INVESTIGATION OF OXYTETRACYCLINE USE AND ABUSE: DETERMINATION OF ITS RESIDUES IN MEAT CONSUMED IN DODOMA AND MOROGORO MUNICIPALITIES

BY

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ABSTRACT

In spite of oxytetracycline (OTC) being a widely used drug in livestock production in Tanzania for many years, there is no published information on its use and occurrence of its residues in the meat consumed in the country. This study was aimed at establishing the mode of OTC use, the occurrence and levels of its residues in the meat consumed in Dodoma and Morogoro regions. The mode of OTC use was studied by the administration of a questionnaire to respondents in some of the slaughter cattle catchment areas. The residue occurrence levels were accomplished by analysis of 131 muscle, liver and kidney samples by using the high performance liquid chromatography (HPLC) technique. OTC sample extraction was done using a pH 4.0 Mcllavaine-EDTA buffer and cleanup by application on Supelclean LC-18 solid phase extraction (S.P.E.) cartridges. OTC quantification was done by using an ATIUNICAM H.P.L.C. system with an electron capture UV detector set at 350nm. The separation of OTCs was carried out by an RP 8-10 Lichrosorb (4.6 mmd x 25cm) column and a Methanol: Acetonitrile: 0.01Maq. Oxalic Acid mobile phase in the ratio 1:2:7 V/V/V, respectively. The study established a high degree of OTC abuse of use especially by the livestock keepers through overdosing by as much as 10-20 times the recommended therapeutic doses, use of wrong routes of administration, arbitrary drug combinations and non-observance of the OTC withdrawal period. Out of the 131 beef samples analysed, 54 (41.2%) had detectable OTC residues. Furthermore 41 (31.3%) samples had violative OTC residues levels compared to the FAO/WHO 1999 OTC maximum residue levels (MRLs) of 0.2 mg/kg (muscle), 0.6 mg/kg (liver) and 1.2mg/kg (kidney). Violative OTC residues ranged from 0.52 mg/kg to 8.98mg/kg of tissue. The 31.3% violative samples, coupled with the high mean OTC residue concentrations observed were very significant compared to those of other

countries. It is an indication of the seriousness of the OTC abuse problem with its attendant residue occurrence and harmful effects on public health, our international trade of animal products and the environment. Deliberate steps need to be taken by the state to redress the situation before it gets worse.

DECLARATION

I, Louis Mshakale Gregory Mmbando do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has not been submitted to any other University for a degree award.

Signature: _____

Date: 29/9/2004.

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LIST OF ABBREVIATIONS

≤	Less than or equal to
≥	Greater than or equal to
®	Trade mark
AC-N	Acetonitrile
ADH	Anti diuretic hormone
AFOs	Agriculture Field Officers
ANOVA	Analysis of Variance
AUC	Area under curve
BQ	Black quarter
CA	Citric acid
CBPP	Contagious bovine pleuropneumonia
CCPP	Contagious caprine pleuropneumonia
CFT	Compliment fixation test
CPss	Plasma steady state concentration
CRD	Chronic respiratory diseases
CSF	Cerebral spinal fluid
CTC	Chlortetracycline
DALDO	District Agriculture and Livestock Development Officer
DHP	Disodium hydrogen phosphate
DNK	Do not know
ECF	East coast fever
EDTA	Disodium ethylenediamine tetracetic acid
FAO	Food and Agricultural Organization

FDA	Food and Drug Administration
Fig	Figure
FMD	Foot and mouth disease
GIT	Gastro-intestinal tract
HCL	Hydrochloric acid
HPLC	High performance liquid chromatography
I/M	Intramuscular
I/V	Intravenous
JECFA	Joint FAO/WHO Codex Expert Committee
KETRI	Kenya Trypanosomiasis Research Institute
LA	Long acting
LC	Liquid chromatography
LFOs	Livestock Field Officers
LSD	Lumpy skin disease
McB	Macllavaine buffer
MeoH	Methanol
Mg/kg	Milligram/kilogram
Ml	Milliliter
mm	Millimeter
MOAC	Ministry of Agriculture and Cooperatives
MRL	Maximum residual level
MWLD	Ministry of Water and Livestock Development
O.E.S	Oxytetracycline elution solution
O.S.S	Oxytetracycline separation or extraction solution

°C	Celsius
OTC	Oxytetracycline
OTC-t_r	Oxytetracycline retention time
OX-AC	Oxalic acid
Ppm	Parts per million
R.E.S	Reticuloendothelial system
SNAL	Sokoine National Agricultural Library
SPE	Solid phase extraction
SUA	Sokoine University of Agriculture
T.V.A	Tanzania Veterianary Association
TC	Tetracycline
TSZ	Tanzanian Shorthorn Zebu
USA	United States of America
UTI	Urinogenital tract infections
UV	Ultraviolet
Vet	Veterinary
VICs	Veterinary Investigation Centers
WHO	World Health Organization
WP	Withdrawal period
µg/l	Microgram/litre

CHAPTER ONE

1.0 INTRODUCTION

The world population which has been doubling almost every two decades reached the 6 billion mark on mid year 2000 (UN Population Declaration, 2000). Correspondingly, the Tanzanian population was 23.1 million in 1988 (National Census, 1988) and with an annual growth rate of 2.8% reached 34.5 million in 2002 (National Census, 2002).

With this rapid increase in population a need arises to use technological means of increasing food production to meet demand. The provision of adequate amounts of protein has therefore necessitated the use of improved animal husbandry techniques to enhance the production of animal food products such as meat, milk and eggs (Kazwala, 2000). This has resulted in the use of either pharmacologically, toxicologically or environmentally active substances such as drugs, feed additives, and pesticides for management of animal diseases and improvement of animal production performance (FAO/WHO, 1984).

However ingestion and administration of drugs or feed additives to food producing animals may result in their accumulation in body tissues, organs and secretions. This may ultimately end up in the human food chain as drug residues (Suliman, 1976; Chewulukei, 1978; Rousdant and Moreitain 1990; Hisao and Keiichi, 1991; Omija *et al.*, 1994; McEnvoy *et al.*, 2000; Aboge *et al.*, 2000; Kurwijila *et al.*, 2000; Muriuki *et al.*, 2001).

Surveys conducted in Germany (Grove *et al.*, 1970), USA (Huber *et al.*, 1971; Tittiger *et al.*, 1980), Canada (Moats *et al.*, 1985), Japan (Oka *et al.*, 1991) and in Kenya (Muriuki *et al.*, 2001) have reported presence of violative antibiotic residues in meat products from cattle, poultry and pig. Causes of violative antibiotic residues were reported to be, among others, use of drugs in wrong species, overdosage (Paige *et al.*, 1997), and non-observance of withdrawal periods (Oka *et al.*, 1991; Paige *et al.*, 1997).

Consumption of animal products containing residues can lead to accummulation in tissues, or to development of chronic effects in humans and animals (Lever, 1972; FAO/WHO, 1984; Hisao and Keiichi, 1991; Omija *et al.*, 1994; Jones, 1999; Omore *et al.*, 1999, Muriuki *et al.*, 2001). Further there may be economic losses and environmental effects (Sozzi and Smiley, 1980; Oberg and Sandine, 1985; Shahani and Whaler, 1986; Marshal *et al.*, 1990; Aalback *et al.*, 1991).

There is currently very scanty information on the extent of misuse or overuse of drugs in Tanzania. Furthermore, not sufficient information is available on the level of residues in products of animal origin. The incidence of chronic non-infectious conditions is on the increase (Kambarage *et al.*, 1992; Kazwala, 2000). It is suspected that natural or synthetic chemicals present in feeds and large-scale misuse of drugs for prophylaxis and therapy cause a large proportion of these conditions.

Neither boiling nor pasteurization of animal food products eliminates residues completely. For example the boiling of milk to 100^0C can deactivate only 50% of residual penicillin, 66% of residual streptomycin and up to 90% of oxytetracyclines. Chloramphenicol is

almost totally resistant to pasteurization and boiling (Hamman *et al.*, 1978; International Dairy Federation, 1979). Normal cooking and roasting of meat degrades tetracycline residues retained in meat products at 5-10ppm to negligible levels of < 0.01ppm (FAO/WHO, 1983). It also degrades 50% of OTC's contained in livers (Katz *et al.*, 1972). However, further studies have to be done on the heat stability of bound tetracyclines' residues (Körner *et al.*, 2001). While freezing temperatures degrade penicillin in tissues gradually retaining detectable levels for about 21 days, freezing temperatures have little effect on oxytetracycline and dihydrostreptomycin (Mercer *et al.*, 1975).

To limit occurrence of harmful effects of drug residues in humans and animals, various regulatory and control measures have been affected. These have included the promulgation of laws, the imposition of monitoring procedures, and setting maximum acceptable residue limits (MRL) in animal food products (Fletouris *et al.*, 1990; FAO/WHO, 1995). Different countries have their own control regulations and MRLs based on the joint FAO/WHO Codex Expert committee or JECFA recommendations. For example while the JECFA 1999 (FAO/WHO 1999) recommended MRLs for oxytetracycline (OTC), chlortetracycline (CTC) and tetracycline (TC) to be 0.2mg/kg for muscle tissue in cattle and pigs, and 1.2mg/kg for kidney tissue in cattle and pigs, corresponding U.S.A recommended values were 0.1mg/kg for muscle tissue and 4.0mg/kg for kidney in cattle and pigs, respectively.

