process but mainly cool bulk milk for delivery to the bigger processors in the area especially Tanga and Dar es salaam cities. The antibiotics screening testing would have been important for safety and quality of milk before going to the consumers or subsequently processing.

5.2 Awareness of small-scale dairy farmers on antibiotic residues in raw milk

The results of the current study showed that 91.8% of respondents' small-scale dairy farmers were aware of the drug residues in milk and that 96.4% of the respondents

were aware of withdrawal periods. These findings are in line with other findings in Tanzania as have been reported by Katakweba *et al.* (2012) and Nonga *et al.* (2009), who both reported 90% awareness, but higher than that of Midenge, (2011) who found 72.2% awareness on drug withdrawal period. Similarly, study conducted in neighbour country Kenya by Ombui, (1994) found that there was high awareness on withdrawal periods among farmers in Kiambu District.

The withdrawal period is the most important parameter in the control of drug residues in animal food products intended for human consumption. Failure to observe OTC withdrawal period of 7 days for milk and 28 days for beef imply that consumers of milk and beef are left to consume OTC residues unknowingly (Omore *et al.*, 2002; Movassagh and Karami, 2010; Pecou and Diserens, 2011). It has been reported that failure to observe withdrawal period has been the main source of drug residues in food of animal origin in many countries (Nisha, 2008 and Alla *et al.*, 2011). Small-scale dairy farmers of Bagamoyo District showed higher level of awareness on withdrawal time and the effect of consuming raw milk containing antibiotic residues, however contrary to this situation the non-compliance to withdrawal period by small-scale dairy farmers in Bagamoyo district could be associated with many reasons including fear of economic losses due to the fact that there is no any scheme which compensate small-scale dairy farmers as incentive to comply with withdrawal time.

Compliance to withdrawal period to antibiotic by small-scale dairy farmers in the study area was proved true by majority of the farmers admitting that they were told not to use milk or sell milk when the dairy lactating animals were under treatment and

after treatment. Awareness of drugs withdrawal period in this study agrees with findings of Syit, (2011) who reported 78.4% of respondents were aware of drugs residues in milk following treatment of sick dairy cattle. The presence of veterinary practitioner or livestock extension officer in each ward in the Bagamoyo District might be the reason for higher level of awareness to the small-scale dairy farmers in the respective area. This is further augmented by the higher use (83.6%) of veterinary officers to treat their animals which showed increasing trend compared to previous report by Kurwijila *et al.*, (2006).

Another important finding was that, 91.8% of small-scale dairy farmers were aware on the health effects associated with consuming milk with antimicrobial residues. The findings concurs with other studies by Midenge, (2011) and Katakweba *et al.*, (2012) in Tanzania, as well as studies in Ethiopia by Syit, (2011) and Widayatiet *et al.*, (2012) in Indonesia. The findings do correlated (10% residues versus 91% awareness) implies that veterinary services should be continue to be used and all farmers should take the advice. However the present level of OTC residues in raw milk from small-scale dairy farmers might be also due to farmers' fear of economic losses and lack of incentive from the Government to compensate small-scale dairy farmers to overcome losses during treatment of animals. Also present antibiotic residues prevalence could be explained by attitude of non-voluntary compliance of small-scale dairy farmers to food safety regulations, since most of raw milk reaches consumers directly without quality and safety checks and in Bagamoyo and elsewhere in Tanzania there is no monitoring and surveillance program which enables raw milk from small scale dairy farmers to be tested for antibiotic residues before can be marketed.

5.3 Oxytetracycline residues in raw milk

Based on high cost of analysis by using HPLC technique protocol to confirm antibiotic residues in milk and also consideration of the result of specific objective number one of this current study which indicated that, oxytetracycline is the mostly common veterinary drug used in the study area. Hence therefore quantification of antibiotic residue levels was confined only to OTC residues by HPLC technique and two different methods, antimicrobial inhibition test and High Performance Liquid Chromatography (HPLC) were used to determine antibiotic residues in raw milk. The study confirmed that antimicrobial inhibition assay did not give any false results for determination of antibiotic residues. False-positive and false-negative samples were not found since all the positive detected samples were all confirmed positive by HPLC technique. The most important thing for screening test is that they cannot give false-negative result, because the first step for confirmation of antibiotics usually consists of rapid test and only presumptive positive results are analyzed by HPLC (Alkan, 2007). According to the international standard for food safety elaborated by Codex Alimentarius Commission (FAO/WHO-CAC, 2012), the recommended MRL for oxytetracycline residue in milk is 100 µg/l. As revealed by the current study 10% of the samples had detectable and violative levels of oxytetracycline residues with an average confirmatory residues level of 766.3 µg/l almost eight times the allowable maximum residue level for milk and therefore indicates alarming situation in food safety and public health concern (Lee *et al.*, 2000 and Oslon *et al.*, 1975).

When administered in accordance with the approved labelling, the prevalence of violative drug residues in milk would be less than 1.0% (Sundlof, 1994). Residues

violation greater than 1.0% generally indicates that the antimicrobial has been used in a manner inconsistent with the labelling. The present findings are in line with the study conducted by Kivaria *et al.*, (2006) who found 7.0% positive antimicrobial residues in marketed milk but considerably lower than the findings by Kurwijila *et al.*, (2006) and Jabbar *et al.*, (2013); which showed 36% and 41.6% respectively. Higher positive antimicrobial residues in the previous study could be because that, in the current study collected samples directly at the small-scale dairy farms while the previous studies collected at the milk collection centers or points and this could be the source of more contamination and possibly deliberate addition of antibiotics in the milk in order to lengthen shelf life before reaching the marketing area particularly for farmers living in rural areas with poor road infrastructure (Kurwijila *et al.*, 2006).

