TETRACYCLINE RESIDUES IN RAW AND COOKED CHICKEN EGGS IN TANZANIA: A CASE STUDY OF DAR ES SALAAM

JOHN CLAUDE MOSHA

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD QUALITY AND SAFETY ASSURANCE OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2017

EXTENDED ABSTRACT

Tetracyclines (TCs) are used in poultry production for prevention and treatment of diseases. The misuse of this drug or poor observance of drugs withdrawal period, may result into unacceptable residue levels in poultry products. Presence of tetracycline residues, just like other antimicrobial drugs, in poultry products, such as, eggs is a concern to the public health. In order to determine tetracycline residues, a total of 291 eggs randomly collected, 3 eggs from 97 layer chicken farms in Dar-es-Salaam, were analyzed for concentration of oxytetracycline (OTC), tetracycline (TTC) and chlorotetracycline (CTC) residues. One raw, boiled and fried egg were analyzed for each parameter. Extraction of TC residues in eggs was done using liquid-liquid extraction in MacIlvaine buffer (pH 4.0) – EDTA, 0.34M Sulphuric acid and 7% sodium tungstate. The quantification of residues was achieved by high performance liquid chromatography (HPLC) with UV detector at 365 nm and C8 column (25cm x 4.6mm x12µm), and the mobile phase was composed of 0.005M oxalic acid and pure acetonitrile. The results indicated that all 97 raw egg samples contained TC residues, 100% OTC residues, 53.6% TTC residues and, 16.5% CTC residues. Out of 97 boiled egg samples, 75.3% had OTC residue, 46.4% had TTC residues and 9.3% had CTC residues. Out of 97 fried egg samples, 96.9% had OTC residue, 15.5% had TTC residue and 7.22 had CTC residue. About 80.4% of raw egg samples contained OTC residue and 2.1% CTC residue levels above the Maximum Residue Limit (MRL), whereas 7.2% of the boiled egg samples contained OTC residue, 2.01% TTC residue and 2.1% CTC residue levels above MRLs and out of the fried egg samples 45.4% contained OTC and 1% CTC levels above MRLs. These findings suggest that commercial egg consumers in Dar-es-salaam are at risk of exposure to tetracycline residues. The major reasons for presence of drug residues in poultry products in Dar-es-salaam is failure to observe drug withdrawal periods, the lack

of understanding of the effects of antimicrobial residues on human consumers and absence of routine programs for monitoring antimicrobial residues in poultry products or in food of animal origin as had been reported by other studies in Tanzania. Furthermore, lack of regulations related to MRLs monitoring programs to control the use of antimicrobials in poultry production, contributed to the high use of antimicrobial drugs.

DECLARATION

I, John Claude Mosha 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 for a degree award.

_____________________________ ____________________

John Claude Mosha Date

(MSc. Food Quality and Safety Assurance)

The above declaration is confirmed by;

_____________________________ ____________________

Prof. Jovin K. Mugula Date

(Supervisor)

COPYRIGHT

ACKNOWLEDGEMENTS

I would like to express my appreciation and the deepest gratitude to my Lord God and Savior, The Father, The Son and The Holy Spirit in the name of Christ Jesus for leading and enabling me to be able to undertake this course and the study to the final stage successfully.

I would like to thank my supervisor, Prof. Jovin K. Mugula, of the Department of Food Technology, Nutrition and Consumer Science, Sokoine University of Agriculture for his guidance from proposal development up to the conclusion of this work. I would like to thank Sokoine University of Agriculture staff, particularly those in the Department of Food Technology, Nutrition and Consumer Science for their cooperation.

I am grateful to the government of the United Republic of Tanzania, especially the Dar es Salaam Regional Commissioner, District Commissioners for all Municipal councils in Dar es Salaam region, and all other local government officials for their support and permission to undertake this study. I would like to thank the Director General of Tanzania Food and Drugs Authority (TFDA) for the sponsorship and permission to pursue the postgraduate studies and access to the TFDA laboratory facilities for sample analyses. I would like to thank the Acting Director of TFDA Laboratory Services, Dr. Danstan Hipolite Shewiyo, the manager of Department of Food Analysis Mr. Rajab Mziray, and all laboratory personnel at TFDA, particularly Mr. Ezekiel P. Mubito, Mr. John Magubila, Mr. Saning'o E. Lenoi, Mr. Oscar B. Kandege, Mr. Adam H. Delloh and Mrs. Neema Mwakalinga for their assistance in the laboratory.

Finally, I would like to thank my wonderful family, my wife; Mariamu M. Msangi, my children; Brian, Irene and Ian for their encouragement and prayers during this period.

DEDICATION

This work is dedicated firstly and foremost to JEHOVAH, my Lord God in Jesus Christ name, then to my wife Mariamu M. Msangi and our beloved children Brian, Irene and Ian John Shara whose support, prayers and perseverance enabled me to conduct and complete this study, to my father, Dr. Claude J.S. Mosha, and my mother Mrs. Rehema Mpangule and Evarista C. Mosha, who have always been my source of inspiration and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BsDA	*Bacillus stearothermophillus* Disc Assay
CAC	Codex Alimentarius Commission
CTC	Chlorotetracycline
EDQM	European Directorate for the Quality of Medicines & HealthCare
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme Linked Immuno-Sorbent Assay
FAO	Food and Agriculture Organization
FPT	Four Plate Test
GARP	Global Antibiotic Resistance Partnership
HPLC	High Performance Liquid Chromatography
JETACAR	Joint Expert Technical Advisory Committee on Antibiotic Resistance
MRL	Maximum Residue Limit
OTC	Oxytetracycline
SD	Standard Deviation
SPE	Solid Phase Extraction
TC	Tetracyclines
TFDA	Tanzania Food and Drugs Authority
TTC	Tetracycline
USA	United States of America
UV	Ultra-Violet rays

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Poultry farming in Tanzania is developing due to increased demand of poultry meat and eggs for protein supplementation (Nonga *et al.*, 2010) consisting of traditional and commercial production system (Goromela *et al.*, 2007). Indigenous chickens contribute to almost 100% of eggs consumed in rural area and 20% of eggs consumed in urban area, while commercial layer chickens contribute to more than 80% of all eggs consumed in urban and peri-urban areas (Ministry of Livestock and Fisheries Development, 2016). Commercial layer production is concentrated in Dar-es-salaam (28%) with a consistent growth rate of 12% per year (The GARP-Tanzania, 2015).

Intensive poultry keeping at high densities of chicken population within the available facilities has potential influence on disease eruption (Mubito *et al.*, 2014). The common chicken diseases found on the study conducted at Morogoro region of Tanzania, were fowl typhoid (85%), infectious bursa disease (Gumboro) (65%) infectious coryza (65%), collibacilosis (55%), coccidiosis (54%), Newcastle disease (50%), helminthiasis (20%) and fowl pox (15%) *(*Nonga *et al.*, 2010). Most intensively farmed food-producing animals including chickens (broilers and layers) often use antimicrobial drugs to herd/flock in feed or water either for improving growth, treatment of diseases or as prophylaxis against threatening infections (JETAC AR, 1998; Nonga *et al.*, 2010). Antimicrobial usage in poultry industry improves well-being and health of birds by reducing or preventing disease outbreak. This usage is of great economic importance since it ensures better production (Mubito *et al.*, 2014; Lohren *et al.*, 2009). The study conducted in Morogoro showed that, the most commonly used antimicrobial drugs include oxytetracycline (75%), amprolium (35%), sulfamethoxypyridazine (35%), sulfanilamide (25%), and chlorotetracycline (10%) (Nonga *et al*., 2010; Katakweba, 2014) and in Dar es Salaam tetracyclines (32.2%) and sulfonamides (20.8%) (Mubito *et al*., 2014).

Improper applications of antibiotics in layer chickens deposit noticeable residues in eggs, particularly when the eggs are harvested and marketed within the withdrawal period of the drug (Nonga *et al.*, 2010; Kan and Petz, 2000). In Tanzania antimicrobial drug residues have been reported in foods of animal origin, in chicken eggs (Mubito *et al*., 2014), in milk (Karimuribo *et al*., 2005; Kurwijila *et al*., 2006), and in beef (Mmbando, 2004).

Heat generated during ordinary cooking procedures can degrade a number of antibacterial drug residues, depending on time and heat treatment involved. Tetracycline (TC) residues are considered relatively unstable compounds. Temperature during cooking had large impact on the loss of antimicrobial drug residues in chicken breast and thigh (Abou-Raya *et al*., 2013; Heshmati, 2015).

1.2 Study Justification

In Tanzania antimicrobials make 85% of the drugs commonly used in animals and there has been excessive prescription, abuse and/or misuse of antimicrobial drugs in intensive layer farms causing pronounced residues in chicken eggs. Tetracyclines rank high among the antimicrobial substances most frequently used in the animal food production (Nonga *et al*., 2010; Mubito *et al*., 2014) and of all antibiotic-associated residues. The occurrence of antibiotic residues in commercial chicken eggs was determined in Morogoro municipality whereby all eggs examined tested positive for antibiotic residues (Darwish *et al*., 2013). The legislation inadequacy, unawareness and ignorance or non-compliance of farmers on the antimicrobial withdraw period have probably contributed to the high rates of antimicrobial residues reported in poultry products (Katabweka, 2014; Mubito *et al*., 2014).

From the fact that, poultry products especially eggs are widely used by the public, there is a high risk of the public to contract health problems such as antibiotic resistance, allergic reactions, autoimmunity, carcinogenicity, mutagenicity and bone marrow toxicity (Pavlov *et al*., 2008: Nisha, 2008) relating to the consumption of eggs containing levels of antimicrobial drug residue and cause its accumulation in the body.

The presence of drug residues at levels above Maximum Residue Limits (MRLs) in foods of animal origin including chicken eggs is one of the most important issues in food safety because of its public health implications. Antibiotic resistance is a global public health concern today. The U.S. Centers for Disease Control and Prevention has described resistance as one of the world's most pressing health problems, because the number of bacteria resistant to antibiotics has increased in the last decade and many bacterial infections are becoming resistant to the most commonly prescribed treatment. The WHO has identified antibiotic resistance as one of the three greatest threats to human health (Swatantra *et al*., 2014).

The Codex Alimentarius Commission (CAC) of the United Nations has stated that the scientific literatures about influence of processing on drug veterinary residues in food are inadequate. Therefore, additional studies are needed in this area because veterinary drug residues vary in their susceptibility to degrade by heating (CAC, 2001; Heshmati, 2015).

Dar-es-salaam is the largest producer of commercial chicken products in Tanzania (Msami, 2008); therefore there is a need to study the prevalence of different antimicrobial drug residues in chicken products. Although tetracycline is one of the highly used antimicrobial drugs in poultry business, up to date only information on sulfonamides residues in eggs is available. To fill the gap, this study was carried out to assess the prevalence, quantity of TCs residues and the effect of heat during cooking on the residue concentration in chicken eggs.

The findings of this study will add to baseline information on the existing data on the prevalence and levels of TCs residues in eggs and the impact of heat on TCs residues during cooking. Also, the study will provide further evidence for the importance of setting and enforcing legislations for management of antimicrobial drugs usage to reduce excessive application of veterinary drugs in animal and poultry production and hence addressing the problem of antibiotic resistance and prevalence of TC residues in foods of animal origin.