Various drugs including antimicrobials, trypanocides, anthelmintics and antitheillerials are extensively used in the control of livestock diseases in Tanzania. According to field

reports, drug use in Tanzania exhibits an apparent large-scale violation of their use procedures.

OTC is apparently the most extensively used drug in food producing animals in Tanzania. It is widely and unlawfully dispensed by the untrained and inadequately trained livestock farmers and extension workers (Gracey, 1986). The development of bacterial resistance and physical persistence of OTC residues in animal food products are the two major concerns in public health (White *et al.,* 1993). Some of the adverse effects such as development of bacterial resistance do not select between the meat consumer and non-consumers when their ineffective therapeutic effects set in. And even when residue levels are kept below the minimum levels that produce hypersensitivity, the long term toxic effects which may result from a continuous low level intake remain unknown (Collins - Thompson, *et al.,* 1988).

The extent of drug (OTC) abuse in Tanzania is not well understood. Factors indicative and leading to this antibiotic abuse are not statistically documented. The impact of the drug abuse on the occurrence of OTC residues is not well known. Recently OTC residue levels have been reported in a survey carried out in the neighbouring country of Kenya in chicken (Omija *et al.,* 1994), and beef (Muriuki *et al.,* 2001). Some samples had violative OTC and CTC levels, well above the recommended FAO/WHO MRLs. Considering the cross border movement of cattle along the northern border between Tanzania and Kenya, it would not be surprising to find comparable OTC residues in meat consumed in especially northern Tanzania.

Nor is qualitative or quantitative data on other individual antimicrobials such as CTC, TC, penicillin, and sulfonamides in milk, meat or eggs available.

Tanzania's annual meat consumption for the year 2000 was 350 000 metric tonnes consisting of 181 000 tonnes of beef, 72 000 tonnes goat meat, 20 000 tonnes of pork and 20,000 tonnes poultry meat (Ministry of Water and Livestock Development, 2001). About 99% of the beef consumed in the country originate from slaughtered Tanzania Shorthorn Zebu (TSZ) cattle.

OTC is one of the most extensively used and probably most abused drug in the country (Kurwijila *et al.*, 2000; Magoma and Magayane, 2000). The relatively inaccessible livestock personnel and livestock farmers whose training on chemotherapy is essentially inadequate or none handle this drug (Gracey, 1986). Meanwhile, inspite of the concerted efforts by the Tanzania Food and Drugs Authority to regulate and control the trading and use of drugs in Tanzania, violation of pharmaceutical laws and regulations has continued. This has led to all sorts of drug abuse with predictable consequences.

To protect the public from the hazardous effects caused by the presence of violative OTC residues in animal food products their monitoring and control in edible animal tissues and organs should be a top priority obligation by State public health agencies. The concentration of OTC residues, as well as other tetracyclines, in the livers and kidneys, has on the average been 4 - 5 times greater than those found in muscles. This has formed the basis of screening for residual tetracycline in livers and kidneys of slaughtered animals as the most effective way of monitoring their violative concentrations (Huber *et al.*, 1975;

Booth and Bocker, 1979). Actually the higher concentrations in those two organs are a direct result of the pharmacokinetics of tetracyclines whereby bile levels can be as much as 20-30 times greater than the corresponding serum levels. For the liver, this is reported to be due to enterohepatic resorption (Huber, 1988; Riviere and Spoo, 2001). The higher kidney concentrations are reported to be a result of its renal excretory function, the main route of elimination of tetracylines except for doxycycline and minocycline (Gibaldi, 1991; Riviere and Spoo, 2001).

In Tanzania there is neither a national maximum acceptable residue level (MRL) for OTC in milk, meat or eggs nor monitoring procedures for its control. These are necessary to protect consumers from a diversity of would be harmful effects. The export of meat to the international markets as envisaged in the National Development Vision 2000-2020 demands knowledge on various drug residue levels and especially of OTC considering its extensive use and abuse nationally. The results obtained will aid in the provision of data for the establishment of a National MRL for OTC, promulgation of new laws and regulations for the pharmaceutical industry and business, pharmaceutical handling and administration procedures in the country.

It is on this background that this study was carried out aiming at investigating the extent of OTC abuse in Tanzania and the extent of occurrence of violative OTC residue levels in meat intended for human consumption. Therefore, the main objectives of this study were to investigate the extent of OTC abuse, the presence, and extent of OTC residues occurrence in meat consumed in the Dodoma and Morogoro Municipalities.

The specific objectives were:

(i) To establish the dosage regime or use pattern, mode of application of OTC by livestock keepers and personnel in Morogoro and Dodoma catchment areas.

(ii) To determine the occurrence and concentration of OTC in raw liver, kidney and muscle tissues from slaughtered cattle in the two municipal abattoirs.

(iii) To relate OTC levels with JECFA (FAO/WHO-1999) MRL values.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definitions of terminology

Throughout this study various terminologies have been used which are used in pharmacology and toxicology and the following definitions as used by the WHO/FAO JECFA committee have been provided.

2.1.1 Antibiotic, drug or chemical residue

This is the deposition or accumulation of a parent compound or its metabolites or decomposition products within cells, tissues or organs following their use for prophylaxis, treatment of disease or as a feed additive to promote growth and feed efficiency. These residues or accumulations have potential toxicological significance (Jackson, 1980). They are expressed as; parts per weight e.g. mg/kg or mg/l i.e. ppm (μg/g or μg/ml), μg/kg or μg/l (i.e. ppb) or ng/kg or ng/l (ie.ppt).

2.1.3 Acceptable daily intake (ADI)

This is the maximum daily intake of a drug or chemical, which can be ingested in a lifetime without appreciable or deleterious effects to health of the consumer considering the facts available at the time (Booth, 1988). It is expressed in mg of the drug (chemical) per kg body weight per day. The values for oxytetracycline and tetracycline are 0.3 μg/kg bwt/day and 0.5μg/kg bwt/day respectively while that of benzylpenicillin is 30μg/kg bwt/day (FAO/WHO, 1990).

2.1.4 Maximum residue level/Tolerance level

This is the maximum permissible level or concentration of a drug or chemical in or on a feed or food at a specified time of slaughter and harvesting, processing, storage and marketing up to the time of consumption by animal or human. They are expressed in parts by weight of the drug/chemical per million i.e. ppm or mg/kg or mg/l or parts per billion ppb i.e. μg/kg or μg/l of the food and are never greater than the permissible level of feed or food in reference. There are three major types of tolerances in defining permissible concentrations of drug residues; finite tolerance, negligible tolerance and zero tolerance (Booth, 1988).

2.1.5 Biovailability of a drug

This refers to both the rate and extent of absorption of a drug. The extent of absorption means the fraction or percentage of the oral or parenteral dosage form, which reaches the sytemic circulation intact (Booth, 1988).

2.1.6 Drug/Chemical withdrawal period

This is the time required for the depletion of a toxicologically potential residue to reach a safe concentration as defined by tolerance (Booth, 1988). It also refers to the time interval between termination of treatment to an animal and the time of consumption of its products such as milk and meat on slaughter.

2.2 General information

Intensification of production of animal protein to meet world human consumption demands in the face of potential world food shortages has necessitated the adoption of improved animal husbandry techniques to optimize production capacities. These techniques have involved the use of various drugs and chemical compounds in the diagnosis, prophylaxis, control and treatment of various diseases. Other uses have included use as feed additives to promote growth, improve feed efficiency or utilization, synchronization or control of the reproductive cycle and breeding performance, enhancement of feed acceptability or palatability to animals and enhancement of human acceptability of the animal end product (Booth, 1988).

In the USA for example by the early 1970's use of drugs had expanded to such an extent that 80% of all the food-producing animals were receiving some kind of medication during, part or most of their lifetime (Shillam, 1974). It was expected that in future most if not all food-producing animals in the USA would receive some kind of a chemotherapeutic, prophylactic or feed additive agent (Booth, 1988). While the use of these drugs and or additives has been necessary for efficient and improved animal production of meat, milk and eggs, their indiscriminate use should not serve as a substitute for good management (Booth, 1998). However the use of these physiologically, pharmacologically and environmentally active substances in animal production has not been without shortcomings some of which could be severe. The main shortcoming consequent to their use has been the occurrence of the parent drugs or compounds or their metabolites or their degradation products as residues in animal food products.

Surveys carried in various countries have confirmed the occurrence of violative levels of OTC and other antibiotics such as penicillin in a substantive number of organs and tissues including milk at Kenya creameries (Chewulukei, 1978), milk in Tanzania (Kurwijila *et al.*, 2000), Chicken meat (Omija *et al.*, 1994), and beef (Muriuki *et al.*, 2001) both in Kenya. Others include those carried out in other countries like the U.S.A (Huber *et al* 1971; Moats *et al* 1995), Germany (Grove *et al.*, 1970), Canada (Tittiger *et al.*, 1980) and Japan (Oka *et al.*, 1991).