Furthermore, the findings from this study is considerably comparable to neighbouring country Kenya (9 to 16%) of antibiotic residues in marketed raw milk (Ombui, 1994; Shitandi *et al.*, 2004 and Kang'ethe *et al.*, 2005). In addition, the findings from this study in combination with the one done by Kivaria *et al.*, (2006) and Kurwijila *et al.* (2006) in the marketed milk along Pwani region and Dar es salaam city imply that community in that areas and other part of the country ingests small doses of antimicrobial from different foods of animal origin. This practice may to a greater extent contribute significantly to the development of microbial resistance.

The degree of contamination of milk and dairy products with antibiotics residues differs, depending on the level of legislation and effectiveness of enforcement methods in different countries (Kaya and Filazi, 2010) therefore the health risk

associated with antibiotics residues in milk will continue to exist in Tanzania which calls for an effective monitoring and surveillance program that is legislated and implemented from the primary production to the consumers.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study show relatively higher prevalence and level of oxytetracycline residues in raw milk of small-scale dairy farms in Bagamoyo District. Lack of proper management, non-compliance to drugs withdrawal period as the major contributing factors to the occurrence of antibiotic residues in milk. From the present findings it is concluded that; there is high level of awareness that consumption of raw milk contaminated with antibiotic residues can predispose the consumers to health hazards, although what was practiced along the milk value chain predisposed milk to contamination with antibiotic residues. Farming practices and attitudes could contribute to the observed level of antibiotic residues in Bagamoyo District. The finding of the abusive level of OTC residues in this study provides alarming situation on the use of not only OTC but also other veterinary drugs by most of small-scale dairy farmers in Bagamoyo District and Tanzania at large.

Inadequate technical infrastructure - in terms of conformity assessment system i.e food laboratories, inspectorate and control authorities, human and financial resources, national legislative and regulatory frameworks, enforcement capacity, management and coordination weakens the ability to confront these challenges of monitoring and ensuring raw milk from not only small-scale dairy farmers but also milk from traditional farmers are safe and free from antibiotic residues. Such systemic weaknesses may not only threaten public health but may also result in compromising food safety and quality in Tanzania.

6.2 Recommendations

To assure the quality and safety of raw milk along the milk value chain it is recommended that;

- The rapid antibiotic screening test i.e microbial susceptibility tests should be performed at the raw milk collection centres to ensure production of antibiotic residue-free milk as the initial step toward addressing the problem.
- Special emphasis on farming practices and knowledge awareness on health risks associated with usage of antimicrobial drugs in general during treating sick animals should be increasingly provided amongst small-scale dairy farmers.
- Also awareness creation on the risks of consumption of raw milk contaminated with antibiotic residues amongst small-scale dairy farmers, rural and urban consumers. Additionally, key players and stakeholders in the milk sector like Veterinary and extension sectors, Tanzania Food and Drugs Authority (TFDA), Tanzania Bureau of Standards (TBS), Tanzania Dairy Board (TDB), Tanzania Milk Processing Association (TAMPA), Tanzania Milk Producers Dairy Association (TAMPRODA) should come-up with harmonized program and strategy to address this challenge of public health.
- Intervention at the farms level is especially important because most of raw milk reaches consumers directly without processing stage.
- Socioeconomic intervention of noncompliance should be introduced such as incentives to promote behavioral changes among small-scale dairy farmers that will enhance voluntary compliance of drug withdrawal periods.

REFERENCES

- Abbasi, M. M., Babaei, H., Ansarin, M., Nourdadgar, A and Nemati, M. (2011). Simultaneous determination of tetracyclines residues in bovine milk samples by solid phase extraction and HPLC-FL method. *Advanced Pharmaceutical Bulletin*,1 (1): 34-39.
- Aila, O., Shitandi, A., Mahungu, S. M. and Sharma, H.K. (2009).Determination of furazolidone residues in chicken tissues using *Bacillis stearothermophilus* test. *Food Control* 20: 543-547.
- Al- Mohana, A.M. G., Nima,A. J., Abd- Alhausen, J. K. and Munahi, A. K. (2010). Determination of cattle milk contamination with antibiotic residues by using microbiological analysis. *Al-Anbar Journal of Veterinary Science* 3: 1999-6527
- Alkan, P. (2007). The confirmation of the commercial kits used in the detection of Antibiotics in milk with HPLC, A Thesis submitted for the degree of Master of Science in Food Engineering, Izmir Institute of Technology.
- Alla, M.B.W., Mohamed, T.E., Abdelgadir, A.E. (2011). Detection of antibiotics residues in beef in Ghanawa Slaughterhouse, Khartoum State, Sudan.*African Journal of Food Science* 5 (10) 574-580.
- Aning, K. G., Donkor, E. S., Omore, A., Nurah, G. K., Osafo, E. L. K. and Staal, S. (2007). Risk of exposure to marketed milkwith antimicrobial drug residues in Ghana.*Open Food Science. Journal.,I: 1-5.*
- AOAC Official Method 995.09,(2000). Chlorotetracycline, Oxytetracycline and Tetracycline in Edible Animal Tissues: Liquid Chromatographic Method.