1.3 Objectives

1.3.1 Overall objective

The overall objective of this study was to quantify TC residues in commercial layer chicken eggs and determine the effect of thermal processing on concentration of TC residues.

1.3.2 Specific objectives

The specific objectives of the study were to:

i. detect and quantify TC residues in commercial chicken eggs,
ii. determine the percentage of eggs with TC residues above MRLs, and
iii. determine effect of thermal processing on concentration of tetracycline residues.

1.3.3 List of manuscripts

i. Tetracycline residues in commercial layer chicken raw eggs,
ii. Tetracycline residues in commercial layer chicken cooked eggs, and
iii. Levels of exposure to tetracyclines and safety of consumers.

1.4 References

Abou-Raya, S., Shalaby, A. R., Salama1, N. A., Emam, W. H. and Mehaya, F. M. (2013). Effect of ordinary cooking procedures on tetracycline residues in chicken meat. *Journal of Food and Drug Analysis* 21: 80-86.

Codex Alimentarius Commission, (2001). Committee on residues of veterinary drugs in foods, document control of veterinary drug residues in milk and milk products. Joint Food and Agriculture Organization of the United Nations – World Health Organization Food Standards Programme, Rome.

Darwish, W. S., Eldaly, E. A., El-abbasy, M. T. and Ikenaka, Y. (2013). Antibiotic residues in food the african scenario.pdf. *Japanese Journal of Veterinary Research 61 (Supplement): S13 - S22*.

Goromela, E., Kwakkel, R., Verstegen, M. and Katule, A. (2007). Identification, characterization and composition of scavengeable feed resources for rural poultry production in Central Tanzania. *African Journal of Agricultural Research* 2 (8): 380- 393.

Heshmati, A. (2015). Impact of cooking procedures on antibacterial Drug Residues in Foods : A Review. *Journal of Food Quality and Hazards Control* 2: 33–37.

Joint Expert Advisory Committee on Antibiotic Resistance - JETAC AR. (1998). *The Use of Antibiotics in Food-producing Animals :* Antibiotic - Resistant Bacteria in Animals and Humans. 249pp.

Karimuribo, E. D., Mdegela, R. H., Kusiluka, L. J. M. and Kambarage, D. M. (2005). Assessment of drug usage and antimicrobial residues in milk from smallholder farms in Morogoro, Tanzania. *Bulletin of* Animal *Health and* Production *in* Africa, 53: 234–241.

Katakweba, A. A. S. (2014). Prevalence and molecular studies of antimicrobial resistance in bacteria from farm animals, wildlife, pets and human in Tanzania. *A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy of Sokoine University of Agriculture. Morogoro, Tanzania*. pp1–243.

Kurwijila, L. R., Omore, A., Staal, S. and Mdoe, N. S. Y. (2006). Investigation of the risk of exposure to antimicrobial residues present in marketed milk in Tanzania. *Journal of Food Protection* 69: 2487–2492.

Mmbando, L. M. G. (2004). Investigation of oxytetracycline use and abuse: Determination of its residues in meat consumed in Dodoma and Morogoro Municipality, M.Sc. dissertation, Depertment. of Veterinary Physiology, Pharmacology, Biochemistry and Toxicology, Sokoine University of Agriculture.

Msami, H. (2008). Poultry sector country review. Central Veterinary Laboratory Dar es Salaam, Tanzania. 61pp.

Mubito, E. P., Shahada, F., Kimanya, M. E. and Buza, J. J. (2014). Antimicrobial use in the poultry industry in Dar-es-Salaam, Tanzania and public health implications. *American Journal of Research Communication 2*(4): 51–63.

Nonga, H. E., Simon, C., Karimuribo, E. D. and Mdegela, R. H. (2010). Assessment of antimicrobial usage and residues in commercial chicken eggs from small-holder poultry keepers in Morogoro municipality, Tanzania. *Zoonoses and Public Health* 57(5): 339–344.

Swatantra, S., Sanjay, S., Neelam, T., Nitesh, K. and Ritu, P. (2014). Antibiotic residues: A global challenge. *Pharma Science Monitor; An International Journal of Pharmaceutical Sciences* 5(3): 184–197.

The GARP-Tanzania (2015). Situation analysis and recommendations: antibiotic use and resistance in Tanzania. *Washington, DC and Nwe Delhi: Center for Disease Dynamics, Economics & Policy* 118pp.

United Republic of Tanzania, (2016). Ministry of Livestock and Fisheries Development. *Budget Speech 2016-17*. 153pp.

CHAPTER TWO

Tetracycline Residues in Commercial Layer Chicken Raw Eggs in Tanzania, a Case Study of Dar es Salaam

John C. Mosha[1, 2], Jovin K. Mugula[1]

[1]Department of Food Technology, Nutrition and Consumer Science, Sokoine University of Agriculture, P.O Box 3006, Morogoro, Tanzania

[2]Tanzania Food and Drug Authority, P.O Box 77150, Dar es Salaam, Tanzania

2.1 Abstract

This study was conducted to quantify tetracycline residue levels in commercial layer chicken eggs collected from farms in Dar es Salaam. Three members of tetracycline group; oxytetracycline (OTC), tetracycline (TTC) and chlorotetracycline (CTC) were quantified in 97 eggs using HPLC. The tetracycline residues: OTC, TTC and CTC were detected in 100, 53.6 and 16.5% of the samples, respectively. The mean levels of OTC, TTC and CTC were: 719.9, 64.4, and 30.8 μg/kg, respectively. The mean level of OTC residue in eggs was above the maximum residue limits for Codex Alimentarius Commission. Therefore, consumers of commercial eggs in Dar es Salaam are at risk of potential health hazard associated with consumption of tetracycline residues especially OTC.

Key words: Commercial layer farms, Antibiotic Residues, Oxytetracycline, Tetracycline, Chlorotetracycline.

2.2 Introduction

Dar es Salaam is Tanzania's most important city for both business and government, with high concentrations of trade and other services and manufacturing compared to other parts of Tanzania. The city experiences tropical climatic conditions, typified by hot and humid weather throughout much of the year with both tropical wet and dry climate. Annual rainfall is approximately 1,100 mm, and in a normal year there are two rainy seasons: "the long rains" in March and May and "the short rains" in November and December.

Worldwide national and international public health agencies have a deep concern about the presence of antibiotic residues in meat, egg and edible viscera of food-producing animal (Ašperger *et al*., 2009; Mahmoudi *et al*., 2014). Antibiotics are substances produced either naturally by living organisms or synthetically in the laboratory, and that are able to kill or inhibit the growth of microorganisms (Darwish *et al*., 2013).

Commercial layer chicken industry in Tanzania faces several constraints, whereby birds are raised in conditions with high level of stress, diseases and poor nutrition (Nonga *et al*., 2010). To overcome these problems, farmers use antimicrobials to enhance growth by using antimicrobial growth promoters (Swatantra *et al*., 2014; Seri, 2013); improve feed efficiency; and to treat and to prevent bacterial infections (Sirdar, 2012). Studies conducted in Dar es Salaam and Morogoro indicated that TCs were the most commonly used antimicrobials (Mubito *et al*., 2014; Nonga *et al*., 2010).

Tetracyclines are highly preferred due to their antimicrobial action on wide range of Gram-positive and Gram-negative bacteria, as well as mycoplasmas, chlamydiae, rickettsia, spirochetes, actinomycetes, and some protozoa (Abdel-mohsein *et al*., 2015) and are easily available at affordable price as single parent drug or in combination with other antibiotic agents, vitamins and minerals (Mubito *et al*., 2014). A chemical residue is either the parent compound or metabolite of the parent compound that may accumulate, deposit, or otherwise be stored within the cells, tissues, organs or edible products of an animal following its improper use to prevent, control or treat animal disease, or to enhance production (Seri, 2013).

In Tanzania, legislations regarding antibiotic drug application in farm animals as well as monitoring and control of their residues are not adequately enforced (Nonga *et al.*, 2009). The inadequacy has probably led to the reported high rates of antimicrobial residues in poultry products. For example, 100% of screened eggs were positive for antimicrobial residues in a study conducted in Morogoro, (Nonga *et al.*, 2010). A quantitative study conducted in Dar es Salaam, indicated that 100% of eggs contained sulphonamide residues (Mubito *et al*., 2014). In addition, most of studies conducted based only on qualitative analysis of antimicrobial residues but not the quantitative analysis.

To address this gap, this quantitative study was conducted to estimate tetracycline residues (tetracycline, oxytetracycline, and chlorotetracycline) in raw eggs collected from commercial layer farms in Dar es Salaam by using the HPLC techniques.

2.3 Materials and Methods

The study was conducted in Dar es Salaam City. Dar es Salaam is the largest city and economic capital of Tanzania, located at 6°48' South, 39°17' East in a quiet bay off the Indian Ocean coast. The City has five municipal councils namely; Ubungo, Ilala, Kinondoni, Temeke and Kigamboni.

2.3.1 Samples

Eggs were collected from commercial chicken layer farmers randomly selected by proportion from the list provided by the respective municipality extension office. A total of 97 farmers from Ilala (38), Temeke (19), Ubungo (18), Kinondoni (13) and Kigamboni (9) municipality were selected. Selection of farmers considered their consent to participate in the study as well as the possession of commercial layers at laying period.

A total of 291 egg samples, 3 eggs from the same flock/batch was collected from 97 commercial layer farmers and assigned the same sample identification number. All randomly selected farmers who consented to participate in the study and had layers chicken at laying period were contacted on the day of sampling. The samples were collected from the farms by the project assistants accompanied with the local government officers.

2.3.2 Quantification of tetracyclines

Tetracycline residues in eggs were extracted and determined using HPLC according to the procedure described by Al-Wabel (2011) with slight modification by introducing sulphuric acid and sodium tungstate to improve protein precipitation.

2.3.2.1 Preparation of standard solutions

Stock standard solutions (2.5 mg/mL) of each tetracycline (OTC, TC and CTC) (EDQM, F-67081 Strasbourg (France) were prepared by transferring 250 mg of each tetracycline into separate weighing dishes then transferred into separate 100 mL volumetric flask with methanol (Carlo Erba Reagents S.A.S, Val de Renil, France), mixed until dissolved the diluted to volume. The stock standards were stable for six months at -10^{o}C to -20^{o}C. Mixed intermediate standard (125ug/ml) was prepared by pipetting 5.0 mL of each tetracycline stock solution to a 100 mL volumetric flask, mixed and diluted to volume with methanol. Intermediate standard solution is stable for six months at at -10^{o}C to -20^{o}C. Thereafter, mixed working standard solution (25 µg/mL) were prepared by pipetting 2 mL of 125 µg/mL mixed intermediate standard solution to a 10 mL volumetric flask and diluted to volume with methanol. Mixed working standard was stable for one week at -10^{o}C to -20^{o}C. Finally, HPLC standard (500 µg/L) was prepared daily by mixing 100 µL of mixed working standard (25 µg/mL), 400 µL methanol, 500 µL MOX (Methanolic oxalic acid (Loba Chemie Pvt. Ltd, Mumbai 400005, India) 0.01M) solution and 1000 µL distilled water (Barloworld Scientific Ltd, Stone, Staffordshire, ST15 0SA, UK); then vortexed before transferred to syringe and filtered into HPLC vials.

2.3.2.2 Sample preparation

One raw egg from each farm was hand mixed and then homogenized by using T18 Digital Ultra Turrax Homogenizer (IKA brand from Germany). 3g of homogenized raw egg sample was transferred into polypropylene centrifuge tube read for extraction.