Their occurrence may be due to direct administration during therapeutic and prophylactic work (Chewulukei, 1978; FAO/WHO, 1984; Booth, 1988; Kazwala, 2000). Alternatively, they could be in feeds as feed additives (Jones, 1978; Booth, 1988; Riviere and Sundlof, 2001). They could also occur due to ingestion of contaminated feed (McEnvoy *et al.*, 2000) or in other cases due to a deliberate adulteration of animal food products e.g. milk in an attempt to extend their shelf life prior to marketing (Aboge *et al.*, 2000). The levels of drug residues in milk, meat and eggs is a measure of the exposure level of an animal to veterinary and livestock production drugs and substances respectively and consequently the level of risk encountered by man upon their consumption.

Violation of drug use procedures include; dispensing to the wrong species and over-dosing (Paige *et al.*, 1997) and non adherence to drug withdrawal periods (Oka *et al.*, 1991; Paige *et al.*, 1997). These are some of the causes, which contribute to the occurrence of antimicrobial and other drug residues in animal food products. In Japan it was found that 49 out of 271 slaughtered animals had violative tetracycline residues due to non-observance of their withdrawal periods (Oka *et al.*, 1991).

Drug misuse in prophylaxis and therapy has been cited as the principal source of drug residues in animal foods especially after market liberalization in Tanzania and elsewhere (Kurwijila *et al.*, 2000; Aboge *et al.*, 2000). Some drugs persist in body tissues beyond their recommended withdrawal times (Huber 1988; Jones, 1999). A pilot study conducted in Mwanza and Dar es Salaam cities to check for the presence of tetracycline and penicillin residues in milk showed that 34.48% and 40.9% respectively out of 402 milk samples tested positive with the Charm AIM-96 antimicrobial inhibition test (Kurwijila *et al.*, 2000). Also, 62.6% of Zebu cattle (TSZ) in Dar es Salaam tested positive indicating a higher contamination in the local breeds than in the exotic ones (Kurwijila *et al.*, 2000).

The severity of the antimicrobial residues contamination in Tanzania can be appreciated when the results of the Mwanza/Dar es Salaam study are compared with those of a similar study conducted in Kenya which had an overall 9.4% of consumer level and 5.7% of market level milk samples responding positive to residue contamination on the same Charm AIM-96 test (Aboge *et al.*, 2000), indicating that the residues originated at farm level. The Mwanza/ Dar es Salaam occurrence levels at 34.48% and 40.9% respectively are comparatively very high. Probably this could be true countrywide. While no substantive studies have been conducted in Tanzania on this problem, more extensive studies are required to identify antimicrobial residues, their extent of occurrence, concentration levels and human and animal pathophysiological effects (Kambarage *et al.*, 1992). Similarly, these parameters in the case of meat consumed in the country are also unknown.

The occurrence of drug residues in animal food products has harmful economic, environmental, animal and public health effects. This has given rise to the increased level of public concern over their occurrence (Lever, 1972; O'Brien *et al.*, 1982; FAO/ WHO, 1984; Katz, 1983; Levy *et al.*, 1988; Marshal *et al.*, 1990; Hisao and Keiichi, 1991; Aalback *et al.*, 1991; Nijsten *et al.*, 1996a).

The human and animal health effects include:

(a) Drug related side effects such as gastrointestinal disturbances by tetracyclines (Katz, 1983; Huber 1988; Riond *et al.*, 1989a; 1992; Riviere and Spoo, 2001; Kamel and Brown, 2002).

(b) Disruption of control mechanisms (Katz, 1983). This may lead to syndromes like nephrogenic diabetes insipidus (Kamel and Brown, 2002) and immunosuppression in the reticuloendothelial system (R.E.S) of food producing animals (Huber, 1988) both of which are caused by tetracyclines.

(c) Direct toxic effects (Katz, 1983) such as hepatotoxicity and nephrotoxicity caused by tetracyclines (Schulz *et al.*, 1963; Moffit *et al.*, 1974; Kamel and Brown, 2002) and phototoxicity (Riviere and Spoo, 2001; Kamel and Brown 2002) both caused by tetracyclines, and permanent tooth growth impairement and discolouration (Grossman *et al.*, 1971).

(d) Hypersensitivity reactions (Oslon and Sanders 1975; Katz, 1983; Burgat, 1984; Dewdney and Edward, 1984). For instance about 5-10% of the human population is allergic to penicillin residues as low as 1ppb of penicillin/ml of milk. The variety of allergies range from minor rashes to anaphylactic shock (Goodman, 1965; Stewart, 1973; Jones, 1999). Even when residue levels are kept below those which can initiate an allergic reaction, their long term cumulative effects from a

continuous low level intake may remain unknown (Collins-Thompson *et al.*, 1988).

(e) The development of bacterial resistance. There has been an increase in resistance in bacteria of human and animal origin due to the extensive use and perhaps misuse of antibiotics (Aalback *et al.*, 1991). This has been precipitated not only from the selection and spread of resistant microorganisms but also from intra and inter-species transfer of antibiotic resistance genes via plasmids (Marshal *et al.*, 1990) and transposons (Chopra, 1986; 1988; Sanchez *et al.*, 1988). This has been revealed by tracking down antibiotic resistant plasmids through the analysis of whole plasmids and those digested by restriction endonucleases (O'Brien *et al.*, 1982; Nijsten *et al.*, 1996b). A common pool of resistant and susceptible microorganisms mutually shared by man and animals exist (Levy et *al.*, 1976; Linton *et al.*, 1977; Mee and Nikolethi, 1983; Nijsten *et al.*, 1996b). From this common pool man acquires resistant bacteria, their genes and plasmids from animals and vice versa directly through contact (Nijsten *et al* 1996a) and indirectly by ingesting meat contaminated with the animals faecal flora (Linton *et al.*, 1977; Nijsten *et al.*, 1996a).

Economic losses incurred include:

(a) Partial or complete inhibition of starter cultures used in milk fermentation in the production of secondary dairy products such as yoghurt and cheese. The inhibition interferes with the production of the desired acidity and flavour of these products reducing their quality and market competitiveness (Marth and Ellickson, 1959; Sozzi and Smiley, 1980; Friuend and Shahani, 1983; Oberg and Sandine, 1985).

(b) The presence of tetracycline residues interferes with the validity of quality control tests such as the milk phosphatase pasteurization test. For example the presence of OTC may cause fully or partially pasteurized milk to appear as non- pasteurized (Manolkidis *et al.*, 1971).

Environmental effects incurred are the increase in the pool of antimicrobial resistant microbe population (Aalback *et al.*, 1991). This may cause an imbalance in the entire ecosystem if unchecked.

The growing concern on drug residues in animal food products led to the formation of a joint FAO/WHO Expert Committee called JECFA committee on Residues of Veterinary Drugs (CCRVD) in 1986 to regulate and control drug residue levels. The FAO/WHO expert committee sets Maximum Residue Limits (MRL'S) for various drugs permissible. The MRL values for oxytetracyclines for all species in 1999 were: 0.2mg/kg for beef, 0.6mg/kg for liver, 1.2mg/kg for kidney, 0.1mg/kg for milk, 0.4 mg/kg for eggs and 0.02mg/kg for fat.

2.3 Tetracycline antibiotics

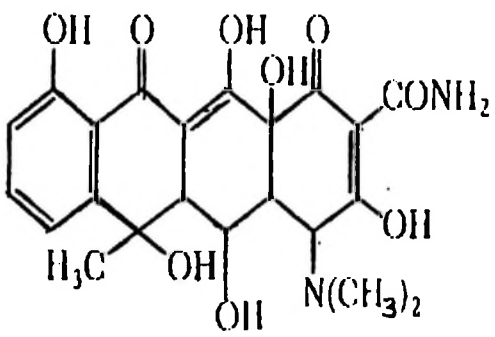
2.3.1 Introduction

Tetracyclines consist of structurally and pharmacologically related semi-synthetic group of antibiotics. They are derived from mould of the genus *Streptomyces*. In addition the term tetracycline also refers to a specific antibiotic within the group of tetracycline

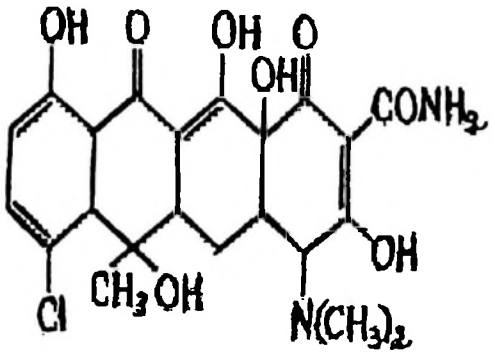
antibiotics (Huber, 1988; Riviere and Spoo, 2001). Historically, the tetracyclines were discovered from a systematic investigation of about 100,000 soil samples world wide during the 1940's and 1950's and about 75 antibiotic producing moulds were discovered (Brander *et al.*, 1991; Riviere and Spoo, 2001). Among them was *Streptomyces (Actinomycetes) aureofaciens, which* was found to produce chlortetracycline and *Streptomyces rimosus*, which produced oxytetracycline (Huber, 1988; Brander *et al.*, 1991).