- Bedada, A. H., Zewde, B. M., and Zewde, M. B. (2012). Tetracycline residues in slaughtered beef cattle from three slaughterhouses in Central Ethiopia. *Journal of Global Veterinary* 8 (6) 546-554.
- Broome, M. C., I. B. Powell and G. K. Y Limsowtin (2002). Starter cultures: specific properties, p. 269–275. *In* Encyclopaedia of dairy science, volume 1. Academic Press, London.
- Codex Alimentarius Commission (CAC) (2012). *Maximum residues limits for veterinary drugs in Foods*. CAC/MRL 2-2012. Rome, Italy. 40pp.
- Darwish, W.S., Eldaly, E.A., El-Abbasy, M.T., Ikenaka, Y., Nakayama, S., Ishizuka, M. (2013). Antibiotic residues in food: the Africa scenario. *Japanese Journal of veterinary research* 61 (sup): S13-S22.
- De Leeuw, P., A. Omore, S. Staal and W. Thorpe (1999). Dairy production systems in the tropics, p. 19–44. *In* L. Falvey and C. Chantalakhana (ed.), Smallholder dairying in the tropics. ILRI/ACC. no. IL-990209. International Livestock Research Institute, Nairobi, Kenya
- Ghidini, S., Zanardi, E., Varisco, G. and Chizzolini, R. (2003): Residues of β -lactam antibiotics in bovine milk: Confirmatory analysis by liquid chromatography tandem mass spectrometry after microbial assay screening. *Journal of Food additives and Contaminants*, 20: 528 – 534
- Goffová, Z. S., Kozárová, I., Máte, D., Marcincák, S., Gondová, Z. and Sopková, D. (2012). Comparison of detection sensitivity of five inhibition tests for the screening of amino glycoside residue in fortified milk. *Journal of Food Science* 30: 314 - 320.

- Grane, M.M. (2000). Analysis of Antibiotic Drug Residues in Biological Matrices, After Evaluation of various Extraction Methodologies and Determination procedures, A Thesis submitted for the degree of Doctor of Philosophy, Dublin City University. High performance thin-layer chromatography-bioautography for multiple antibiotic residues in cow's milk. *Journal of Chromatography Analyte Technology Biomed Life Science*. 784 (2):315-22
- Hillerton, J. E., Halley, B. I., Neaves, P. and Rose, M. D (1999). Detection of antimicrobial substances in individual cow and quarter milk samples using Delvotest microbial inhibitor tests. *J. Dairy Science*. 82: 704-711.
- Hossain, T. J., Alam, M. K. and Sikdar, D. (2011). Chemical and microbiological quality assessment of raw and processed liquid market milks of Bangladesh. *Journal of Food Science and Technology* 5: 6 - 17.
- Ivona, K. and Mate, D. (2002). Evaluation of the sensitivity of individual test organisms to residual concentrations of selected types of drugs. *Slov. Veterinary. Residues.*, 9: 78-82.
- Jabbar, A., Ur-Rehman, S (2013). Microbiological Evaluation of Antibiotic Residues in meat, milk and eggs: 2(5) 2349-2354. *Journal of Microbiology, Biotechnology and Food Sciences*.
- Jevinova, P., Dudrikova, E., Sokol, J., Nagy, J., Mate, D., Pipova, M. and Cabadaj, R. (2003). Determination of oxytetracycline residues in milk with the use of HPLC method and two microbial inhibition assays. *Bull. Vet. Inst. Pulawy* 47: 211-216

- Kang'ethe, E. K., G. O. Aboge, S. M. Arimi, L. W. Kanja, A. O. Omore, and J. J. McDermott. 2005. Investigation of risk of consuming marketed milk with antimicrobial residues in Kenya. *Food Control* 16:349–355.
- Karimuribo, E. D., Mdegela, R. H., Kusiluka, L. J. M and Kambarage, D. M (2005). Assessment of drug usage and antimicrobial residues in milk on smallholder farms in Morogoro, Tanzania. *Bulletin of Animal Health and Production in Africa* 53(4): 234–241.
- 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* 24(10), Morogoro Tanzania, Art number 170, <http://www.lrrd.org/lrrd24/10/kata24170.htm> (site visited on 04/03/2014).
- Katla, A.K., H. Kruse, G. Johnsen and H. Herikstad (2001). Antimicrobial susceptibility of starter culture bacteria used in Norwegian dairy products. *International Journal of Food Microbiology*. 67:147–152.
- Kaya, E. S. and Filazi, A. (2010). Determination of antibiotic residue in milk samples. *Research Article* 16: 31 - 35.
- Kivaria, F. M., Noordhuizen, J. P. T. M. and Kapaga, A. M. (2006). Evaluation of the hygienic quality and associated public health hazards of raw milk marketed by smallholder dairy producers in the Dar es Salaam region, Tanzania. *Tropical Animal Health Production* 38: 185 - 94.