2.3.2.3 Extraction of tetracycline residues

2.3.2.3.1 Liquid-liquid extraction

The McIlvaine buffer (20 mL, pH 4.0) - EDTA (S.D. Fine – Chem Ltd, Mumbai 400025, India) solution (1.625 L of McIlvaine buffer (mixture of 625 mL of 28.4 g of anhydrous disodium hydrogen phosphate (Loba Chemie Pvt. Ltd, Mumbai 400005, India) in 1L of ultra-pure distilled water + 1L of 21.0 g of citric acid monohydrate (Sigma-Aldrich, Saint Louis, Missouri 63103, USA) in 1L of distilled water) and 60.5 g of EDTA) was added into the polypropylene centrifuge tube containing 3g of homogenized raw samples, capped and shaken for 10 min on flat-bed shaker at speed of 300 rpm. Centrifuge tubes were uncapped and 1mL of 0.34M sulphuric acid (Loba Chemie Pvt. Ltd, Mumbai 400005, India) and 7mL of 7% sodium tungstate (VWR International ltd, Poole, BH15 1TD, England) were added, capped and gently hand-shaken for 7 seconds, centrifuged at 4000 rpm for 25-30 min at 15^{o}C. The supernatants from the extraction were filtered by side arm flask under vacuum through Whatman No. 2 filter paper pre-wetted with McIlvaine buffer-EDTA solution in Büchner funnel.

2.3.2.3.2 Solid phase extraction

Filtrates were subjected to solid phase extraction Oasis Max Cartridge (Waters Corporation Milford, Massachusetts USA) attached to an SPE vacuum manifold capable of processing up to 20 solid phase extraction cartridges (SPE cartridges were not allowed to dry from pre-wash, sample addition and sample elution). Each cartridge was conditioned with 20 mL of methanol followed by 20 mL of ultra-pure distilled water. Test extract was loaded to SPE cartridge and vacuumed at rate of 5 mL/min. Subsequently, the elution took place with 6 mL of methanolic acid into 15 mL test tubes as eluate collection vessel. When the flow stopped, vacuum was increased to remove residual solvent from the cartridge. Then tubes were removed from the vacuum manifold and vortexed. These tubes containing the methanolic eluate were placed in a Nitrogen-Evaporator with a water bath temperature of 40-50^{o}C reduced to 0.5 – 1 mL under a stream of dry nitrogen. The final eluate was adjusted to 1 mL with methanol, vortexed and then diluted to 2 mL with distilled water and vortexed again. The final extract (0.5 – 1mL) was poured into a 3 mL syringe and filtered through acrodisc filter into HPLC auto-sampler vial.

2.3.2.4 High performance liquid chromatography

Extracts were chromatographed on a Shimadzu LC-20AT (Kyoto, Japan) HPLC system quipped with a Shimadzu DGU-20A$_5$ degasser, Shimadzu SPD-M20A Diode Array

Detector, Shimadzu CBM-20A Communication System and Shimadzu LC Solution Software. The type of column used was Thermo-Scientific, BDS Hypersil C8 (125 x 4) mm, Partial size 5μ, Part No. 28205-124030, SN: 10058200. Mobile phase solutions used were 0.005 M Oxalic acid (Solvent A) and pure Acetronitrile HPLC Grade (Solvent B) (Carlo Erba Reagents, Chaussee du Vexin, France). The volume (100 µL) of tetracyclines was injected under low pressure gradient mode of 75% A and 25% B and detected at wavelength (λ) of 365 nm with flow rate of 0.8 mL/min. The column temperature was kept at 30 – 45°C.

2.3.2.5 Calibration linearity

The HPLC response was linear for OTC (r^2 = 0.967), TTC (r^2 = 0.973) and CTC (r^2 = 0.998) in the range of 100 to 2000 µg/kg with correlation coefficient (r) equals to 0.983, 0.986 and 0.999 respectively. The retention time for OTC, TTC, and CTC was found to be 3.109, 3.732 and 6.641 min, respectively, under the described method.

2.3.3 Recovery of tetracyclines in egg sample

The mixed working standard was added to 3g whole-homogenized egg from which the concentrations of 200, 300, 400, 500 and 600 µg/kg were prepared, to meet the MRLs of Codex Almentarius Commission standard (CAC/MRL2-2015) and then vortexed to ensure proper mixing of the standard solution. It was extracted using the method described in section 2.3.2.3 and applied to the HPLC system (Table 2.1).

Table 2.1: Recovery of oxytetracycline, tetracycline and chlorotetracycline in spiked samples

Spiked Concentrations (µg/kg)	Average recovery (%)		
	Oxytetracycline	Tetracycline	Chlorotetracycline
200	69	70	71
300	77	87	83
400	73	98	85
500	70	61	69
600	67	79	76

2.3.4 Statistical analysis

The statistical comparison of data was performed by one-way analysis of variance (ANOVA) using R-Commander software version 3.3.0 © 2009-2014 R Studio to reveal significant differences among the samples studied. Determination of significance of differences among residues was obtained by Duncan multiple range tests. Residue levels with P-values less than 0.05 were considered significant.

2.4 Results and Discussion

2.4.1 Results

A total of 97 eggs samples collected from commercial layers production farms in Dar-es-salaam were analyzed for oxytetracycline (OTC), tetracycline (TTC) and chlorotetracycline (CTC) residues by using the HPLC system. The quantitative results indicated that all analyzed samples had detectable levels of tetracycline residues such that 100% of the samples had detectable levels of OTC, 53.6% had detectable levels of TTC and 16.5% had detectable levels of CTC residues. Oxytetracycline, tetracycline and chlorotetracycline residue concentrations in raw eggs ranged from 3.23 – 1655 µg/kg (mean, 719.90 µg/kg), 0.0 – 255 µg/kg (mean, 64.35 µg/kg) and 0.0 – 1176 µg/kg (mean, 30.79 µg/kg), respectively. The prevalence and mean levels of OTC, TTC and CTC residues are indicated in Table 2.2.

Table 2.2: Prevalence and levels of oxytetracycline, tetracycline and chlorotetracycline residues in raw eggs

Residues	Overall range (µg/kg)	Mean levels (µg/kg)	Prevalence (%)
Oxytetracycline	3.23 – 1655	719.90 ± 373.30	100
Tetracycline	0.0 – 255	64.35 ± 79.08	53.6
Chlorotetracycline	0.0 – 1176	30.79 ± 144.99	16.5

2.4.2 Discussion

Antimicrobial classes used to treat poultry are similar to those used in human medicine and they include aminoglycosides, tetracyclines, beta-lactams, quinolones, macrolides, polypeptides, amphenicols and sulphonamides (Stolker and Brinkman 2005). Tetracycline compounds are increasingly being used in farm animal production as growth promoters and for treatments of diseases.

The findings of this study indicated that the majority of the commercial chicken raw eggs sold for human consumption in Dar es Salaam city, Tanzania, contained antimicrobial residues. Most of the samples analyzed had detectable levels of tetracycline residues. The observed high prevalence of residues raised the public health concern due to the potential health effects which can be experienced by the consumer. Similarly, higher antimicrobial residues in eggs have been reported in Saudi Arabia, Solvenia and Trinidad (Al-Ghamdi *et al*., 2000; Cerkvenik, 2002; Adesiyuni *et al*., 2005). Antimicrobial drug residues in foods of animal origin in Tanzania have been reported in milk (Karimuribo *et al*., 2005; Kurwijila *et al*., 2006), and in beef (Mmbando, 2004). Tetracycline residues levels in animal products depend on the initial dosage, the duration between the drug administration and animal product collection, the extent of drug use control and drug residue monitoring (Nonga *et al,* 2010; Abbasi *et al*., 2012). In Tanzania, legislation regarding drug use and veterinary drug residue control is lacking and basic facilities for determination of residues are also unavailable at the level of farms and markets (Nonga *et al.,* 2010).

Also the study revealed that, oxytetracycline (OTC, 100%) is the most used antimicrobial drug among the tetracycline group of antibiotics followed by tetracycline (TTC, 54%) and chlorotetracycline (CTC, 17%). Similar results were observed in the study conducted in Morogoro municipality (Nonga *et al*., 2010) oxtetracycline was the most used antimicrobial drug by 75%. This was mainly due to the poorly regulated supply of antibiotics, low knowledge on public health effects, poor attitudes and wrong practices of poultry farmers (Mubito *et al*., 2014).

These findings clearly indicate that consumers in Dar es Salaam are probably at high risk of contracting potential health effects and urgent attention is needed. As of year 2017, in Dar es Salaam, there were no routine programs for monitoring antimicrobial residues in poultry products or in animal derived food. Furthermore, the lack of knowledge on the effects of antimicrobial residues on human consumers and the lack regulations related to MRLs contributed to the high use of antimicrobial drugs.

2.5 Conclusion

In conclusion, the commercial raw eggs distributed into the markets of Dar es Salaam were found to contain high levels of tetracycline residues. Despite the fact that eggs are consumed cooked, and thermal processing stated to reduce residue levels, its high levels in raw eggs still poses health risks to the commercial egg consumers. Therefore, the control of veterinary antibiotics used to ensure safer animal food products is needed in developing countries to minimize the initial levels of residues in commercial eggs. Observation of drug withdrawal periods and extension programs for farmers will be highly beneficial to reduce levels of residues in products of animal origin including chicken eggs (Nonga *et al.*, 2010).

Acknowledgements

I am grateful for the financial support provided by the Tanzania Food and Drugs Authority.

2.6 References

Abbasi, M. M., Babaei, H., Ansarin, M., Nourdadgar, A. and Nemati, M. (2012). Phase extraction and simultaneous determination of tetracycline residues in edible cattle tissues using an HPLC-FL Method. *Iranian Journal of Pharmaceutical Research* 11(3): 781-787.

Abdel-mohsein, H. S., Mahmoud, M. A. M. and Ibrahim, A. (2015). Tetracycline residues in intensive broiler farms in upper egypt: Hazards and risks. *Journal of World's Poultry Research* 5(3): 48–58.

Adesiyuni, A., Offish, N., Lashley, V., Seepersadsingh N., Rodrigo S., and Georges, K. (2005). Prevalence of antimicrobial residues in table eggs in Trinidad. *Journal of Food Protection* 68: 1501–1505.

Al-Ghamdi, M. S., Al-Mustafa, Z. H., Al-Faky, A., El-Morsy, I. H. and Essan, H. (2000). Residues of tetracycline compounds in poultry products in eastern province of Saudi Arabia. *Journal of Public Health* 114: 300–304.

Al-Wabel, N. A. (2011) Monitoring of tetracycline residues in table eggs collected from Qassim region, Kingdom of Saudi Arabia. *Journal of Agriculture and Veterinary Science*, 4(2): 109–123.

Ašperger, D., Babić, S., Pavlović, D. M., Dolar, D., Košutić, K., Horvat, A.J. and Kaštelan-Macan M. (2009). SPE-HPLC/DAD determination of trimethoprim, oxytetracycline and enrofloxacin in wa-ter samples. *International Journal of Environmental and Ana-lytical Chemistry* 89: 809-819.

Cerkvenik, V. (2002). Analysis and monitoring of chloramphenicol residues in food of animal origin in Slovenia from 1991 to 2000. *Journal of Food Additives and Contaminants* 4: 357–367.