2.3.2 Chemistry

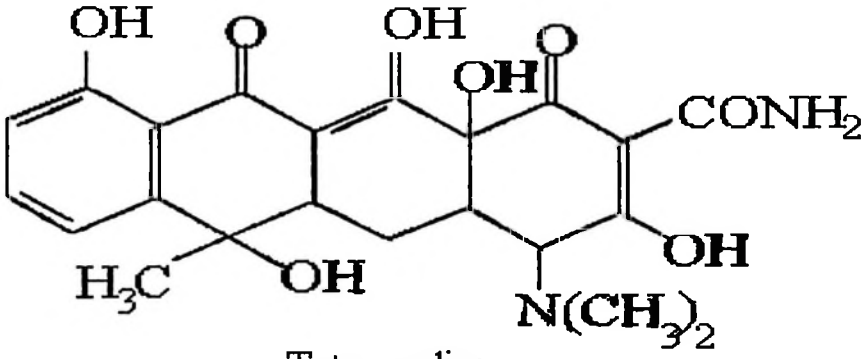
Tetracyclines occur as tawny yellowish, odorless, slightly bitter and powdery crystalline bases. Chemically they (Fig. 1) are four ringed amphoteric compounds differentiated by radical substitutions on the rings. All tetracyclines form salts with both acids and bases (Huber, 1988; Brander *et al.*, 1991; Riviere and Spoo, 2001). They are hygroscopic exhibiting an acid pH in aqueous solutions (Riviere and Spoo, 2001) a crucial factor in the preparation of their extraction buffers from various tissues for analytical purposes. For therapeutic use they are mainly prepared as their hydrochloride salts (Riviere and Spoo, 2001) especially for parenteral injection. The chemical structure of the commonly used tetracyclines are shown in Figure 1 (Huber, 1988; Riviere and Spoo, 2001).



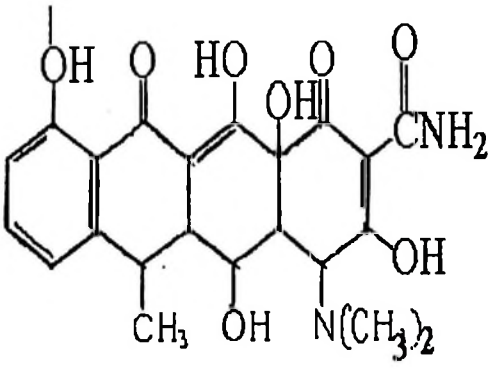
Oxytetracycline



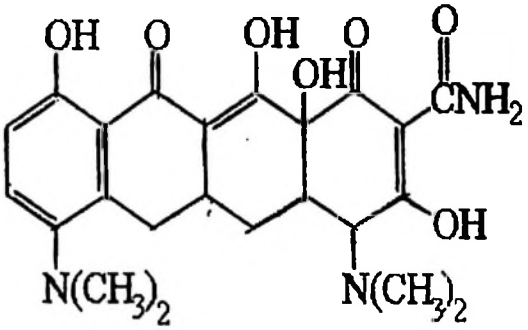
Chlortetracycline



Tetracycline



Doxycycline



Minocycline

Figure 1: Molecular structures of commonly used tetracyclines

The physical and chemical properties of the commonly used tetracyclines are shown on Table 1.

Table 1: Physical and chemical properties of the commonly used tetracyclines

Drug	Molecular weight	PK_a
Chlortertracyline	478.88	3.3, 7.4, 9.3
Doxycycline	462.46	NA
Minocycline	457.48	2.8, 5.0, 7.8, 9
Oxytetracycline	460.44	NA
Tetracycline	444.43	8.3, 10.2

NA = information not available

2.3.2.1 Oxytetracycline ($C_{22}H_{24}N_2O_9$)

Oxytetracycline occurs mainly in two forms, i.e. the dihydrate form or amphoteric OTC and the hydrochloride form or OTC salt (Brander *et al.*, 1991). It is very stable compared to chlortetracycline (Brander *et al.*, 1991). This is an important property with respect to its extraction and analytical processes.

(1) Oxytetracycline dihydrate ($C_{22}H_{24}N_2O_9.2H_2O$)

Synonyms: Oxytetracycline, Terrafungine

Chemical name: 4-Dimethylamino - 1, 4, 4a, 5, 5a, 6, 11, 12a - Octahydro - 3, 5, 6 10, 12, 12a - hexahydroxy - 6- methyl-l, 11-dioxo aphthadene-2-carboxyamide dihydrate. Its Molecular weight (Mwt) is 496.5 while its molecular structure is as shown in Figure 2.

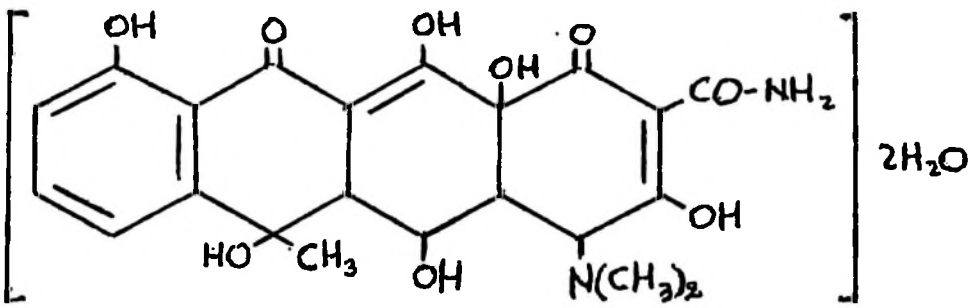


Figure 2: Molecular structure of oxytetracycline dihydrate

Physically it is a tawny crystalline powder with a specific rotation of -188^0 to -120^0 of a 1.0% solution in 0.1N hydrochloric acid. It has a solubility of 1 in 2000 in water and 1 in 100 in ethanol. It deteriorates in solution with a pH < 2 and is rapidly destroyed by alkalis (White, 1984).

(2) Oxytetracycline hydrochloride (C_{22} H_{24} N_2 O_9. HCl)

This is the most common form of preparation for commercial use (Huber, 1988; Riviere and Spoo, 2001). Its chemical name is; 4-Dimethylamino- 1,4,4a, 5, 5a, 6,11,12a octahydro–3,5,6,10,12,12a–hexahydroxy–6-methyl–1,11-dioxonaphthacene–2carboxyamide Hydrochloride (White, 1984). Its molecular structure is shown on Figure 3.

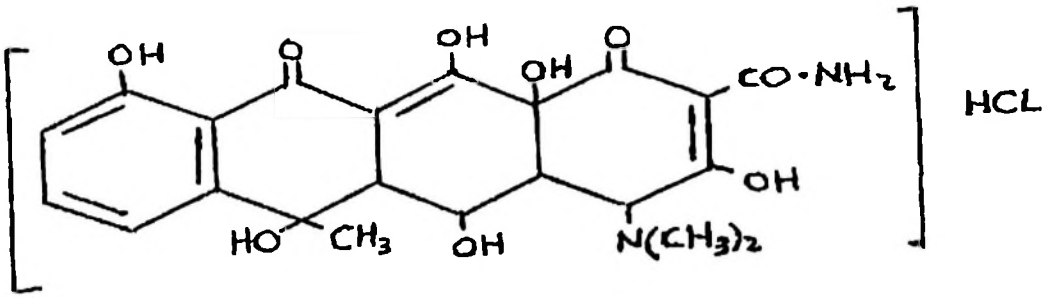


Figure 3: Molecular structure of oxytetracycline hydrochloride

Physically it is a yellow hygroscopic crystalline powder. While its solubility is 1 in 2 of water and 1 in 45 of ethanol it is insoluble in ether and chloroform (White, 1984).

2.3.2.2 Extraction

OTC is extracted by organic solvents from aqueous alkaline solutions (White, 1984).

2.3.2.3 Analytical tests

2.3.2.3.1 Qualitative tests

Several qualitative tests are available for the qualitative analysis of oxytetracycline, and have various sensitivities. The tests include the colour tests at both the micro and the macro level, crystal tests at the micro and macro level, ultra-violet absorption spectrum, and Charm microbial inhibition tests including the Charm AIM- 96 and the Charm-ROSA tests (Kurwijila *et al.*, 2000). However, these are not specific for tetracycline. Other tests include chromatography tests using either paper, thin layer, or High Performance Liquid Chromatography (HPLC).

(1) Colour tests

The Micro-tests include the sulfuric acid-formaldehyde combination test (Margin test). A dull orange colour indicates a positive test. Its sensitivity is on the concentration range higher than 0.5 μg/ml. The sulfuric acid -ammonium molybdate test (Fröhde's test) is another colour test, but again its sensitivity is low, giving positive results in ranges greater than 0.5 μg/ml. A black purple colour indicates a postive test. The sulfuric acid – ammonium vanadate test (Mandelin's test) is sensitive on the range greater than 0.5μg/ml. A positive test is indicated by a change to a purple→ red → yellow colour. The concentrated nitric acid test (Vitalis test) also has sensitivity at concentrations greater than 0.5μg/ml. Macro-tests include concentrated sulfuric acid test, the sulfuric acid - nitrous acid (Liebermanns reagent) test, and the sulfuric acid – phenol (sulfonal) test (White, 1984). With the concentrated sulfuric acid test, addition of 2ml of the acid into 0.5ml of a sample produces a grey to purple colour for a positive test. A further addition of 2 to 4ml water produces a yellowish colour.