- Komba, E. V. G., Komba, E. V., Mkupasi, E. M., Mbyuzi, A. O., Mshamu, S., Luwumba, D., Busagwe, Z and Mzula, A. (2012). Sanitary practices and occurrence of zoonotic conditions in cattle at slaughter in Morogoro Municipality, Tanzania: implications for public health. *Tanzania Journal of Health Research* 14 (2) 1-12.
- Kurwijila, R. L., Omore, A., Staal, S. and Mdoe, N. S. Y. (2006). Investigation of the risk of exposure to antimicrobial residue present in marketed milk in Tanzania. *Journal of Food Protection* 69: 2487 - 2492.
- Kurwijila, L.R., Omore, A. and Staal, S.(2009). Dairy Sub Sector Development Strategy, East Africa Regional Initiatives in Value Chains.Lesson from ongoing R & D initiatives in Dairy value chains, Rural Livelihood Development Company.*Version Board 17 - 04 - 2009*.
[www.rldc.co.tz/docs/rldcdairy] site visited on 20/9/2013
- Lee, M .H., H. J. Lee and P.D Ryo (2000). Public health risks: chemical and antibiotic residues—review. *Asian-Australian Journal of Animal Science* 14:402–413.15.
- Mdegela, R. H., Ryoba, R., Karimuribo, E. D., Phiri, E. J., Løken, T., Reksen, O., Mtengeti, E., Urio, N. A. (2009). Prevalence of clinical and subclinical mastitis and quality of milk in smallholder dairy farms in Tanzania.*Journal of the South African Veterinary Association* 80: 163 - 168.
- Mellau, L. S. B., Nonga, H. E and Karimuribo, E. D. (2010).A slaughterhouse survey of lung lesions in slaughtered stocks at Arusha, Tanzania.*Journal of Preventive Veterinary Medicine* 97: 77-82.

- Michalova, E., Novotna, P and Schlegelova, J. (2004). Tetracyclines in veterinary medicine and bacterial resistance to them. *Czechoslovakia Journal of Veterinary Medicine* 49 (3) 79-100.
- Midenge, B. Y. (2011). Awareness on recommended veterinary drugs withdrawal period among small scale dairy cattle farmers in Kinondoni Municipality. Dissertation for Award of MSc Degree at Muhimbili University of Health and Allied Sciences, Dar es salaam, Tanzania. 90pp.
- Mitchell, J M.; Griffiths, M W.; Mcewen, W B and Yee A J (1998). Antimicrobial drug residues in milk and meat; causes, concerns, prevalence, regulations, tests and test performance. *Journal of Food Protection.*, 61:742-756.
- Mmbando, L.M.G. (2004). Investigation of Oxytetracycline use and abuse: Determination of its residues in meat consumed in Dodoma and Morogoro Municipality. Unpublished Dissertation for Award of MVM Degree at Sokoine University of Agriculture, Morogoro, Tanzania, pp 12-25.
- Mosalagae, D., Pfukenyi, D. M. and Matope, G. (2011). Milk producer's awareness of milk-borne zoonoses in selected smallholder and commercial dairy farms of Zimbabwe. *Tropical Animal Health and Production* 43: 733 - 739.
- Movassagh, M. H. and Karami, A. R. (2010). Determination of antibiotics in bovine milk in Tabrin, Iran. *Global Veterinarian* 5(3): 195 - 197.
- Msangi, B. S. J. (Ed.) (2006). Value-chain analysis and socio-economic assessment of the dairy industry in Tanzania. In: *Proceeding of the Tanzania Dairy Board, The six National Dairy Development*. (Edited by UNDP/ Business care services/ Tetrapak), 2 June, 2006, Morogoro Hotel, Morogoro, Tanzania. 14 – 25pp.

- Muriuki, F.K., Ogara, W.O., Njeruh, F.M and Mitema, E.S. (2001). Tetracycline residues levels in cattle meat from Nairobi slaughter house in Kenya. *Journal of Veterinary Science* 2: 97-101.
- Navratilova, P., Borkovcova, I., Drackova, M., Janstova, B and Vorlova, L. (2009). Occurrence of Tetracycline, Chlortetracyclin, and Oxytetracycline Residues in Raw Cow's Milk. *Czechoslovakia Journal of Food Science* 27: 379 – 385.
- NCCLS, (2002). Performance Standards for Antimicrobial Disk Diffusion and Dilution Susceptibility Tests for Bacteria Isolated from Animals. National Committee for Clinical Laboratory Standards (NCCLS), Pennsylvania. *American Society of Microbiology Journal* 9: 405–422
- Nijsten, R., N. London, A. Van de Bogaard, and E. Stobberingh (1996). Antibiotic resistance among *Escherichia coli* isolated from faecal samples of pig farmer and pig. *Journal of Antimicrobial Chemother.* 37:1131–1140.
- Nisha, A.R. 2008. Antibiotic Residues - A Global Health Hazard. *Veterinary World*, 1(12): 375 - 377.
- Njombe, A. P. and Msanga, Y. N. (2009). Livestock and Dairy Industry Development in Tanzania. [<http://www.mifugo.go.tz>] site visited on 12/07/2013.
- Njombe, A.P., Msanga, Y., Mbwambo.N and Makembe.N. (2011). The Tanzania Dairy Industry: Status Opportunities and prospects; *A paper presented to the 7th African Dairy Conference and Exhibition held at Dar es salaam.*
- Nollet., Loe, M.L., ed. (1992). *Food analysis by HPLC*. New York: Marcel Dekker Inc.