Codex Alimentarius Commission, (2001). Committee on residues of veterinary drugs in foods, document control of veterinary drug residues in milk and milk products. Joint Food and Agriculture Organization of the United Nations – World Health Organization Food Standards Programme, Rome.

Darwish, W. S., Eldaly, E. A., El-abbasy, M. T. and Ikenaka, Y. (2013). Antibiotic residues in food the african scenario. *Japanese Journal of Veterinary Research* 61 (Supplement): S13 - S22.

Joint Expert Advisory Committee on Antibiotic Resistance - JETAC AR. (1998). *The Use of Antibiotics in Food-producing Animals :* Antibiotic - Resistant Bacteria in Animals and Humans. 249pp.

Karimuribo, E. D., Mdegela, R. H., Kusiluka, L. J. M. and Kambarage, D. M. (2005). Assessment of drug usage and antimicrobial residues in milk from smallholder farms in Morogoro, Tanzania. *Bulletin of* Animal *Health and* Production *in* Africa 53: 234– 241.

Katakweba, A. A. S. (2014). Prevalence and molecular studies of antimicrobial resistance in bacteria from farm animals, wildlife, pets and human in Tanzania. A Thesis

Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy of Sokoine Urniversity of Agriculture. Morogoro, Tanzania. 243pp.

Kurwijila, L. R., Omore, A., Staal, S. and Mdoe, N. S. Y. (2006). Investigation of the risk of exposure to antimicrobial residues present in marketed milk in Tanzania. *Journal of Food Protection* 69: 2487–2492.

Mmbando, L. M. G. (2004). Investigation of oxytetracycline use and abuse: determination of its residues in meat consumed in Dodoma and Morogoro municipalities. M.Sc. Thesis, Sokoine University of Agriculture, Morogoro, Tanzania. 85pp.

Mubito, E. P., Shahada, F., Kimanya, M. E. and Buza, J. J. (2014). Antimicrobial use in the poultry industry in Dar-es-Salaam , Tanzania and public health implications. *American Journal of Research Communication* 2(4): 51–63.

Nonga, H. E., Simon, C., Karimuribo, E. D. and Mdegela, R. H. (2010). Assessment of antimicrobial usage and residues in commercial chicken eggs from small-holder poultry keepers in Morogoro municipality, Tanzania. *Zoonoses and Public Health* 57(5): 339–344.

Sczesny, S., Nau, H. and Hamscher, G. (2003). Residue analysis of tetracyclines and their metabolites in eggs and in the environment by HPLC coupled with a microbiological assay and tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, 51(3): 697–703.

Seri, H. I. (2013). Introduction to veterinary drug residues : Hazards and Risks. *Veterinary Drug Residues in Food Derived from Animals*. pp1–7.

Sirdar, M. M., Picard, J., Bisschop, S., Jambalang, A. R. and Gummow, B. (2012). A survey of antimicrobial residues in table eggs in Khartoum State, Sudan, 2007–2008. *Onderstepoort Journal of Veterinary Research* 79(1): 9.

Stolker, A. A. and Brinkman, U. A. (2005). Analytical strategies for residue analysis of veterinary drugs and growth-promoting agents in food-producing animals – review. *Journal of Chromatography* 1067: 15–53.

Swatantra, S., Sanjay, S., Neelam, T., Nitesh, K., and Ritu, P. (2014). Antibiotic residues: A global challenge. *Pharma Science Monitor; An International Journal of Pharmaceutical Sciences* 5(3): 184–197.

The GARP-Tanzania (2015). Situation analysis and recommendations: antibiotic use and resistance in Tanzania. *Washington, DC and Nwe Delhi: Center for Disease Dynamics, Economics & Policy*. 118pp.

United Republic of Tanzania, (2016). Ministry of Livestock and Fisheries Development. *Budget Speech 2016-17*. 153pp.

CHAPTER THREE

Tetracyclines residue in Commercial Chicken Cooked Eggs in Tanzania: A Case Study of Dar es Salaam

John C. Mosha[1, 2], Jovin K. Mugula[1]

[1]*Department of Food Technology, Nutrition and Consumer Science, Sokoine University of Agriculture, P.O Box 3006, Morogoro, Tanzania*

[2]*Tanzania Food and Drug Authority P.O Box 77150, Dar es Salaam, Tanzania*

3.1 Abstract

Antibiotics are used in poultry farms to enhance growth, feed efficiency, reduce diseases and used as prophylactic treatment at periods of stress. Tetracyclines are the most commonly used antimicrobial drugs in food-producing animals. In Tanzania veterinary antibiotics are easily accessible with the increased intensive poultry production. However, most foods of animal origin including eggs are not eaten raw; they require thermal processing before consumption. This study estimated tetracyclines' residue in cooked commercial layer chicken eggs. Egg samples (194) were collected from layer farms in Dar es Salaam and then of these, 97 were boiled and the other 97 were fryied. Then these thermally-processed samples were analyzed for: oxytetracycline (OTC), tetracycline (TTC), and chlorotetracycline (CTC) resides using HPLC technique. For case of boiled egg samples; OTC residue was detected in 75.26 %, TTC residue in 46.39 % and CTC residue in 9.28 % of all analyzed samples. Among the fried eggs; OTC residue was detected in 96.91 %, TTC residue was detected in 15.46 % and CTC residue in 7.22 % of all analyzed samples. The mean level of OTC was higher in fried samples (360.05 μg/kg) than in boiled samples (115.75 μg/kg). However, the mean levels of TTC and CTC were low in both boiled and fried, 18.85 μg/kg and 18.69 μg/kg and 3.69 μg/kg and 14.58

μg/kg respectively. The mean level of OTC residue in both fried and boiled egg samples was lower than the maximum residue limits of CAC standards (400 μg/kg). Therefore, we see that the potential risk of consumers to public health hazards are reduced when chicken eggs are thermal processed.

Keywords: Commercial layer farms, antibiotics, residues, thermal processing, oxyxtetracycline, tetracycline, chlorotetracycline.

3.2 Introduction

Most foods of animal origin are not eaten raw, but require thermal processing before consumption. Thermal processing involves combination of time and temperature depending on the type of food and the intended use of food. There are very few studies conducted on the thermal stability or effect of ordinary cooking procedures on tetracycline residues in chicken eggs.

Stability of antibacterial drug residues under cooking conditions is an important research field, which provides valuable information related to health safety aspects and is very important from a safety and toxicological point of view (Hseih *et al.*, 2011). Most available information on drug residues in foods of animal origin is mostly related to the concentration of these drugs or their metabolites in raw samples. For years, many researchers have been interested in determining whether antibiotic residues can be destroyed or concentration reduced by different cooking procedures, pasteurization, or canning processes (Isidori *et al*., 2005). Abou-Raya *et al*. (2013) studied the changes by different cooking procedures on TCs including oxytetracycline (OTC), tetracycline (TTC), chlorotetracycline (CTC) and doxycycline (DOC) in chicken and milk respectively, and showed varying significant ($p < 0.05$) reduction of TCs concentration. This study managed to quantify the levels of TC residues in boiled and fried commercial chicken eggs.

3.3 Materials and Methods

The study was conducted in Dar es Salaam City. Dar es Salaam is the largest city and economic capital of Tanzania, located at 6°48' South, 39°17' East in a quiet bay off the Indian Ocean coast. The City has five municipal councils namely, Ubungo, Ilala, Kinondoni, Temeke and Kigamboni.

3.3.1 Samples

Eggs were collected from commercial chicken layer farmers which were randomly selected by proportion using a list provided by the respective municipality extension office. A total of 97 farmers from Ilala (38), Temeke (19), Ubungo (18), Kinondoni (13) and Kigamboni (9) municipalities. Selection of farmers considered their consent to participate in the study as well as the possession of commercial layers at laying period.

A total of 291 egg samples, 3 eggs from the same flock/batch were collected from 97 commercial layer farmers and assigned the same sample identification number. All the randomly selected farmers who consented to participate in the study and whohad layer chickens at laying period were contacted on the day of sampling. The samples were collected from the farms by the project assistants accompanied with the local government officers.

3.3.2 Quantification of tetracycline in boiled and fried eggs

Tetracycline residues in eggs were extracted and determined using HPLC according to the procedure described by Al-Wabel (2011) with slight modifications by introducing sulphuric acid and sodium tungstate to improve protein precipitation.

3.3.2.1 Preparation of standard solutions

Stock standard solutions (2.5 mg/mL) of each tetracycline (OTC, TC and CTC) (EDQM, F-67081 Strasbourg (France) were prepared by transferring 250 mg of each tetracycline into separate weighing dishes then transferred into separate 100 mL volumetric flask with methanol (Carlo Erba Reagents S.A.S, Val de Renil, France), mixed until dissolved the diluted to volume. The stock standards are stable for six months at -10^{o}C to -20^{o}C. Mixed intermediate standard (125 µg/mL) was prepared by pipetting 5.0 mL of each tetracycline stock solution to a 100 mL volumetric flask, mixed and diluted to volume with methanol. Intermediate standard solution is stable for six months at at -10^{o}C to -20^{o}C. Thereafter, mixed working standard solution (25 µg/mL) were prepared by pipetting 2 mL of 125 µg/mL mixed intermediate standard solution to a 10 mL volumetric flask and diluted to volume with methanol. Mixed working standard is stable for one week at -10^{o}C to -20^{o}C. Finally, HPLC standard (500 µg/L) was prepared daily by mixing 100 µL of mixed working standard (25 µg/mL), 400 µL methanol, 500 µL MOX (Methanolic oxalic acid (Loba Chemie Pvt. Ltd, Mumbai 400005, India) 0.01M) solution and 1000 µL distilled water (Barloworld Scientific Ltd, Stone, Staffordshire, ST15 0SA, UK) then vortexed before transferred to syringe and filtered into HPLC vials.

3.3.2.2 Sample preparation

3.3.2.2.1 Boiled egg samples

The egg samples were boiled at 85-95^{o}C for 10min, and blended. Then 3g was weighed and transferred into polypropylene centrifuge tube. Then 5 mL of McIlvaine buffer (pH 4.0) - EDTA solution was added to the tube. Then the mixture was homogenized by using T18 Digital Ultra Turrax Homogenizer (IKA brand from Germany) ready for extraction procedures.

3.3.2.2.2 Fried egg samples

The egg samples were fried by using Non-Stick Pan without the application of cooking oil at 110-120^{o}C for 3min and blended. Then 3g of blended fried eggs were transferred into polypropylene centrifuge tube. Five (5) mL of McIlvaine buffer (pH 4.0) - EDTA solution was added into the polypropylene centrifuge tube containing 3g of sample and then the mixture was homogenized ready for extraction procedures.

3.3.2.3 Extraction of tetracycline residues

3.3.2.3.1 Liquid – liquid extraction

Tetracycline residues were extracted as previously described by Al-Wabel, (2011) with slight modifications. The volume (15 mL) of McIlvaine buffer (pH 4.0) - EDTA solution (1.625 liter of McIlvaine buffer (mixture of 625mL of 28.4 g of anhydrous disodium hydrogen phosphate in 1L of ultra-pure distilled water + 1L of 21.0 g of citric acid monohydrate in 1L of distilled water) and 60.5 g of EDTA) was added into the polypropylene centrifuge tube containing 3g of homogenized cooked samples, capped and shaken for 10 min on flat-bed shaker at speed of 300 rpm. Centrifuge tubes were uncapped then 1mL of 0.34M sulphuric acid (Loba Chemie Pvt. Ltd, Mumbai 400005, India) and 7mL of 7% sodium tungstate (VWR International ltd, Poole, BH15 1TD, England) were added, capped, gently hand-shaken for 7 seconds and centrifuged at 4000 rpm for 30 min at 15^{o}C. The supernatants from the extraction were filtered by side arm flask under vacuum through Whatman No. 2 filter paper pre-wetted with McIlvaine buffer-EDTA solution in Büchner funnel.