(2) Crystal tests

Micro tests: the gold bromide-hydrochloric acid solution test. Dense rosettes are produced within a sensitivity of 1 in 440; sodium phosphate solution-rods. They are best viewed under polarized light with a sensitivity of 1 in 100 (White, 1984).

(3) Ultra-violet absorption spectrum

OTC dihydrate in solution of a standard pH 2.0, maximum absorption at 353 nm wavelength (E1% 1cm 270 to 280); in 0.1 N sulfuric acid maximum at 269 nm

(E1%, 270-280); in 0.1N sulfuric acid, maximum at 269 nm (E1%, 1cm 400), about 320 nm (E1%, 1 cm 203), and 352 nm (E1% 1cm 2 72) (White, 1984).

(4) Chromatography

(i) Paper: System p1-Rf 0.24. Location under UV light, orange flurescence; location reagents; bromine–starch-potassium iodide, a strong reaction produced; potassium permanganate spray, a reaction is produced.

(ii) Thin Layer: System T1-Rf 0.05, streaking (Location under UV light, orange fluorescence location reagent potassium permanganate ($KMn0_4$) spray, positive reaction.

(iii) High performance liquid Chromatography (HPLC) (Mac Neil, 2000; Muriuki *et al.*, 2001).

2.3.3 Antimicrobial action

At therapeutic levels the main OTC mode of action is bacteristatic on sensitive microorganisms. At higher concentrations, it may be bacteriocidal (Brander *et al.*, 1991). The antimicrobial activity of OTC is effected by the binding of OTC to base number A 892 and protein S7 on the 30S ribosomal subunit of the 70S bacterial ribosome of susceptible organisms. This in turn interferes with the folding of the base A 892-1400 region on the rRNA (Russel and Chopra, 1990). This OTC/30S ribosomal subunit binding effectively interferes with the binding of the amino-acyl-tRNA or activated amino acid to the mRNA/ribosome complex molecule. This action effectively blocks the elongation of a microbial protein peptide chain. This blocking of the protein peptide chain elongation results in the inhibition of microbial protein synthesis of susceptible fast proliferating

microorganisms such as bacteria (Suzuka *et al.*, 1966; Russel and Chopra, 1990). OTC and other tetracyclines form stable chelate complexes with polyvalent cations such as Ca^{++}, Mg^{++}, Fe^{++} and Al^{+++} (Huber, 1988; Riviere and Spoo, 2001). This chelating effect is reported to be antimicrobial, as a result of removal of essential metallic ions from access by bacterial cells (Huber, 1988). Tetracyclines, especially chlortetracycline (CTC) interfere with the conversion of glutamate into bacterial cell protein. High concentrations inhibit bacterial cell glutamate accumulation completely (Huber, 1988), consequently exhausting the microbial glutamate pool. OTC acts synergistically with polymyxins, a property, which is useful in combination therapy.

Oxytetracycline and others in the tetracycline group have a much lesser affinity for mammalian cell ribosome, and two main reasons have been reported to account for that. The first is their higher affinity for 70S over the 80S ribosome. The second one is their accumulation in bacterial cells due to a higher efflux of tetracycline from mammalian cells by active transport (Russel and Chopra, 1990). This has resulted in a much more diminished OTC/mammalian ribosomal binding. As a consequence mammalian protein synthesis inhibition or catabolic effect is reduced significantly (Riviere and Spoo, 2001). Pharmacodynamically, this phenomenon is advantageous to mammals; it is one of the reasons for the relatively high therapeutic index of tetracyclines compared to other antimicrobials.

2.3.4 Microbial resistance to tetracyclines

Microbial resistance to tetracyclines is reported to occur. It is conferred by the tetracycline - R factor or gene which acts in 3 mechanisms. The first mechanism of resistance

development to tetracyclines is caused by plasmid and transposon encoded tetracycline efflux systems (Chopra, 1988). The expression of the plamid and transposon encoded efflux system on the bacterial membrane prevents tetracycline accumulation in the cell which prevents tetracyclines from reaching and interacting with the ribosomes. These tetracyclines encoded efflux systems found in both the G-ve and G+ve bacteria consists of several plasmid and transposon encoded membrane located resistance proteins (Russel and Chopra, 1990), which form a comprehensive network of tetracycline efflux proteins. The efflux of tetracyclines from bacterial cells is by active transport (Russel and Chopra, 1990). This expulsion of tetracyclines from bacterial cells prevents their accumulation in bacterial cells precipitating into their being blocked from interacting with base A892 and protein S7 on the 30S subunit of the 70S ribosomes. This effectively inhibits the bacteriostatic action of tetracyclines (Russel and Chopra, 1990).

The second mechanism of resistance to tetracycline is by production of plasmid and transposon coded ribosomal protection factors (Russel and Chopra, 1990). These protection factors act as alternative binding sites to tetracyclines, again inhibiting tetracycline interaction with base A892 on the 30S subunit of 70S ribosome. A good example of these protection factors is the 'tet M' resistance system found naturally on *Streptococci* and 'tet O' system found on *Campylobacter jejuni* (Russel and Chopra, 1990).

The third mechanism of microbial resistance to tetracycline is plasmid-mediated detoxification of tetracylines as reported in *Escherichia coli* bacteria grown aerobically. Transposon coded resistance transmission is the more notorious form because of the

versatility of its transmission. Unlike plamids, transposons do not need sophisticated DNA binding mechanisms (Russel and Chopra, 1990).

The main transposons involved in mediating tetracycline bacterial resistance are; T_n 10, T_n 1721 found in G-ve bacteria and T_n 916^d found in *Streptococci* (Russel and Chopra 1990). Microbial resistance against OTC normally means resistance against the other tetracyclines with the possible exception of minocycline (Brander *et al.*, 1991; Kamel and Brown, 2002). Penicillinase producing gonococci are relatively resistant to tetracyclines (Kamel and Brown, 2002). This has a clinical significance therapeutically.

2.3.5 Pharmacokinetics

The physical and chemical properties of tetracyclines allow them to be available as parenterals, boluses, capsules, powders, feed additives and ointments for administration in veterinary medicine and animal production (Huber, 1988). While the pharmacology of tetracycline bases and their salts is essentially the same, the relatively insoluble base renders it unsuitable for parenteral use. However its less bitter taste makes it more suitable for oral administration (Brander *et al.*, 1991) although encapsulated hydrochloride salts are also commonly used orally (Huber, 1988).

2.3.5.1 Administration

Various routes of administration can be used for tetracyclines depending on the type of tetracycline and animal species involved (Riviere and Spoo, 2001). The routes include topical, oral and parenteral. While most tetracyclines can be administered parenterally by intravenous (IV), only OTC can be administered by intramuscular (IM) route due to its

low-level tissue irritation. Tetracycline can also be administered by intramuscular route but only if formulated with a small amount of local anesthetic. For deep IM administration, both OTC and TC are mixed with 5% Mg Cl_2 and 2% procaine to reduce post injection pain at the injection site (Huber, 1988). However OTC in a propylene glycol and water solution can be administered without procaine (Huber, 1988). In contrast, due to its severe tissue irritation, CTC is not administered by the IM route (Huber, 1988; Riviere and Spoo, 2001). OTC and other tetracyclines can also be administered orally and topically (Huber, 1988; Brander *et al.*, 1991; Riviere and Spoo, 2001). The oral route is the most preferred for tetracyclines in order to minimize their adverse effects (Riviere and Spoo, 2001). Oral administration to herbivores leads to severe disruptions of intestinal microflora. However low subtherapeutic doses of tetracyclines especially chlortetracycline can be administered in-feed as additives to promote production (Richey *et al.*, 1977; Quarles *et al.*, 1977; Dawson *et al.*, 1983; Jones *et al.*, 1983; Zinn, 1993).

Generally the recommended IV and IM dosage for all tetracyclines is 4.4 - 11.0mg/kg Bwt per day (24 hrs). Depending on the type of disease, the dose can be increased up to 3 times (Huber, 1988). Huber (1988) recommended administration in two divided doses at 12 hour intervals in acute illness. This regime improves the therapeutic effect and reduces the possibility of shock or toxaemia from excess bacterial debris. Baggot *et al.* (1977) recommended administration of 10mg/kg as loading dose, followed by 7.5mg/kg at 12 hourly intervals for management of systemic infections caused by tetracycline sensitive bacteria.

Formulations: OTC is unique among tetracyclines in that it has both conventional and long-acting (LA) formulations. The differences in formulation are inherent to the type of solvent carriers and OTC concentrations used in the OTC injectable solutions. However, within the long acting formulations there are several types of solvent carrier systems with the same OTC concentration level i.e. of 20% or recently of 30% OTC solution.