- Nonga, H.E., Mariki, M., Karimuribo, E.D and Mdegela, R.H. (2009).Assessment of Antimicrobial Usage and Antimicrobial Residues in Broiler Chickens in Morogoro Municipality, Tanzania.*Pakistan Journal of Nutrition* 8: 203-207.
- Nonga, H.E., Simon, E.D, and Mdegela, R.H. (2009).Assessment of Antimicrobial Usage and Residues in Commercial Chicken Eggs from Smallholder Poultry Keepers in Morogoro Municipality, Tanzania.*Journal of Zoonoses and Public Health* 57: 339–344.
- Okerman, L., Croubels, S., Cherlet, M., De Wash, K., De Backer, P. and Van Hoof, J. (2004). Evaluation and establishing the performance of different screening tests for tetracyclines residues in animal tissues.*Food additives and contaminants, Vol. 21 No. 2 PP 145-153*.
- Olatoye, I. L and Basiru, A. (2013).Antibiotic Usage and Oxytetracycline Residue in African Catfish (*Clarias gariepinus*) in Ibadan, Nigeria.*Journal of Fish and Marine Science* 5 (3) 302-309.
- Olatoye, I.O and Ehinmowo, A.A. (2010). Oxytetracycline Residues in Edible Tissues of Cattle Slaughtered in Akure, Nigeria. *Journal of Nigerian Veterinary* 31 (2): 93-102.
- Oliver, S. P., Boor, J. K., Murphy, C. S. and Murinda, E. S. (2009). Food safety hazards Associated with consumption of raw milk. *Food-borne Pathogens and Disease* 6: 793 - 806.
- Ombui, J. N. (1994). Antibiotic residues in milk received by dairy cooperatives societies in Kiambu district, Kenya.*East African Medical Journal* 71:628 - 630.

- Omija, B., Mitema, S. and Maitho, E. 1994. Oxytetracyclines residue levels in chicken of medicated drinking water to laying chickens. *Food Additives and Contaminants* 11: 641-647.
- Omoro, A. O., Arimi, S. M., Kang'ethe, E. K., McDermott, J. J. and Staal, S. J. (2002). Analysis of milk-borne public health risks in milk markets in Kenya. In: *Proceeding of the Annual Symposium of the Animal Production Society of Kenya*, 9 - 10 May, 2002, Kari-nahrs, Naivasha. 21 – 30pp.
- Oslon, J. C., and A. C. Sanders (1975). Penicillin in milk and milk products: some regulatory and public health considerations. *Journal of Milk Food Technology*. 38:630–633.
- Pecou, A. and Diserens, J. (2011). Strategies for detecting antibiotic residue in milk. In: *Guidance on the Application of Screening and Confirmatory Methods in Integrated Dairy Chain Management*. (Edited by SCAMAC – project group), 27 may 2011, Lyon, France. 1 – 17pp.
- Pena, A., Lino, C.M., Alonso, A., and Barceloa, D. (2007). Determination of Tetracycline Antibiotic Residues in Edible Swine Tissues by Liquid Chromatography with Spectrofluorometric Detection and Confirmation by Mass Spectrometry. *Journal of Agriculture and Food Chemistry* 55: 4873–4979.
- Ramirez, A., Gutiérrez, R., Diaz G., González, C., Pérez, N., Vega, S., Noa, M. (2003). High-performance thin-layer chromatography -bioautography for multiple antibiotic residues in cow's milk. *Journal of Chromotogram B Analyt Technol Biomedical Life Science*, 784 (2): 315322.

- Shitandi, A. (2004). Risk factors and control strategies of antibiotic residues in milk at farm level in Kenya. Thesis for Award of PhD Degree at the Swedish University of Agricultural Sciences, Uppsala, Sweden. 36pp.
- Shitandi, A., and A. Sternesjö. (2004). Factors contributing to the occurrence of antimicrobial drug residues in Kenyan milk. *J. Food Protection*. 67:399–402.
- Shitandi, A., and Sternesjö, A. (2001). Detection of Antimicrobial drug residues in Kenyan milk. *Journal of Food Safety*, 21: 205–214.
- Sundlof, S.F., 1994. Antimicrobial drug residues in food producing animals. In: J.F. Prescott, and J.D. Baggot (eds), *Antimicrobial Therapy in Veterinary Medicine*, 2nd edn, (Iowa State University Press, Ames, IA), 569–584
- Swai, E. S. and Schoonman, L. (2011). Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania. *Tanzania Journal of Tropical Biomedicine* 11: 217-222.
- Syit, A. D. (2011). Detection and determination of Oxytetracycline and penicillin antibiotic residue levels in Bovine bulk milk from Debrezeit and Nazareth dairy farms in Ethiopia. In: *Proceedings of the 1st International Technology, Education and Environment Conference African Society for Scientific Research*. (Edited by Human Resource Management Academic Research Society). May 2008, Addis Ababa, Ethiopia. 325 – 337pp.
- Thrusfield, M. (2005): *Veterinary epidemiology*. 2nd Edition, Oxford, Blackwell Science Ltd., Pp 182-198.

- USA Department of Agriculture, Food safety and inspection services, Office of public Health science: CLG-TET2.04 test method, (2011): Quantitative identification of Tetracyclines by HPLC Technique.
- Widayati, A., Suryawati, S., De Crespigny, C. and Hiller, J. E. (2012). Knowledge and beliefs about antibiotics among people in Yogyakarta City, Indonesia. *Antimicrobial Resistance and Infection Control* 1: 1 - 38.
- Zwald, A. P., L. Ruegg, J. B. Kaneene, L. D. Warnick, S. J. Wells, C. Fossler, and L. Halbert(2004). Management practices and reported antimicrobial usage on conventional and organic dairy herds. *J.Dairy Sci.* 87:191–201.