3.3.2.3.2 Solid phase extraction

Filtrates (from 3.3.2.3.1 and 3.3.2.2.2) were subjected to an SPE Oasis Max Cartridge (Waters Corporation Milford, Massachusetts USA) attached to an SPE vacuum manifold capable of processing up to 20 solid phase extraction cartridges (SPE cartridges were not allowed to dry from pre-wash, sample addition and sample elution). Each cartridge was conditioned with 20 mL of methanol followed by 20 mL of ultra-pure distilled water. Test extract was loaded to SPE cartridge and vacuumed at rate of 5 mL/min. Subsequently, the elution was done with 6 mL of methanolic acid collected into a 15 mL test tube. When the flow stopped, vacuum was increased to remove residual solvent from the cartridge and the tubes containing the eluates were removed from the vacuum manifold and vortexed. Then the tubes were placed in a Nitrogen-Evaporator with a water bath (40-50^{o}C) to reduce their volumes to 0.5 – 1 mL under a stream of dry nitrogen. The final eluate was adjusted to 1 mL with methanol, vortexed and then diluted to 2 mL with distilled water and vortexed again. The final extract (0.5 – 1 mL) was poured into a 3 mL syringe and filtered through acrodisc filter into HPLC auto-sampler vial.

3.3.2.4 High performance liquid chromatography

Extracts were analyzed using a Shimadzu LC-20AT (Kyoto, Japan) HPLC system quipped with a Shimadzu DGU-20A$_5$ degasser, Shimadzu SPD-M20A Diode Array Detector, Shimadzu CBM-20A Communication System and Shimadzu LC Solution Software. The type of column used was Thermo-Scientific, BDS Hypersil C8 (125 x 4) mm, Partial size 5µ, Part No. 28205-124030, SN: 10058200. Mobile phase solution used were 0.005 M Oxalic acid (Solvent A) and pure Acetronitrile HPLC Grade (Solvent B) (Carlo Erba Reagents, Chaussee du Vexin, France). Injection volume, 100 µL of Tetracyclines were injected under low pressure gradient mode of 75% A and 25% B and detected at wavelength (λ) of 365 nm with flow rate of 0.8 mL/min. The column temperature was kept at 30 – 45°C.

3.3.3 Statistical analysis

The statistical comparison of data was performed by one-way analysis of variance (ANOVA) using R-Commander software version 3.3.0 (2009-2014 R Studio, Inc.) to reveal significant differences among the samples studied. Determination of significance of differences among residues was obtained by Duncan multiple range tests. Residue values whose P values were < 0.05, were considered different significantly.

3.4 Results and Discussion

3.4.1 Results

A total of 97 eggs samples collected from commercial layer farmers in Dar-es-salaam were boiled at 85-95^{o}C for 10min and then analyzed for oxytetracycline (OTC), tetracycline (TTC) and chlorotetracycline (CTC) residues by using the HPLC system. The quantitative results indicated that 90.72% of analyzed samples had detectable levels of tetracycline residues, such that 75.3% of the samples had detectable levels of OTC, 46.4% of the samples had detectable levels of TTC and 9.3% of the samples had detectable levels of CTC residues. OTC, TTC and CTC residue concentrations in boiled eggs ranged from 0.0 - 780.4 µg/kg (mean, 115.7 µg/kg), 0.0 – 635.8 µg/kg (mean, 18.8 µg/kg) and 0.0 – 598.4 µg/kg (mean, 18.7 µg/kg), respectively. The summary of the prevalence and mean levels of OTC, TTC and CTC residues in boiled egg samples is as shown in Table 3.1.

Furthermore, the other 97 eggs samples collected from commercial layers production farms in Dar-es-salaam were fried on the non-stick pan at 110 – 130^{o}C for 2 – 2.5 min and then analyzed for oxytetracycline (OTC), tetracycline (TTC) and chlorotetracycline (CTC) residues by using the HPLC system. The quantitative results indicated that 96.9% of the analyzed samples had detectable levels of tetracycline residues, such that 96.8% of the samples had detectable levels of OTC, 15.5% of the samples had detectable levels of TTC and 7.2% of the samples had detectable levels of CTC residues. OTC, TTC and CTC residue concentrations in fried eggs ranged from 0.0 – 852.6 µg/kg (mean, 360.1 µg/kg), 0.0 – 166.5 µg/kg (mean, 3.69 µg/kg) and 0.0 – 590.4 µg/kg (mean, 14.58 µg/kg), respectively. The summary of the prevalence and mean levels of OTC, TTC and CTC residues in fried egg samples is shown in Table 3.2.

Table 3.1: Prevalence and levels of oxytetracycline, tetracycline and chlorotetracycline residues in boiled eggs

Residues	Overall range (µg/kg)	Mean levels (µg/kg)	Prevalence (%)
OTC	0.0 – 780.42	115.75 ± 146.64	75.26
TTC	0.0 – 635.78	18.85 ± 88.50	46.39
CTC	0.0 – 598.38	18.69 ± 83.58	9.28

Table 3.2: Prevalence and levels of oxytetracycline, tetracycline and chlorotetracycline residues in fried eggs

Residues	Overall range (µg/kg)	Mean levels (µg/kg)	Prevalence (%)
OTC	0.0 – 852.56	360.05 ± 219.93	96.89
TTC	0.0 – 166.50	3.69 ± 17.64	15.46
CTC	0.0 – 590.37	14.58 ± 72.70	7.22

3.4.2 Discussion

The study revealed the potential reduction of tetracycline residues when treated by heat during ordinary cooking procedures. The residue levels in boiled and fried egg samples were low compared to that of raw egg samples. The similar observation was made by Gratacós-Cubarsí *et al*. (2007) who mentioned that ordinary cooking procedures, i. e. microwave and boiling reduced the initial concentrations of TC residues by 56 to 82%. The findings also indicated that high potential reduction of TC residues were obtained as a result of boiling procedure leading to lower TC residue levels (7.2%) compared to 45.4% of fried. Temperature during cooking has the largest impact on the loss of tetracycline residues (Abou-Raya *et al*., 2013; Du *et al*., 1997; Fedeniuk *et al*., 1997; Ibrahim, 1994;). Also the same observation was made by Abou-Raya *et al*. (2013) that with regard to cooking time, it was observed that regardless of the cooking procedures and TC agents, prolonged cooking time reduced the content of TCs significantly ($p < 0.01$). In addition, Al-Ghamdi *et al*. (2000), stated that OTC was more stable than TTC, CTC and DOC in meat and liver samples after cooking by boiling. Therefore it is clear that both cooking temperature and time are the important factors to cause reduction of the initial levels of tetracycline residues. The study indicated that, thermal stability within Tetracycline group could be very different despite their structural similarity. Therefore, it is not appropriate to predict thermal stability simply by class of antibiotics (Hsieh *et al*., 2011).

3.5 Conclusion

The present study implies that TC residues are unstable drugs that can be degraded during cooking, rendering chicken eggs apparently safe for human consumption according to the maximum residual limit cited by the Codex Almentarius Commission Standard (CAC/MRL2-2015). It can be concluded that cooking of eggs can have a great effect on TC residual losses and provides an additional margin of safety for consumers. This result shall help the processors and quality control institutions to concentrate more on enforcing farmers to adhere to withdraw periods and evaluate the presence of these antibiotics in heat-processed products rather than the raw material. This will also be useful for restaurants and housewives to prepare safe meals.

Acknowledgements

I am grateful for the financial support provided by the Tanzania Food and Drugs Authority.

3.6 References

Abdel-mohsein, H. S., Mahmoud, M. A. M. and Ibrahim, A. (2015). Tetracycline residues in intensive broiler farms in upper egypt: Hazards and risks. *Journal of World's Poultry Research* 5(3): 48–58.

Abou-Raya, S. H., Shalaby, A. R., Salama, N. A., Emam, W. H. and Mehaya, F.M. (2013). Effect of ordinary cooking procedures on tetracycline residues in chicken meat. *Journal of Food and Drug Analysis* 21(1): 80-86.

Al-Ghamdi, M. S., Al-Mustafa, Z. H., El-Morsy, F., Al-Faky, A., Haider, I. and Essa, H. (2000). Residues of tetracycline compounds in poultry products in the eastern province of Saudi Arabia. *Public Health* 114: 300-304.

Al-Wabel, N. A. (2011) Monitoring of tetracycline residues in table eggs collected from Qassim region, Kingdom of Saudi Arabia. *Journal of Agriculture and Veterinary Science* 4(2): 109–123.

Biswas, A. K., Kondaiah, N., Anjaneyulu, A. S. R. and Mandal, P. K. (2010). Food safety concerns of pesticides, veterinary drug residues and mycotoxins in meat and meat products. *Asian Journal of Animal Sciences* 4(2): 46.

Chowdhury, S., Hassan, M. M., Alam, M., Sattar, S. and Bari, S. (2015). Antibiotic residues in milk and eggs of commercial and local farms at Chittagong, Bangladesh. *Veterinary World* 8(3): 467–471.

Codex Alimentarius Commission, (2001). Committee on residues of veterinary drugs in foods, document control of veterinary drug residues in milk and milk products. Joint Food and Agriculture Organization of the United Nations – World Health Organization Food Standards Programme, Rome.

Darwish, W. S., Eldaly, E. A., El-abbasy, M. T. and Ikenaka, Y. (2013). Antibiotic residues in food the african scenario.pdf. *Japanese Journal of Veterinary Research* 61 (Supplement): S13 - S22.

Du, W. X., Marshall, M. R., Xu, D. H., Santerre, C. R. and Wei, C. I. (1997). Retention of oxytetracycline residues in cooked channel catfish fillets. *Journal of Food Science* 62: 119-122.

Fedeniuk, R. W., Shand, P. J. and Mccurdy, A. R. (1997). Effect of thermal processing and additives on the kinetics of oxytetracycline degradation in pork muscle. *Journal of Agricultural and Food Chemistry* 45: 2252-2257.

Gratacós-Cubarsí, M., Fernandez-García, A., Picouet, P., Valero-Pamplona, A., García-Regueiro, J. A. and Castellari, M. (2007). Formation of tetracycline degradation products in chicken and pig meat under different thermal processing conditions. *Journal of Agriculture and Food Chemistry* 55: 4610-4616.

Heshmati, A. (2015). Impact of cooking procedures on antibacterial drug residues in foods : A review. *Journal of Food Quality and Hazards Control* 2: 33–37.

Heshmati, A., Kamkar, A., Salaramoli, J., Hassan, J. and Jahed, G. H. (2013). The effect of two methods cooking of boiling and microwave on tylosin residue in chicken meat. *Iranian Journal of Nutrition Sciences and Food Technology* 8: 61-71.