Both formulations have their own characteristic properties in the various domestic animal species. These include the route of administration, peak serum concentrations, serum half-life (t ½), tissue irritation, tissue residual retention, etc. This has resulted in various advantages and disadvantages for each type of formulation. For example when a conventional OTC formulation was administered IM or IV to horses at 4.4mg/kg bwt two times daily, plasma concentration of this dosage regime sustained therapeutic concentrations for about 48 hrs. The same dose administered IV does not maintain the same concentrations even for 24hrs (Huber, 1988). And when a 5.0mg/kg bwt dose was administered IV, levels peaked up within 30 minutes post injection. The levels then declined steadily within 36 hrs, and could not be detected 48 hrs, post injection (Brown *et al.*, 1981). In contrast when a 10 mg/kg bwt dose was administered IV in the same species, plasma concentrations reached 16.85μg/ml within 30 minutes, declining steadily to a still relatively high concentration of 4.67μg/ml within 4 hrs (Larson and Stowe, 1981). Similar concentration versus time profiles were found in other extracellular fluids such as synovial, peritoneal and urine fluids upon IV administration. This indicates that OTC passes across those membranes readily enough to provide therapeutically adequate concentrations in those tissues for various infections (Riviere and Spoo, 2001).

The use of LA formulations has the advantage of producing prolonged (3 - 5 days) serum and tissue levels. This eliminates the need for frequent dosing (Riviere and Spoo, 2001) but is, unfortunately, characterized by persistent OTC residues posing a public health hazard (Huber, 1988). The most commonly used LA formulations in the USA consist of a combination of OTC and 2-pyrrolidone as the solvent carrier (Riviere and Spoo, 2001). Various pharmacokinetic studies of this LA formulation versus the conventional formulation have been conducted in different domestic animals other than the horse. In one study in young beef cattle, the LA formulation was administered IM. Serum concentrations reached 4 μg/ml within 60 - 90 minutes post injection and were sustained for 12 hrs. The serum $t_{½}$ was 21.8 hrs while the bioavailability was 51.5% (Toutain and Raynaud, 1983).

In a second study in cattle treated with the LA formulation serum concentrations were greater than 0.5 μg/ml about 87 hrs after administration, compared with 52 hrs for the conventional formulation (Mevius *et al.*, 1986a). A third study involving the administration of a 20mg/kg dose of either the conventional or LA OTC hydrochloride formulations produced lower LA peak levels but had a longer $t_{½}$ of 36.9hrs. The serum concentrations remained at greater than 0.5 μg/ml for 86.8 hrs (Davey *et al.*, 1985). In contrast the conventional formulation produced higher peak levels but much shorter serum $t_{½}$ of 11.1hrs (i.e.1/3 that of the LA formulation). The serum concentrations remained at greater than 0.5 μg/ml for 51.5 hrs only (Davey *et al.*, 1985) i.e. 0.58 or 3/5 that of the LA formulation. Similar findings have been observed in dairy cows & calves (Nouws and Vree, 1983), pigs (Xia *et al.*, 1983; Nouws; *et al.*, 1990), dogs (Immelman and Dreyer, 1981), and sheep (Nouws *et al.*, 1990).

Another LA formulation used in the USA consists of a combination of OTC and povidone as the solvent carrier system. Povidone is used both in the short acting and long acting formulations but in variable ratios (Huber, 1988). The short-acting formulation (i.e. the conventional one) called Liquamycin 100 consists of 100mg of OTC per ml, i.e. a 10% OTC solution, with 19% povidone. In contrast, the most commonly used long-acting Liquamycin known as Liquamycin LA-200 consists of 200 mg OTC per ml (i.e. a 20% OTC solution) with 5% povidone (Huber, 1988). The recommended dose for Liquamycin is 7-11mg/kg bwt, but may be higher depending on the disease condition, e.g. up to 20mg/kg bwt in cattle (Huber, 1988).

Parenteral administration of tetracyclines causes some tissue irritation. Various solvent systems induce variable degrees of local irritation at the injection sites (Huber, 1988; Riviere and Spoo, 2001). Hence the inclusion of local anesthetics in some IM preparations of OTC and TC (Huber, 1988). IM preparations should not be used IV to avoid cardiovascular problems. The level of tissue irritation is a function of the concentration of OTC and vehicle or carrier solvent system used (Huber, 1988; Riviere and Spoo, 2001). For example, the administration of OTC in pigs by the IV route using the short and long acting formulations indicated that the irritation at the 20% solution was greater than at the 10% formulation (Nouws, 1984). The same observations were made in dogs (Huber, 1988).

Use of OTC has sometimes resulted in tissue damage and persistent residues. When the LA formulation was used in cattle IM at the neck and thigh regions, residues persisted in those regions for more than 5 and 7 weeks, respectively (Huber, 1988). In pigs use of the

20% OTC formulation produced more persistent residues than with the 10% formulation (Nouws, 1984).

This brings into question the implication of their recommended withdrawal periods to public health concerning the meat consumed. Most LA formulation manufacturers recommend 21- 28 days as the withdrawal time before slaughter. While OTC bioavailability following S/C injection is 83-91% (Huber, 1988), its oily injections meant for S/C poultry administration should not be used parenterally in mammals (Brander *et al.*, 1991). Oral administration of OTC is mainly done to small animals at a dose rate of 33 - 110 mg/kg /day, preferably divided into 2 or 3 doses. In large animals OTC and CTC are used mainly as feed additives at sub-therapeutic levels to improve feed efficiency while acting prophylactically against some diseases at the same time (Huber, 1988).

OTC and other tetracyclines are used in the form of their hydrochloride salts topically to treat bovine mastitis. They are applied directly into the udder as an intramammary infusion of 400mg of the hydrochloride salt (Huber, 1988; Brander *et al.*, 1991). OTC administered IV diffuses in the udder into milk in therapeutically adequate concentrations for mastitis; IM administration does not (Brander *et al.*, 1991). However these parenteral administrations are not adequately effective because their milk concentrations decline sharply after the first milking. Hence an effective treatment of mastitis must be local by an intramammary infusion. It is reported that for some forms of mastitis both parenteral and intramammary treatment may be necessary (Brander *et al.*, 1991). Some OTC and CTC absorption into the blood stream and untreated udder quarters occur from treated quarters (Huber, 1988).

In ophthalmology OTC is also used locally as an ointment containing 1 mg OTC/g of ointment in the treatment of conjunctivitis. Alternatively it is used as eye drops of a buffered aqueous solution containing OTC at 5mg/ml (Huber, 1988).

2.3.5.2 Absorption

Upon absorption, much of the OTC is reversibly bound to blood proteins. The unbound portion is the one responsible for its antimicrobial activity (Huber, 1988). Irrespective of the route of administration, OTC in whatever forms (dihydrate, hydrochloride or sodium salt form) rapidly attains the pH of blood or tissue. This property by OTC is responsible for the essentially similar clinical or pharmacodynamic effect by its various forms (Huber, 1988).

Generally the gastrointestinal tract (GIT) absorption of OTC and other tetracyclines is good and fast reaching therapeutic blood levels in 2-4 hrs. Their $t_{½}$ ranges from 7-19 hrs (Brander *et al.*, 1991; Riviere and Spoo, 2001). GIT absorption varies between species and formulations (Riviere and Spoo, 2001). For example in carnivores OTC absorption reaches Cp_{ss} within 2-4hrs, persists for approximately 6hrs, declining to undetectable levels within 24 hrs (Huber, 1988).

Though GIT absorption is fast, absorption via this route is not complete. This has resulted in a wide variation of bioavailability values ranging from 52 - 92% (Schifferli *et al.*, 1982; Toutain and Raynaud, 1983). And while OTC is readily absorbed from the GIT in most mammals, its GIT absorption in poultry is more restricted (Brander *et al.*, 1991).

Due to the fact that tetracyclines chelate easily with polyvalent ions for example Ca^{++}, Mg^{++}, Fe^{++} Al^{+++} etc, this effect may partially impair their gut absorption. This can occur when the tetracyclines are administered concurrently with food, dairy products, the polyvalent ions, kaolin/pectin preparations and antacids (Neuvonen *et al.*, 1970; Gothoni *et al.*, 1972; Hagermark and Hoglund, 1974; Aronson, 1980). This is very important therapeutically for it may have a significant bearing on the pharmacodynamic response by tetracyclines when used therapeutically via the oral route.

When administered parenteraly peak OTC Cp_{ss} concentrations are reached rapidly by the IV route. Peak plasma Cp_{ss} following IM administration are reached within 1-2hrs (Huber, 1988; Brander *et al.*, 1991). They persist at therapeutic levels for about 12 hrs, and only traces remain 24 hrs post injection (Huber, 1988). In food producing animals peak Cp_{ss} levels are achieved by injectingnot more than 10mls of OTC injectable solution at one IM injection site. One interesting OTC absorption aspect following IM administration is that its bioavailability has a regional or body injection site variability. In one study the bioavailability values at 52 hrs post injection were 79% following an IM administration on the buttocks, 86% on the neck region, and 98% on the shoulder (Nouws and Vree, 1983). This again may be important during OTC IM administration for a maximum pharmacodynamic response. Tetracylines in contrast to other antibiotics are able to persist in blood plasma much longer in significant concentrations due to their enterohepatic resorption. Enterohepatic concentration of OTC has also led to its bile concentrations to be as high as 20 to 30 times the plasma concentrations (Acoccela *et al.*, 1968; Huber, 1988; Riviere and Spoo, 2001) rendering it useful in the treatment of liver infections (Brander *et al.*, 1991).