APPENDICES

Appendix 1: Semi-Structured Questionnaire for Small-Scale Dairy Farmers:

Assessment of availability of veterinary services, accessibility, handling practices and use of veterinary drugs in treatment of cattle diseases

Section one: Location

Ward

Date

Section two: Respondent characteristics

1. Respondent name (Option)

2. Sex of the respondents
 i) Male () ii) Female ()
3. What is your age?
 i) 15-35 years ()
 ii) 36- 55 years ()
 iii) Above 55 years ()
4. Marital status
 i) Single ()
 ii) Married ()
 iii) Divorced, Separated, Widowed()
5. What is your education level? **Tick only one**
 i) Informal education&Adult education ()
 ii) Primary school education ()
 iii) Secondary school education ()
 iv) College &University education ()
6. Rank the most important source of income for your household starting from number **1,2,3,4, and 5**. Number 1 meaning the most source followed by other numbers.
 i. Livestock keeping/Dairy farming ()
 ii. Crop farming ()
 iii. Farming and livestock keeping/Dairy farming ()
 iv. Business ()
 v. Paid employment ()

Section Three: General knowledge/information on access to veterinary services, handling/practices and use of veterinary drugs.

1. What is the commonest type of production system practiced (**Choose one**).
 - i) Communal grazing
 - ii) Zero grazing/Intensive ()
 - iii) Semi intensive grazing
 - iv) Extensive grazing system

2. How do you recognize sick animals? (clinical signs)
 - i)
 - ii)
 - iii)
 - iv)
 - v)

3. What are the common diseases affecting cattle in this area?
 - i) ECF
 - ii) Anaplasmosis
 - iii) Trypanosomiasis
 - iv) Mastitis
 - v) FMD
 - vi) Lumpy skin diseases
 - vii) CBPP
 - viii) Worms

4. What type of antibiotics are given to your dairy animals?(The research may ask/request to see if there are any empty bottle/pack)
 - i) Tetracyclines (CTC &OTC)
 - ii) Pen Streptomycin
 - iii) Sulphonamide
 - iv) Gentamycin
 - v) Others (specify)

5. Where do you normally obtain drugs for treating animal? **Choose only one**
 - i) Pharmacy
 - ii) Market e.g cattle market ()
 - iii) From livestock officer
 - iv) Any other (State).....

6. Who treat your animal when they get sick?
 - a) Conduct own treatment ()
 - b) Call livestock officer

c) Any other means (State).

.....

7. If own treatment do you read instruction before administration? **Put a tick (√)**
 i) Yes () ii) No ()

8. If own treatment, how do you quantify the amount to administer? **State in brief.**

.....

.....

9. If own treatment, how frequently do you use drugs per day? **Choose only one answer**

- a) Once per day
- b) Twice per day
- c) Three times per day ()
- d) Four times per day
- e) More than four times per day

10. If own treatment, what are the key issues/aspects that you consider before drug administration?

- i. Type of drugs to be administered 0) Yes 1) No
- ii. Proper dose for age and weight of animal 0) Yes 1) No
- iii. Expiry date of the drugs 0) Yes 1) No
- iv. Withdrawal period 0) Yes 1) No

11. At what period /time do you treat your animal? **Choose only one.**

- i) When they are sick
- ii) Continue for some day after recovery ()
- iii) At any time even if they are not sick

12. Do you sell milk immediately after last dose of cattle treatment?

- i) Yes.... ii) No.....

13. If the answer in question 12 is NO, why not selling milk immediately after last dose of treatment?

- i) Observe veterinary withdrawal period/time
- ii) Milk contains veterinary drug residues
- iii) Others (specify)

14. If the answer in question 12 is NO, how long do you start milking an animal that has been treated with antimicrobials? **Choose only one and put a tick (√)**

- a) Two days
- b) Four days

- c) Five days ()
- d) After seven days
- e) Stop from milking as per drug manufacturer's recommendations
- f) Other (specify)

15. Do you know the drug withdrawal period? (The researcher should clarify the term)

- i) Yes..... ii) No

16. If the answer is Yes in question 15, do you follow it? i) Yes.... ii) No.....

17. Is there any health effects if a person consume milk with antibiotic drug residues? i)Yes.... ii) No..... iii) I don't know

18. If answered Yes in question 17, list down the health effects you know which may be caused by antibiotic drug residues in milk.

- i) Allergic reactions to some sensitive individual
- ii) Bacterial resistance to antibiotics
- iii) Direct toxic effects
- iv) Others (specify).....

19. Have you received any training on how to handle veterinary drugs, administration and handling sick animals? **Put a tick (√)**

- a) Yes () b) No ()

20. Are the livestock services available at any time when needed? **Put a tick (√)**

- a) Yes () b) No ()

21. How do you store drugs used to treat your animals?

- i)
- ii)
- iii)

22. Is there any other way in which drugs uses to treat animals are used?

- i)
- ii)
- iii)
- iv)
- v)

23. Is there any problem associated with use of drugs to animals? State them

- i)
- ii)
- iii)
- iv)
- v)

24. How often do you drink milk in your family

- a) Every day
- b) Two times per week ()
- c) Four times per week
- d) Any other (Please State)

25. What is the amount of milk drunk per meal per person (approximate average)

- Put a tick (√)**
- a) About $\frac{1}{4}$ of a litre
 - b) About $\frac{1}{2}$ of a litre ()
 - c) About $\frac{1}{8}$ of a litre
 - d) More than $\frac{1}{2}$ a litre