Hsieh, M. K., Shyu, C. L., Liao, J. W., Franje, C. A., Huang, Y. J,. Chang, S. K., Shih, P. Y. and Chou, C. C. (2011). Correlation analysis of heat stability of veterinary antibiotics by structural degradation, changes in antimicrobial activity and genotoxicity. *Journal of Veterinarni Medicina* 56(6) : 274–285.

Ibrahim, A. and Moats, W. A. (1994). Effect of cooking procedures on oxytetracycline residues in lamb muscle. *Journal of Agriculture and Food Chemistry* 42: 2561-2563.

Idowu, F., Junaid, K., Paul, A., Gabriel, O., Paul, A., Sati, N., Maryam, M. and Jarlath, U. (2010). Antimicrobial screening of commercial eggs and determination of tetracycline residue using two microbiological methods. *International Journal of Poultry Science* 9(10): 959–962.

Isidori, M., Lavorgna, M., Nardelli, A., Pascarella, L. and Parella, A. (2005). Toxic and genotoxic evaluation of six antibiotics on non-target organisms. *Science of the Total Environment* 346: 87-98.

Joint Expert Advisory Committee on Antibiotic Resistance - JETAC AR. (1998). *The Use of Antibiotics in Food-producing Animals :* Antibiotic - Resistant Bacteria in Animals and Humans. 249pp.

Katakweba, A. A. S. (2014). Prevalence and molecular studies of antimicrobial resistance in bacteria from farm animals, wildlife, pets and human in Tanzania. *A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy of Sokoine Urniversity of Agriculture. Morogoro, Tanzania*. pp1–243.

Ministry of Livestock and Fisheries Development (2011). Tanzania Livestock Sector Development Programme. *7th African Dairy Conference and Exhibition, MovenPick Palm Hotel*. 123pp.

Mubito, E. P., Shahada, F., Kimanya, M. E. and Buza, J. J. (2014). Antimicrobial use in the poultry industry in Dar-es-Salaam , Tanzania and public health implications. *American Journal of Research Communication*, 2(4): 51–63.

Narrod, C., Tiongco, M. and Costales, A. (2012). Global poultry sector trends and external drivers of structural change. *Poultry in the 21st Century: Avian Influenza and Beyond*. pp1–28.

Nonga, H. E., Simon, C., Karimuribo, E. D. and Mdegela, R. H. (2010). Assessment of antimicrobial usage and residues in commercial chicken eggs from small-holder poultry keepers in Morogoro municipality, Tanzania. *Zoonoses and Public Health* 57(5): 339–344.

Sczesny, S., Nau, H. and Hamscher, G. (2003). Residue analysis of tetracyclines and their metabolites in eggs and in the environment by HPLC coupled with a microbiological assay and tandem mass spectrometry. *Journal of Agricultural and Food Chemistry* 51(3): 697–703.

Seri, H. I. (2013). Introduction to veterinary drug residues: Hazards and risks. *Veterinary Drug Residues in Food Derived from Animals*. pp1–7.

Sirdar, M. M., Picard, J., Bisschop, S., Jambalang, A. R. and Gummow, B. (2012). A survey of antimicrobial residues in table eggs in Khartoum State, Sudan, 2007–2008. *Onderstepoort Journal of Veterinary Research* 79(1): 9.

Swatantra, S., Sanjay, S., Neelam, T., Nitesh, K. and Ritu, P. (2014). Antibiotic residues: A global challenge. *Pharma Science Monitor; An International Journal of Pharmaceutical Sciences* 5(3): 184–197.

The GARP-Tanzania (2015). Situation analysis and recommendations: Antibiotic use and resistance in Tanzania. *Washington, DC and Nwe Delhi: Center for Disease Dynamics, Economics and Policy*. 118pp.

United Republic of Tanzania, (2016). Ministry of livestock and fisheries development. *Budget Speech 2016-17*. 153pp.

CHAPTER FOUR

Levels of Exposure to Tetracyclines and Safety of Consumers from Commercial Layer Chicken Eggs in Tanzania: A Case Study of Dar es Salaam.

John C. Mosha[1, 2], Jovin K. Mugula[1]

[1]*Department of Food Technology, Nutrition and Consumer Science, Sokoine University of Agriculture, P.O Box 3006, Morogoro, Tanzania*

[2]*Tanzania Food and Drug Authority P.O Box 77150, Dar es Salaam, Tanzania*

4.1 Abstract

The abundant use of tetracycline antibiotics in veterinary medicine may result in the presence of their residues in commercial layer chicken eggs at unsafe concentrations that can adversely affect public health. The aim of the current study was to determine the levels of exposure to tetracycline residues for the safety of consumers in Dar es Salaam. A total of 291 samples, 3 samples from each layer farm, were collected from 97 layer farms in Dar es Salaam. The batch of 97 samples was analyzed raw and the other two batches were analyzed boiled (97) and fried (97). The tetracycline residues were quantified by using HPLC system. The prevalence of raw egg samples (80.41% OTC, 0% TTC and 2.06% CTC) detected with TC residue levels above MRL was higher than of boiled (7.22 % OTC, 2.06 % TTC and 2.06% CTC) and fried (45.36 % OTC, 0 % TTC and 1.03 % CTC) egg samples. Also, the mean concentration levels of tetracycline residues above MRL was higher (850.10 µg/kg OTC, 0 µg/kg TTC and 976.46 µg/kg CTC) in raw egg samples than it was in boiled (554.24 µg/kg OTC, 623.45 µg/kg TTC and 499.81 µg/kg CTC) and fried (565.05 µg/kg OTC, 0 µg/kg TTC and 590.37 µg/kg CTC).

Keywords: Commercial layer chicken, Antibiotics, Residues, Oxytetracycline, Tetracycline, Chlorotetracycline, Maximum residue limits

4.2 Introduction

Withdrawal of medication is necessary so that levels of drug residue above MRL are avoided in foods of animal origin e.g. meat, milk, and eggs marketed for human consumption thereby safeguarding humans from unnecessary exposure to antimicrobials (Nisha, 2008). For example, all interviewed poultry farmers in Dar es Salaam were aware of drug withdrawal period but none of them declared to observe this requirement because they fear investment losses (Nonga *et al*., 2010).

In developed countries, governments and stake holders are involved in routine monitoring of farms for observance of withdrawal periods and slaughter houses and food industries for drug residues (Sundlof *et al*., 2000; Dey *et al*., 2003). In Africa, agricultural sectors consume a large portion (>50%) of antibiotics in animal farming (Miller *et al*., 2003). However, in many African countries including Tanzania there are no legislations which regulate antimicrobial drugs dispensing and usage in poultry farms in order to monitor and control prevalence residues in foods of animal origin (Darwish *et al*., 2013; Nonga *et al*., 2010).

The use of antibiotics in food-producing animals including chickens may leave residues in foodstuffs of animal origin like meat, milk, and eggs. Abuse or misuse of veterinary drugs is one of the causes of drug residues in animal products (Pavlov *et al*., 2008; Idowu *et al*., 2010). The occurrence of antibiotic residues in commercial chicken eggs was determined in Morogoro whereby all eggs examined tested positive for antibiotic residues (Darwish *et al*., 2013).

The occurrence of antimicrobial residues may be due to different factors such as failure to observe the withdrawal periods of each drug, extra-label dosages for animals, improper maintenance of treatment records, feeding, the use of unlicensed antibiotics drug dosage, type and age of animal, disease status, and route of administration (Heshmati, 2015). For example in Dar es Salaam about 90% of poultry farmers have no knowledge of antibiotic restrictions and adverse effects of residues to public health (Mubito *et al*., 2014).

Antibiotic residues in foods of animal origin potentially cause numerous health concerns in humans. These problems include toxic effects, transfer of antibiotic resistance in bacteria to humans making treatment of human infection more difficult, immune-pathological effects, carcinogenicity, mutagenicity, nephropathy, hepatotoxicity, reproductive disorders, bone marrow toxicity, and allergy in sensitive or sensitized individuals (Nisha, 2008). The resistance could be transferred from non-pathogenic microorganisms to pathogenic ones, which would then no longer respond to normal drug treatment (Heshmati *et al*., 2015) and uncontrolled antibiotic dispensing for animals presents high risk for spread of antimicrobial resistance among domestic animals with potential to contribute antimicrobial resistance to human beings (The GARP-Tanzania, 2015).

Antimicrobial residues such as tetracycline may have a direct toxic effect on consumers, for example allergic reactions in hypersensitive individuals, gastrointestinal disturbances, poor fetal development and skin allergies in eggs containing sulfonamide residues (Sirdar *et al*., 2012; Idowu *et al*., 2010). Tetracyclines have also been reported to cause hyperuricemia, hypokalemia, proximal and distal renal tubular acidosis (Goldfrank *et al*., 2002).

4.3 Materials and Methods

The study was conducted in Dar es Salaam City. Dar es Salaam is the largest city and economic capital of Tanzania, located at 6°48' South, 39°17' East in a quiet bay off the Indian Ocean coast. The City has five municipal councils namely, Ubungo, Ilala, Kinondoni, Temeke and Kigamboni.

4.3.1 Samples

Eggs collected from commercial chicken layer farmers were randomly selected by proportion using a list provided by the respective municipality extension office. A total of 97 farmers from: Ilala (38), Temeke (19), Ubungo (18), Kinondoni (13) municipal and Kigamboni (9) were selected. The selection of farmers considered their consent to participate in the study as well as the possession of commercial layers at laying period.

A total of 291 egg samples, 3 eggs from the same flock/batch were collected from 97 commercial layer farmers and assigned the same sample identification number. All the randomly selected farmers who consented to participate in the study and had layers chicken at laying period were contacted on the day of sampling. The samples were collected from the farms by the project assistants accompanied with the local government officers.

4.3.2 Quantification of tetracycline residues

Tetracycline residues in eggs were extracted and determined using HPLC according to the procedure described by Al-Wabel (2011) with slight modification by introducing sulphuric acid and sodium tungstate to improve protein precipitation.

4.3.2.1 Preparation of standard solutions

Stock standard solutions (2.5 mg/mL) of each tetracycline (OTC, TC and CTC) (EDQM, F-67081 Strasbourg (France) was prepared by transferring 250 mg of each tetracycline into separate weighing dishes then transferred into separate 100 mL volumetric flask with methanol (Carlo Erba Reagents S.A.S, Val de Renil, France), mixed until dissolved the diluted to volume. The stock standards are stable for six months at -10^{o}C to -20^{o}C. Mixed intermediate standard (125 µg/mL) was prepared by pipetting 5.0 mL of each tetracycline stock solution to a 100 mL volumetric flask, mixed and diluted to volume with methanol. Intermediate standard solution is stable for six months at -10^{o}C to -20^{o}C. Thereafter, mixed working standard solution (25 µg/mL) were prepared by pipetting 2 mL of 125 µg/mL mixed intermediate standard solution to a 10 mL volumetric flask and diluted to volume with methanol. Mixed working standard is stable for one week at -10^{o}C to -20^{o}C. Finally, HPLC standard (500 µg/L) was prepared daily by mixing 100 µL of mixed working standard (25 µg/mL), 400 µL methanol, 500 µL MOX (Methanolic oxalic acid 0.01M (Loba Chemie Pvt. Ltd, Mumbai 400005, India)) solution and 1000 µL distilled water (Barloworld Scientific Ltd, Stone, Staffordshire, ST15 0SA, UK) then vortexed before transferred to syringe and filtered into HPLC vials.