2.3.5.3 Distribution

Upon absorption OTC and other tetracyclines largely bind reversibly to plasma proteins. The binding is variable depending on the species involved. Tetracyclines are widely distributed in body tissues, organs and fluids. However higher concentrations occur in the liver and kidney (Huber, 1988; Riviere and Spoo, 2001). Relatively high concentrations are also found in the spleen, lungs and centers of ossification (Huber, 1988). Generally OTC is most useful therapeutically due to its wide body distribution. Therapeutic levels can be attained even by oral dosage (Brander *et al.*, 1991).

The body distribution of tetracyclines is a function of their individual lipid solubilities. So that unlike minocycline and doxycycline which penetrate the brain, CSF and prostate gland with relative ease, OTC and CTC do so only in small sub-therapeutic levels (Riviere and Spoo, 2001). This is due to their 5-10 times lower lipid solubility (Riviere and Spoo, 2001; Kamel and Brown, 2002). Occasional therapeutic OTC levels occur in CSF only during meningitis (Brander *et al.*, 1991). Minocycline and doxycycline can be used to treat prostate gland infections due to their high distribution in prostatic fluid (Huber, 1988). Tetracyclines pass through the bovine placenta entering fetal circulation. Fetal plasma concentrations are ½ those of maternal plasma. In geese a single oral dose of 50mg/kg of OTC produce a peak plasma concentration of 3.4 μg/ml, 4 hrs post injection declining to 0.8μg/ml after 24 hrs.

2.3.5.4 Metabolism

With the exception of minocycline, OTC and the remaining tetracyclines are not metabolised significantly (Aronson, 1980). Hence metabolism is not an important route of

OTC elimination. This is unlike minocycline and doxycycline, which undergo some biotransformation into inactive conjugates or chelates. The metabolism of minocycline is more complete than the rest of the tetracyclines (Huber 1988; Riviere and Spoo, 2001; Kamel and Brown, 2002).

2.3.5.5 Elimination

Tetracyclines undergo elimination mainly by renal and faecal excretion (Huber, 1988; Brander *et al.*, 1991; Riviere and Spoo, 2001). Renal Excretion accounts for between 25 - 30% (Huber, 1988) and 60% (Riviere and Spoo, 2001) of an OTC dose by glomerular filtration in urine. Faecal elimination constitutes between 10% (Huber, 1988) and 40% (Riviere and Spoo, 2001) of an OTC dose disposition via secretion by hepatocytes into bile. However the renal route appears to be of minor importance in doxycycline and minocycline disposition (Riviere and Spoo, 2001). Pharmacokinetically, OTC distribution and disposition obey first-order kinetics. Its elimination at therapeutic doses is neither dose dependent nor a function of the route of administration and exhibits the three compartment-open kinetic model (Baggot, 1977; Baggot *et al.*, 1977).

So that upon administration of a single IV injection of OTC, its plasma concentration (C_p) at any time t is given by:

$$Cp = Pe^{-\quad t} + Ae^{-\alpha t} + Be^{-\beta t}$$

Where
- Cp is the OTC plasma concentration at time t,
- A, B and P are the y-axis intercepts of the Disposition curve by the method of residuals by y-axis extrapolation.

- α, β and γ are coefficients of the slope or gradient of the disposition curve during the distribution and elimination phases by the method of residuals.
- e is the base logarithm.

It is fortunate and a blessing in disguise to man and the food producing animals that OTC elimination obeys first-order disposition kinetics both in man and the food-producing animals. If OTC exhibited zero-order kinetics, this would have precipitated into an additive effect and accumulation of OTC much faster in both rendering them more prone to OTC toxic effects by reducing the OTC LD_{50}. For example in the case of the food producing animals in which OTC and other tetracyclines are fed as feed additives their accumulation would have been more accelerated and at much higher concentrations. And even for those food animals receiving OTC therapeutically especially the long acting formulations, OTC residue accumulation would have been very high depending on the value of the α and β elimination phase coefficients. This would have resulted in a high intake of OTC residues by humans. This in turn would have made humans more prone to OTC residue toxicity due to a high intake of the already accumulated OTC residues in animal food products and also due to a zero elimination kinetic order if this were the case. This is because in zero-kinetic order eliminations only a certain constant amount of drug or chemical can be eliminated within any fixed duration of time. The value of the elimination phase coefficient (β) in the triexponential expression above is the determinant factor on OTC residue accumulation level and length of its withdrawal time upon the consumption of animal food products such as milk, meat and eggs.

In man when a single IV or IM dose is given OTC occurs in urine within 1hr, peaking at 4hrs post injection. Urinary concentrations can reach approximately 400μg/ml of urine and persist for about 24 hrs (Brander *et al.*, 1991). In domestic animals orally administered OTC produce optimal urine concentrations within 2 – 8 hrs. However antibacterial activity can persist for up to 3 days post cessation of dosing. With repeated therapy, that is two times or three times daily urinary OTC levels can be greater than 100 μg/ml which are above the minimum inhibitory concentration (MIC) levels for most susceptible bacteria (Huber, 1988). Faecal elimination of OTC occurs irrespective of the route of administration (Huber 1988). In man faecal concentrations can be as high as 2.5mg/g of faeces (Brander *et al.*, 1991).

Elimination of OTC from milk also occurs in some domestic animal species irrespective of the route of administration. Concentrations of OTC found in milk are 50% those of maternal serum. However no OTC has been detected in the milk of suckling pigs (Huber, 1988). Administration of OTC at 2 – 4 mg/kg bwt IM or IV produces peak milk concentrations of 0.9 -1.9 μg/ml within 6 hrs. Traces can be detected 48 hours post injection (Huber, 1988). Oral administration of chlortectracyline at doses equal or greater than 400 mg produces some detectable amounts in milk. By comparison cows receiving oral doses of 2 - 12g of OTC daily, produce detectable residues in milk (Huber, 1988). Use of OTC intrauterine pessaries to treat reproductive conditions such as retained placenta and endometritis produced milk OTC residues in about 66% of treated animals, which disappeared after the 6^{th} milking (Huber, 1988).

Studies have shown that OTC persists longer and is eliminated more slowly in sick animals than in healthy ones. Considering the high safety margin of OTC, persistence is obviously advantageous to sick animals for it provides sustained therapeutic OTC levels necessary to eliminate susceptible infectious agents (Huber, 1988). Given the residue occurrence pattern in milk and meat, and the kinetics of OTC in animals, it is imperative to strictly observe the OTC withdrawal periods.

2.3.6 Toxicity

Tetracyclines, OTC inclusive, are relatively safe antibiotics (Huber, 1988; Brander *et al.*, 1991; Riviere and Spoo, 2001). The IV LD_{50} in mice is about 150-180 mg/kg bwt. Dogs can tolerate an IV dose of 30mg/kg daily for 17 days. They can also tolerate IM doses of 50-100 mg/kg bwt or oral daily doses of 75-465 mg/kg bwt for about 8 weeks without any signs of toxicity (Huber, 1988). Acute toxicity is not common with OTC (Brander *et al.*, 1991). Generally tetracyclines can be used safely in hepatic and renal dysfunction without significant toxic effects (Huber, 1988).

Inspite of their relative high safety margin, they have various side effects and long term toxicities if not handled properly. Since glomerular filtration is their principal route of somatic disposition except for doxycycline and minocycline, animals with severe renal insufficiency may succumb to some tetracycline toxicosis (Riviere and Spoo, 2001). Some of the toxic effects include the following:

(1) **Gastrointestinal disturbances due to gastric and upper ileal irritation:** OTC and other tetracyclines with the exception of doxycycline and minocycline are orally absorbed in the upper ileum (Riviere and Spoo, 2001). And when they are orally or in the case of doxycycline and minocycline parenterally administered, they cause a massive GIT microflora suppression and alteration in its constitution (Riviere and Spoo, 2001; Kamel and Brown, 2002).

In herbivores this promotes the growth of resistant microbial species such as fungi causing extensive digestive disturbances. A good example is the occurrence of a severe pseudomembranous colitis in the horse upon administration of OTC at 4.4-mg/kg post surgery. This is due to proliferation of *Clostridium difficile/perfringens* and *Candida* spp., i.e. superinfections (Huber, 1988; Kamel and Brown, 2002). This is why OTC and other tetracyclines, especially doxycycline, are contraindicated in this species (Riond *et al.*, 1989a; Riviere and Spoo, 2001). Acute tympanitis may also occur in post weaning calves as reported for chlorteracycline (Huber, 1988).

(2) **Renal Nephrotoxicity:** This has been observed in feedlot calves (Huber, 1988). All tetracyclines, are antianabolic and increase protein breakdown which can worsen uremia cases in man and animals with renal insufficiency (Kamel and Brown, 2002). Hence excessive blood levels can cause renal necrosis and hepatic degeneration as observed in dehydrated calves (Teuscher *et al.*, 1982). Nephrotoxicity has also been observed in dogs administered two doses of OTC at 130mg/kg once every 24 hours (Stevenson, 1980).