26. Who take large share of the milk in a family **Choose only one and put a tick**

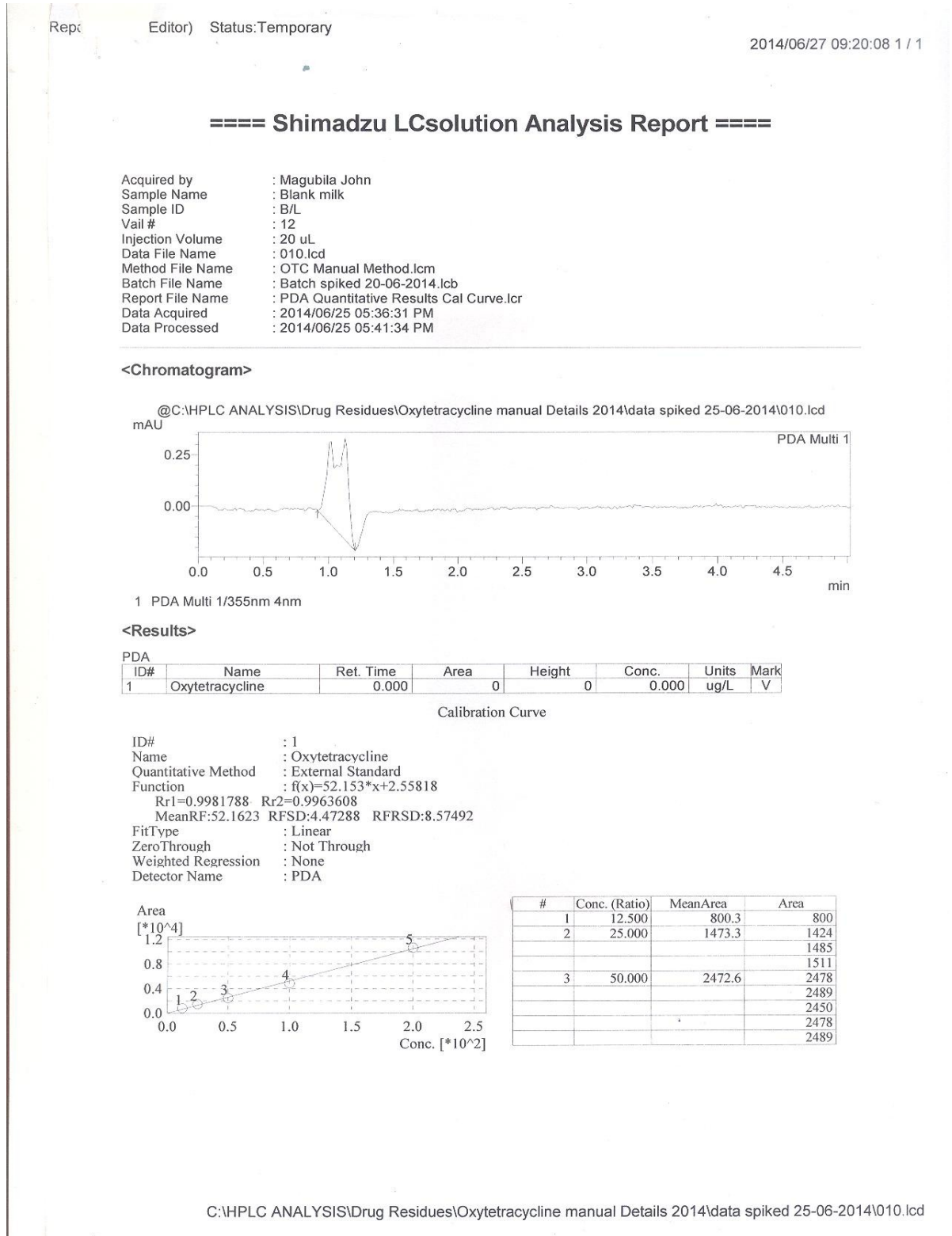
- (√)
- a) Children
 - b) Young women (15-25 years)
 - c) Young men (15-25 years)
 - d) Middle aged women (26-50 years)
 - e) Middle aged men (26-50 years)
 - f) Older women (above 50 years)
 - g) Older men (above 50 years)