4.3.2.2 Sample preparation

4.3.2.2.1 Preparation of raw egg

Tetracyclines residues were extracted by the solid phase technique (Al-Wabel, 2011). The raw egg samples were homogenized by using T18 Digital Ultra Turrax Homogenizer (IKA brand from Germany). 3g of homogenized raw egg sample was transferred into polypropylene centrifuge tube, 20 ml of McIlvaine buffer (pH 4.0) - EDTA (S.D. Fine – Chem Ltd, Mumbai 400025, India) solution (1.625 liter of McIlvaine buffer (mixture of 625 mL of 28.4 g of anhydrous disodium hydrogen phosphate (Loba Chemie Pvt. Ltd, Mumbai 400005, India) in 1L of ultra-pure distilled water + 1L of 21.0 g of citric acid monohydrate (Sigma-Aldrich, Saint Louis, Missouri 63103, USA) in 1L of distilled water) and 60.5 g of EDTA) was added and then centrifuge tubes were capped, shaken for 10 min on flat-bed shaker at speed of 300. Centrifuge tubes were uncapped and 1mL of 0.34M sulphuric acid and 7mL of 7% sodium tungstate were added, capped and gently hand-shaken for 7 seconds, centrifuged at 4000 rpm for 25-30 min at 15^{o}C. Supernatant from the extraction were filtered by side arm flask under vacuum through Whatman No. 2 filter paper pre-wetted with McIlvaine buffer-EDTA solution in Büchner funnel.

4.3.2.2.2 Preparation of boiled eggs

The egg samples were boiled at 95^{o}C for 10min, blended then 3g was weighed and transferred into polypropylene centrifuge tube then 5 mL of McIlvaine buffer (pH 4.0) - EDTA solution was added then homogenized by using T18 Digital Ultra Turrax Homogenizer (IKA brand from Germany) ready for extraction procedures.

4.3.2.2.3 Preparation of fried eggs

The egg samples were fried by using Non-Stick Pan without the application of cooking oil at 110-120^{o}C for 3min, blended then 3g of blended fried egg was transferred into polypropylene centrifuge tube. 5mL of McIlvaine buffer (pH 4.0) - EDTA solution was added into the polypropylene centrifuge tube containing 3g of sample then homogenized ready for extraction procedures.

4.3.2.3 Extraction of tetracycline residuals

4.3.2.3.1 Liquid – liquid extraction

20ml and 15 ml of McIlvaine buffer (pH 4.0) - EDTA solution (1.625 liter of McIlvaine buffer (mixture of 625mL of 28.4 g of anhydrous disodium hydrogen phosphate in 1L of ultra-pure distilled water + 1L of 21.0 g of citric acid monohydrate in 1L of distilled water) and 60.5 g of EDTA) was added into the polypropylene centrifuge tube containing 3g of homogenized raw and cooked samples respectively, capped and shaken for 10 min on flat-bed shaker at speed of 300 rpm. Centrifuge tubes were uncapped then 1mL of 0.34M sulphuric acid (Loba Chemie Pvt. Ltd, Mumbai 400005, India) and 7mL of 7% sodium tungstate (VWR International ltd, Poole, BH15 1TD, England) were added, capped, gently hand-shaken for 7 seconds and centrifuged at 4000 rpm for 30 min at 15^{o}C. Supernatant from the extraction were filtered by side arm flask under vacuum through Whatman No. 2 filter paper pre-wetted with McIlvaine buffer-EDTA solution in Büchner funnel.

4.3.2.3.2 Solid phase extraction (SPE)

Filtrates were subjected to an SPE Oasis Max Cartridge (Waters Corporation Milford, Massachusetts USA) attached to an SPE vacuum manifold capable of processing up to 20 solid phase extraction cartridges (SPE cartridges were not allowed to dry from pre-wash, sample addition and sample elution). Each cartridge was conditioned with 20 mL of methanol followed by 20 mL of ultra-pure distilled water. Test extract were loaded to SPE cartridge and vacuumed at rate of 5 mL/min. Subsequently, the elution took place with 6 mL of methanolic acid into 15 mL test tubes as eluate collection vessel. Once the flow stopped, vacuum was increased to remove residual solvent from the cartridge, tubes removed from the vacuum manifold and vortexed. The tubes containing the methanolic eluate were placed in a Nitrogen-Evaporator with a water bath temperature of 40-50^{o}C reduced to 0.5 – 1 mL under under a stream of dry nitrogen. The final eluate was adjusted to 1mL with methanol, vortexed then diluted to 2 mL with distilled water and vortexed again. 0.5 – 1 mL of the final extract was poure into a 3 mL syringe and filtered through acrodisc filter into HPLC autosampler vial.

4.3.2.4 High performance liquid chromatography

Extracts were chromatographed on a Shimadzu LC-20AT (Kyoto, Japan) HPLC system quipped with a Shimadzu DGU-20A$_5$ degasser, Shimadzu SPD-M20A Diode Array Detector, Shimadzu CBM-20A Communication System and Shimadzu LC Solution Software. The type of column used was Thermo-Scientific, BDS Hypersil C8 (125 x 4) mm, Partial size 5µ, Part No. 28205-124030, SN: 10058200. Mobile phase solution used were 0.005 M Oxalic acid (Solvent A) and pure Acetronitrile HPLC Grade (Solvent B) (Carlo Erba Reagents, Chaussee du Vexin, France). Injection volume, 100 µL of Tetracyclines were injected under low pressure gradient mode of 75% A and 25% B and detected at wavelength (λ) of 365 nm with flow rate of 0.8 mL/min. The column temperature was kept at 30 – 45°C.

4.3.2.5 Calibration linearity

The HPLC response was linear for OTC (r^2 = 0.967), TTC (r^2 = 0.973) and CTC (r^2 = 0.998) in the range of 100 to 2000 µg/kg with correlation coefficient (r) equals to 0.983, 0.986 and 0.999 respectively. The retention time for OTC, TTC, and CTC was found to be 3.109, 3.732 and 6.641 minutes respectively.

4.3.3 Recovery of tetracyclines in egg sample

The mixed working standard was added to the 3g whole homogenized egg whose concentrations were 200, 300, 400, 500 and 600 µg/kg, to meet the MRLs of Codex Almentarius Commission standard (CAC/MRL2-2015) and then vortexed to ensure proper mixing of the standard solution. It was extracted with the above-stated analytic method and applied to the HPLC system (Table 4.1).

Table 4.1: Recovery of oxytetracycline, tetracycline and chlorotetracycline in spiked samples

Spiked Concentrations (μg/kg)	Average recovery (%)		
	Oxytetracycline	**Tetracycline**	**Chlorotetracycline**
200	69	70	71
300	77	87	83
400	73	98	85
500	70	61	69
600	67	79	76

4.3.4 Statistical analysis

The statistical comparison of data was performed by one-way analysis of variance (ANOVA) using R-Commander software version 3.3.0 © 2009-2014 R Studio, Inc. to reveal significant differences among the samples studied. Determination of significance of differences among residues was obtained by Duncan multiple range tests. Residue levels with P-values less than 0.05 were considered different significantly.

4.4 Results and Discussion

4.4.1 Results

Of 97 raw egg samples analyzed during this study, 78 (80.4%) had detectable levels for oxytetracycline residues above MRL and 2 (2.06%) had detectable levels for chlorotetracycline above MRL. The mean levels and overall range of the raw egg samples positive for tetracycline residues above MRL are shown in Table 4.2.

Furthermore, out of 97 boiled egg samples analyzed during this study 7 (7.2%) had detectable levels for tetracycline residues above MRL. The prevalence of tetracycline groups that comprised the samples with levels above MRL were oxytetracycline, which was found in 7 (7.2%), tetracycline in 2 (2.06%) and chlorotetracycline in 2 (2.06%) of the analyzed samples. The mean levels and overall range of the boiled egg samples found with tetracycline residues above MRL are shown in Table 4.3.

Out of 97 fried egg samples, 44 (45.4%) had detectable levels for tetracycline residues. The two tetracycline groups that were found in samples above the MRL were oxytetracycline, which was found in 44 (45.4%) and chlorotetracycline in 1 (1.03%) of the samples. The mean levels and range of the fried egg samples found with tetracycline residues above MRL are shown in Table 4.4.

Table 4.2: Prevalence of raw egg samples with tetracycline residual levels above MRL

Residues	**Overall range (µg/kg)**	**Mean levels (µg/kg)**	**Prevalence (%)**
Oxytetracycline	434.25 – 1655.09	850.10 ± 284.53	80.41
Tetracycline	ND	ND	ND
Chlorotetracycline	776.45 – 1176.47	976.46 ± 282.86	2.06

Table 4.3: Prevalence of boiled egg samples with tetracycline residual levels above MRL

Residues	**Overall range (µg/kg)**	**Mean levels (µg/kg)**	**Prevalence (%)**
Oxytetracycline	441.29 – 780.42	554.24 ± 135.82	7.22
Tetracycline	611.11 – 635.78	623.45 ± 17.44	2.06
Chlorotetracycline	401.25 – 598.38	499.81 ± 139.39	2.06

Table 4.4: Prevalence of fried egg samples with tetracycline residual levels above MRL

Residues	**Overall range (µg/kg)**	**Mean levels (µg/kg)**	**Prevalence (%)**
Oxytetracycline	410.80 – 852.56	565.05 ± 113.90	75.26
Tetracycline	ND	ND	ND
Chlorotetracycline	590	590.37 ± 0	1.03

ND = Not Detected

4.4.2 Discussion

4.4.2.1 Levels of exposure to tetracyclines

About 44% of the total number of samples detected for tetracyclines had residue levels above CAC/MRL 2-2015 standards. The maximum residue limit (MRL) for tetracyclines (OTC, TTC, and CTC) in poultry eggs is 400 µg/kg (CAC, 2015). The findings show that there is a considerable large proportion of eggs containing tetracycline residue levels, which exceeded the maximum tolerable limit. The presence of drugs or antibiotics residues in food above the maximum permissible level has been a worldwide public health concern (Kempe and Verachtert, 2000). The number of samples with tetracycline residues' levels above MRL was high in raw egg samples (80.4%) followed by fried egg samples (45.4%) and low in boiled egg samples (7.2%). This has been explained by Abou-Raya (2013) who revealed that cooking could lead to a reduction of TCs contents in the samples, and microwave cooking had a more pronounced effect on TCs than frying and boiling. Mean tetracycline residues' levels as detected from raw, boiled and fried egg samples were significantly different ($p<0.05$). The mean levels of tetracycline residues above MRL were mostly prominent to oxytetracycline, 850 µg/kg in raw eggs, 565 µg/kg in fried eggs and 554 µg/kg in boiled eggs, followed by chlorotetracycline, 976.5 µg/kg in raw eggs, 590 µg/kg in fried eggs and 499.8 µg/kg in boiled eggs. Incidences of higher levels of Oxytetracycline than those of TTC and CTC have been observed in other studies. In the study by Bedada and Zewde (2012), it was revealed that out of 384 samples that they analyzed for tetracycline residues, 71.3% (274/384) had detectable oxytetracycline residues while tetracycline and doxycycline residues were not detected in any of the samples analyzed.