27. Do you have any comment/suggestion in regard to antimicrobial usage? State

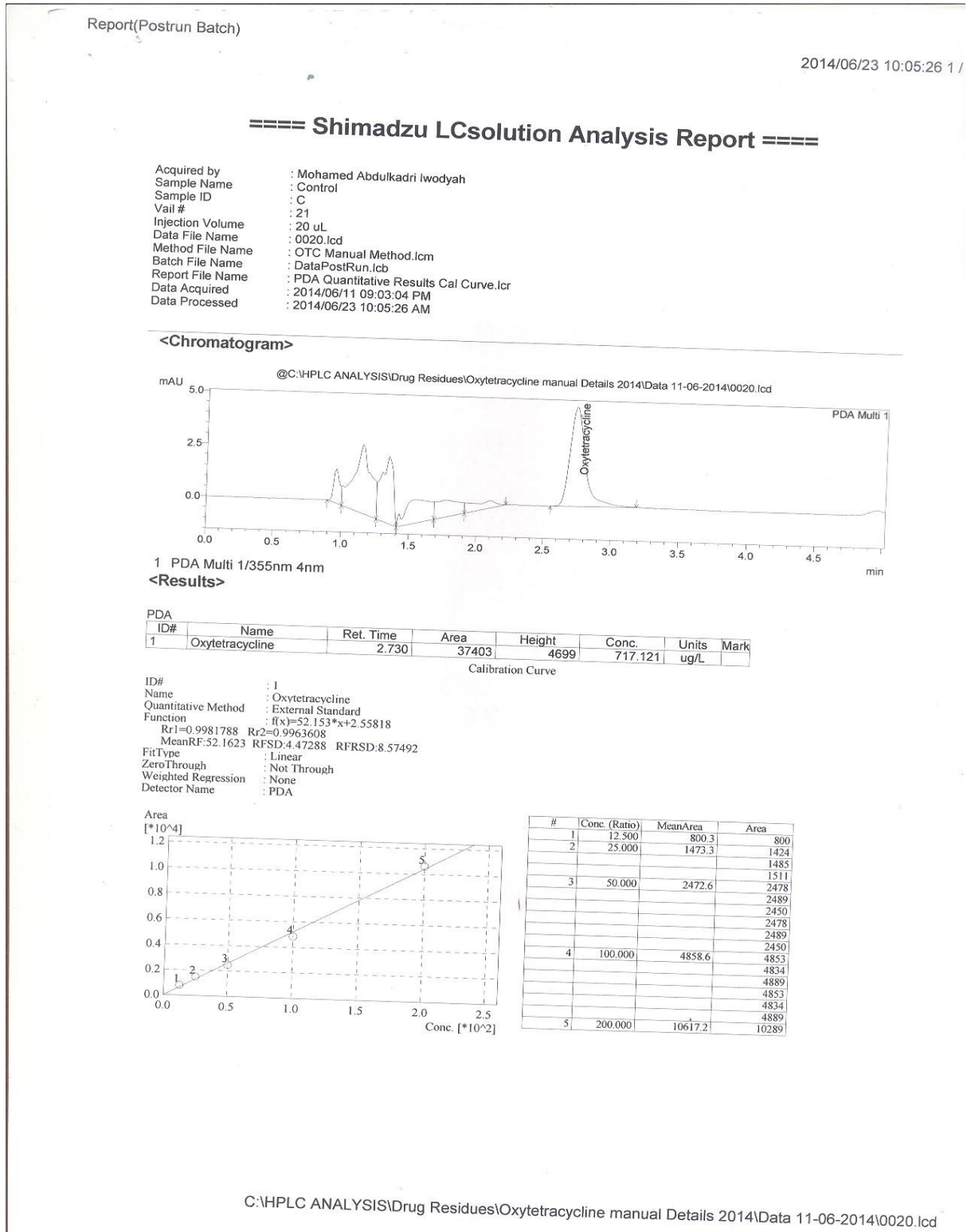
- i)
- ii)

Thank you very much for your Cooperation

Appendix 2: Chromatogram of OTC standard solution of Blank Spiked.



Appendix 3: Chromatogram of known control milk sample with OTC Residues



Appendix 4: Chromatogram of positive OTC Spiked at a concentration of 1000µg/l

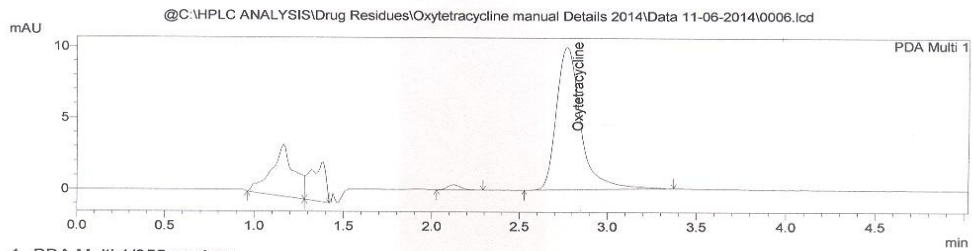
Report(Report Editor)

2014/06/12 16:34:02 1 / 1

==== Shimadzu LCsolution Analysis Report ====

Acquired by : Mohamed Abdulkadri Iwodyah
 Sample Name : Oxytetracycline Std
 Sample ID : 1000 ug/L
 Vial # : 7
 Injection Volume : 20 uL
 Data File Name : 0006.lcd
 Method File Name : OTC Manual Method.lcm
 Batch File Name : Batch 3.lcb
 Report File Name : PDA Quantitative Results Cal Curve.lcr
 Data Acquired : 2014/06/11 07:40:47 PM
 Data Processed : 2014/06/12 04:32:24 PM

<Chromatogram>

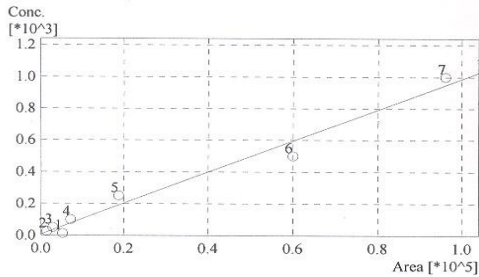


<Results>

ID#	Name	Ret. Time	Area	Height	Conc.	Units	Mark
1	Oxytetracycline	2.765	96091	10007	950.356	ug/L	

Calibration Curve

ID# : 1
 Name : Oxytetracycline
 Quantitative Method : External Standard
 Function : f(x)=0.00980415*x+8.26512
 Rr1=0.9885426 Rr2=0.9772165
 MeanRF:0.0118042 RFSD:0.00527763 RFRSD:44.7099
 FitType : Linear
 Zero Through : Not Through
 Weighted Regression : None
 Detector Name : PDA



#	Conc. (Ratio)	MeanArea	Area
1	12.500	5271.7	5272
2	25.000	1467.9	1468
3	50.000	2889.2	2889
4	100.000	7208.1	7208
5	250.000	18784.2	18784
6	500.000	60007.0	60007
7	1000.000	96091.0	96091

Appendix 5: Chromatogram of OTC Standard solution at a concentration of 200µg/l