TCs are important class of antibiotics in food, animal health and production. These antibiotics have been used for many decades in the treatment of diseases, promote growth and to maintain animals health (Olatoye and Ehinmowo 2010; Bedada and Zewde 2012). Katakweba *et al.* (2013) reported that OTC is one of the most commonly used antibiotics in livestock production in Tanzania. The easy access to these antibiotics that is unauthorized use of these antibiotics, the failure to follow label directions and lack of awareness may lead to improper management of these drugs (Donoghue, 2003). The MRL depends on the withdrawal period of the particular antibiotics, in this case tetracyclines in which sometimes the presence of the antibiotic could drop to tolerable or undetectable levels. On the study conducted in Dar es Salaam by Mubito *et al*. (2014) showed that, all interviewed chicken producers reported to be aware of withdrawal period but none of them declared to observe it due to fear of capital losses. It was further found that about 90% of farmers had no knowledge about health effect associated with antibiotic drugs residues. In developed countries, such as USA, Japan and EU, regulations and strict monitoring measures on the use of antimicrobials have been established (Salehzadeh *et al*., 2006, Reig and Toldrá, 2008, Passantino and Russo, 2008, Donoghue, 2003, Sirdar *et al*., 2012c) but in developing countries such strict regulations are either not in place or not enforced (Sirdar *et al*., 2012b).

4.4.2.2 Antibiotic residues and risks to human health

Over the years, the presence of antimicrobial drug residue in the foodstuffs at levels above MRLs and their effect on human health has been a major concern to public health (McEwen and Fedorka-Cray, 2002). The present study demonstrated that tetracycline residues in the commercial eggs marketed in Dar es Salaam exceeded the safety limits recommended by the CAC and put consumers at risk of exposures to health effects associated with consumption of high levels of antibiotic drugs. Today, in addition to the adverse effects that can occur as a result of the use of veterinary drugs, antibiotic resistance is considered to be a major threat to human health. The presence of antibiotic residues in foods of animal origin has attracted attentions as to whether their long-term intake could be contributing to the development of antibiotic resistance in humans. Some of risks associated with human exposure to antimicrobial residues are (i) Allergic/toxic reactions (ii) chronic toxic effects occurring with prolonged exposure to low levels of antibiotics, (iii) development of antibiotic-resistant bacteria and (iv) disruption of normal flora in human intestine.

4.5 Conclusion

In the present study, the average concentrations of total tetracycline residues in commercial layer chicken eggs (80.4%) were determined greater than the maximum residue limit (MRL) set by CAC. OTC had the highest share in this contamination because is the most commonly used (75%) antimicrobial drug. This situation exposes consumers to the risks of public health issues associated with consumption of antimicrobial residues in foods of animal origin including eggs.

Acknowledgements

I am grateful for the financial support provided by the Tanzania Food and Drugs Authority.

4.6 References

Abdel-mohsein, H. S., Mahmoud, M. A. M. and Ibrahim, A. (2015). Tetracycline residues in intensive broiler farms in upper egypt: Hazards and risks. *Journal of World's Poultry Research* 5(3): 48–58.

Abou-Raya, S., Shalaby, A. R., Salama1, N. A., Emam, W. H. and Mehaya, F. M. (2013). Effect of ordinary cooking procedures on tetracycline residues in chicken meat. *Journal of Food and Drug Analysis* 21: 80-86.

Bedada, A. H. and Zewde, B. M. (2012). Tetracycline residue levels in slaughtered beef cattle from three slaughterhouses in central Ethiopia. *Journal of Global Veterinaria* 8(6): 546-554.

Biswas, A. K., Kondaiah, N., Anjaneyulu, A. S. R. and Mandal, P. K. (2010). Food safety concerns of pesticides, veterinary drug residues and mycotoxins in meat and meat products. *Asian Journal of Animal Sciences* 4(2): 46.

Chowdhury, S., Hassan, M. M., Alam, M., Sattar, S. and Bari, S. (2015). Antibiotic residues in milk and eggs of commercial and local farms at Chittagong, Bangladesh. *Veterinary World* 8(3): 467–471.

Codex Alimentarius Commission, (2001). Committee on residues of veterinary drugs in foods, document control of veterinary drug residues in milk and milk products. Joint Food and Agriculture Organization of the United Nations – World Health Organization Food Standards Programme, Rome.

Darwish, W. S., Eldaly, E. A., El-abbasy, M. T. and Ikenaka, Y. (2013). Antibiotic residues in food the african scenario.pdf. *Japanese Journal of Veterinary Research* 61 (Supplement): S13 - S22.

Donoghue, D. J. (2003). Antibiotic residues in poultry tissues and legs. Human Health Concerns? *Poultry Science* 82: 618–621.

Heshmati, A. (2015). Impact of cooking procedures on antibacterial drug residues in foods : A review. *Journal of Food Quality and Hazards Control* 2: 33–37.

Idowu, F., Junaid, K., Paul, A., Gabriel, O., Paul, A., Sati, N., Maryam, M. and Jarlath, U. (2010). Antimicrobial screening of commercial eggs and determination of tetracycline residue using two microbiological methods. *International Journal of Poultry Science* 9(10): 959–962.

Joint Expert Advisory Committee on Antibiotic Resistance - JETAC AR. (1998). *The use of antibiotics in food-producing animals :* Antibiotic - Resistant Bacteria in Animals and Humans. 249pp.

Katakweba, A. A. S. (2014). Prevalence and molecular studies of antimicrobial resistance in bacteria from farm animals, wildlife, pets and human in Tanzania. A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy of Sokoine Urniversity of Agriculture. Morogoro, Tanzania. 243pp.

Kempe, M. and Verachtert, B. (2000) Cartridges with molecularly imprinted recognition elements for antibiotic residues monitoring in milk cream. Pure and Applied Biochemistry, Lunds Universitét Centre for Chemistry and Chemical Engineering Getingevagen, Lund. pp1-10.

McEwen, S. A. and Fedorka-Cray, P. J. (2002). Antimicrobial use and resistance in animals. *Clinical Infectious Diseases* 34 (Supplement 3): S93-S106.

Ministry of Livestock and Fisheries Development (2011). Tanzania Livestock Sector Development Programme. *7th African Dairy Conference and Exhibition, MovenPick Palm Hotel*. 123pp.

Mubito, E. P., Shahada, F., Kimanya, M. E. and Buza, J. J. (2014). Antimicrobial use in the poultry industry in Dar-es-Salaam , Tanzania and public health implications. *American Journal of Research Communication* 2(4): 51–63.

Narrod, C., Tiongco, M. and Costales, A. (2012). Global poultry sector trends and external drivers of structural change. *Poultry in the 21st Century: Avian Influenza and Beyond*. pp1–28.

Nisha, A. R. (2008). Antibiotic residues – A global health hazard. *Veterinary World* 1(12): 375-377.

Nonga, H. E., Simon, C., Karimuribo, E. D. and Mdegela, R. H. (2010). Assessment of antimicrobial usage and residues in commercial chicken eggs from small-holder poultry keepers in Morogoro municipality, Tanzania. *Zoonoses and Public Health* 57(5): 339–344.

Olatoye, I. O. and Ehinmowo, A. A. (2010). Oxytetracycline residues in edible tissues of cattle slaughtered in Akure, Nigeria. *Nigerian Veterinary Journal* 31(2): 93-102.

Passantino, A. and Russo, C. (2008). Maximum residue levels of veterinary medicines in relation to food safety: european community legislation and ethical aspects. *Journal für Verbraucherschutz und Lebensmittelsicherheit* 3(4): 351- 358.

Reig, M. and Toldrá, F. (2008). Veterinary drug residues in meat: Concerns and rapid methods for detection. *Meat Science* 78 (1-2): 60-67.

Salehzadeh, F., Madani, R., Salehzadeh, A., Rokni, N. and Golchinefar, F. (2006). Oxytetracycline residue in chicken tissues from Tehran slaughterhouses in Iran. *Pakistan Journal of Nutrition* 5(4): 377-381.

Sczesny, S., Nau, H. and Hamscher, G. (2003). Residue analysis of tetracyclines and their metabolites in eggs and in the environment by HPLC coupled with a microbiological assay and tandem mass spectrometry. *Journal of Agricultural and Food Chemistry* 51(3): 697–703.

Seri, H. I. (2013). Introduction to veterinary drug residues : Hazards and Risks. *Veterinary Drug Residues in Food Derived from Animals*, 1–7.

Sirdar, M. M., Picard, J., Bisschop, S., Jambalang, A. R. and Gummow, B. (2012). A survey of antimicrobial residues in table eggs in Khartoum State, Sudan, 2007–2008. *Onderstepoort Journal of Veterinary Research* 79(1): 9.

Swatantra, S., Sanjay, S., Neelam, T., Nitesh, K. and Ritu, P. (2014). Antibiotic residues: A global challenge. *Pharma Science Monitor; An International Journal of Pharmaceutical Sciences* 5(3): 184–197.

The GARP-Tanzania (2015). Situation analysis and recommendations: Antibiotic use and resistance in Tanzania. *Washington, DC and Nwe Delhi: Center for Disease Dynamics, Economics & Policy*. 118pp.

United Republic of Tanzania, (2016). Ministry of livestock and fisheries development. *Budget Speech 2016-17*. 153pp.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Concentrations of tetracyclines in commercial chicken eggs samples collected from layer farms in Dar es Salaam and, levels of exposure to tetracyclines' residue in commercial chicken eggs for Dar es Salaam consumers were determined with consideration to the set MRL by CAC to assess safety of commercial chicken egg consumers. This study reflects the outcomes of improper usage and absence of antibiotic (tetracyclines) legislation and monitoring systems in intensive poultry farms in Dar es Salaam. There is a great potential health risk of exposure to high levels of tetracycline residues for Dar es Salaam consumers specially kids, who consume commercial chicken eggs. OTC residue was much higher in raw eggs than in fried and boiled. Nevertheless, TC residues were much reduced in boiled egg samples than in fried egg samples. Meanwhile, higher levels of tetracycline residues above MRL were observed in raw eggs.

Indigenous chicken eggs are mostly produced and consumed in peri-urban and rural areas though this study could not consider them due to financial constrain. Therefore, this remains as the research gap that should be considered for future studies.

5.2 Recommendations

There is no doubt that neither humans nor animals can live without antibiotics as they are some of the most effective antimicrobial treatments. However, the misuse of antibiotics may result in the afore-mentioned health hazards. Thus, the reduction of antibiotic use constitutes a challenge for the world. In order to achieve such a reduction, the following steps should possibly be considered with regard to all antibiotics;

i. The effective prevention of infectious diseases, adoption of strict hygiene standards and rearing skills may reduce our need for antibiotics, particularly in the veterinary field.

ii. The use of alternatives to antibiotics, such as vaccination against some bacterial diseases may be of great value in the near future.

iii. To establish and strengthen the regulatory system in terms of safety standards, proper monitoring, surveillance, testing and public education, will avoid the unnecessary use of antibiotics and ensure the proper antibiotic use that safeguards public health and food safety.

iv. Antibiotics use in feed additives should be ceased.

v. Avoid using antibiotics in the veterinary field without a veterinarian's prescription and prohibition of sale of veterinary medicinal formulas in kiosks and supermarkets.

vi. Strict observation of antibiotic cessation (withdrawal periods) periods should be made.

However, more studies should be conducted to quantify tetracycline residues in indigenous chicken eggs. This will provide more baseline information on whether this kind of eggs also contains tetracycline residues or not for the public health concern.