(3) **Hepatotoxicity:** This occurs as a result of excessive blood levels which could be a result of absolute over dosage or a higher than required frequency of dosing. Excessively high blood levels could also be due to renal insufficiency (Riviere and

Spoo, 2001; Kamel and Brown, 2002). This can cause a fatal acute fatty liver degeneration (Teuscher *et al.*, 1982). Pregnant humans and animals are more prone to this syndrome (Kamel and Brown, 2002). In mice hepatotoxicity was observed as an increase in transaminases, alkaline phosphatase, urea, total and conjugated bilirubin and a reduction in cholesterol (Bocker *et al.*, 1982; Hopf *et al.*, 1985).

(4) **Shock:** This occurs during rapid IV administration of OTC. This is probably due to the chelation of Ca^{++} resulting in a reduction in cardiac performance (Gyrd-Hansen *et al.*, 1981). This can be avoided by administering/infusing the drug very slowly, i.e. 5 - 10min, or dilute the dose with normal saline or fluid free of polyvalent ions.

(5) **Tissue irritation:** OTC is only slightly irritating. However the other tetracyclines are so irritating that they should be administered IV or orally only (Huber, 1988; Brander *et al.*, 1991; Riviere and Spoo, 2001). Chlortetracycline is the most irritating. When used as an intramammary infusion in dry non-lactating cows its irritation led to the formation of a chronic granuloma in the udder (Huber, 1988).

(6) **Hypesensitivity:** Although allergy to tetracyclines is rare it has been reported in humans (Kamel and Brown, 2002).

(7) **Thrombophlebitis:** This occurs as a sequel to the IV administration of OTC and other tetracyclines (Kamel and Brown, 2002).

(8) **Growth inhibition:** Poor foetal development exemplified as growth inhibition in prematures (Cohlan *et. al.*, 1963).

(9) **Teeth abnormalities:** Mottling of teeth especially if OTC is administered in early pregnancy or postpartum due to chelation of Ca^{++} from dentine and hypoplasia of enamel in developing teeth (Hamp, 1967; Grossman *et al* 1971; Moffit *et al.*, 1974).

(10) **Effects in bone:** Abnormal bone growth in children aged 8 years has been reported (Kamel and Brown, 2002).

(11) **Occurrence of pseudotumor cerebri** with increased intra-cranial pressure and bulging fontanelles (Kamel and Brown, 2002).

(12) **Phototoxicity:** This has been reported in man especially with the use of demecycline though the most commonly reported lesion is dermatitis (Riviere and Spoo, 2001; Kamel and Brown, 2002).

(13) **Onycholosis:** This has been reported to occur in man (Segal, 1963; Harber *et al.*, 1961; Riviere and Spoo, 2001).

(14) **Nephrogenic diabetes insipidus:** Some tetracyclines especially demeclocycline has been found to cause nephrogenic diabetes insipidus (Kamel and Brown, 2002).

(15) **Vertigo:** Minocycline has been found to cause vertigo (Kamel and Brown, 2002).

(16) **Immunosuppression in food producing animals:** OTC and other tetracyclines have been found to suppress immunological responses in food producing animals. In a study carried out in pigs, aged 2-3 weeks, and 2-6 months, receiving OTC for 10 - 14 days, it was found that there was a reduction in immune response as shown by a reduced serum antibody titre. This was found to occur due to the inhibition of lymphoid tissue proliferation and reduced splenic live nuclei. However passive colostral immunity remained (Huber, 1988).

In another study, when chlortetracycline was fed to pigs at a dose of 1g/day, there was antibody reduction. And when they were given a dose of 15 mg/kg, there was a reduced immune response against swine *Erysipelas* (Lyashenko, 1966); the antibody titre was reduced and slower to build up. When poultry were given feed containing

50-200 mg of chlortetracycline per 900 kg of feed, immunological response against *Mycoplasma synoviae* was reduced (Huber, 1988).

(17) **Teratogenic effects:** OTC and other tetracyclines are potential teratogens (Carson, 1997); hence when handling them care should be taken. For example when weighing on balances gloves should be worn.

(18) **Fanconis syndrome:** Expired OTC and other tetracyclines can degenerate into compounds, which cause Fanconis syndrome (Kamel and Brown, 2002).

2.3.7 Clinical indications

Tetracyclines are bacteriostatic broad spectrum antibiotics which inhibit the growth and proliferation of a wide range of microorganisms. They act against gram-positive and gram-negative bacteria and other intracellular organisms (Huber, 1988; Riviere and Spoo, 2001; Kamel and Brown, 2002). These include some mycoplasms and other pathogens not affected by other antibiotics such as rickttsiae (Huber, 1988; Riviere and Spoo, 2001; Kamel and Brown, 2002). Some large viruses of the psittacosis group in animals and the human lympho venereum virus (Huber, 1988) and *Chlamydia* (Huber, 1988; Riviere and Spoo, 2001; Kamel and Brown 2002). At high doses tetracycline exhibit some antiprotozoal action such as *Anaplasma* spp.(Huber, 1988; Riviere and Spoo, 2001). The broad-spectrum nature of tetracyclines as a group is essentially the same, although generally their potency against gram-positive bacteria is greater than that of gram negative bacteria (Huber, 1988). Individual variations in potency are mainly due to differences in their lipid solubilities (Riviere and Spoo, 2001; Kamel and Brown, 2002).

Tetracyclines have a good to moderate activity against *Corynebacterium spp, Steptococci spp* especially the β-hemolytic *streptococci, Pasteurrella multocida,* some mycoplasmas, Anaplasma and some other protozoa, *Bacillus spp., Erysipelothrix rhusiopathiaea, Listeria monocytogenes, Actinobacillus spp., Brucella spp., Hemophillus spp., Campylobacter fetus, Borrelia spp.., Leptospira spp., Fusobacterium spp., Actinomyces* and *Bordetella spp.*,(Brender *et al.*, 1991; Riviere and Spoo, 2001). Also vulnerable to tetracycline are *Francisella tularensis and Yersinia* (Huber, 1988; Brander *et al.*, 1991; Riviere and Spoo, 2001).

Some bacterial species have variable susceptibility to tetracycline as shown by some *Staphytococcal* and *Enterococcal* species, some *Entrobacter spp., E.coli*, Klebsiella *spp., Proteus spp., Salmonella spp.*(Huber, 1988; Riviere and Spoo, 2001) and *Bacillus anthracis* (Huber, 1988). Also found in this group of variable susceptibility are some anaerobes such as *Bacteriodes spp.* and *Clostridium spp.* (Huber, 1988; Riviere and Spoo, 2001). However some bacterial species are relatively resistant to OTC and other tetracyclines. These include *Mycobacterium spp., Proteus vulgaris, Pseudomonas aeruginosa, Serratia spp.* and some *Mycoplasma spp.* (Prescott and Baggot, 1993). Others in this group include *Aerobacter aerogenes,* group A-hemolytic *Streptococci, Streptococcus feacalis* and many strains of *Staphylococci* (Huber, 1988; Kamel and Brown, 2002). Within the tetracycline group of antibiotics doxycycline and minocycline are more effective due to their higher lipid solubility.

The antimicrobial effects of OTC and other tetracyclines observed above are made use of clinically for therapy and prophyxis as follows.

(1) **Treatment of anaplasmosis:** OTC is used in the treatment of anaplasmosis (Huber, 1988; Brander *et al.*, 1991; Riviere and Spoo, 2001). The dose ranges from 11 mg/kg/day (Huber, 1988) to 22mg/kg/day (Brander *et al.*, 1991). An IM administration of OTC at 11mg/kg/day for 12 days was found to eliminate *Anaplasma marginale* infections in cows and sheep (Huber *et al.*, 1978). A higher frequency of the maintenance dose enhanced the effectiveness of the therapy and elimination of the carrier state (Huber, 1988).

In another treatment trial of 23 cattle with a natural subclinical anaplasmosis, OTC was administered at 20mg/kg IM. A total of 4 injections were given at 3-day intervals. All the 23 cows were cured effectively and produced a negative Compliment Fixation Test (CFT). Similar results were obtained for CTC and rolicycline (Huber, 1988).

(2) **Treatment of brucellosis:** Cows with less than 5 months of pregnancy were administered two doses of OTC at 10g per dose IM at 12 day interval between them (Plommet, 1974; Kamel and Brown, 2002). They got protected against brucellosis. This treatment can be combined with subsequent vaccination. However OTC protection is ineffective after the 5th month of pregnancy.

(3) **ECF immunization of cattle by the "Immunization at treatment method" (ITM):** This has been achieved by the concomitant administration of an active infective stabilate of *Theilleria parva* with OTC 30% LA at 1ml/10kg bwt (single dose). About 80-90% of immunized animals acquires a life long immunity (East Coast Fever Infection and Treatment control project report by MoWLD 2002).

(4) **Bovine hoof diseases:** Stubborn bovine hoof diseases can be overcome by the IV administration of OTC via the common dorsal digital veins. This reduces the convalescence period accelerating recovery (Huber, 1988)

(5) **Porcine atrophic rhinitis:** Administration of OTC at 40 mg/kg/day at day 3, day 6 and day 12 of age cured all clinically affected pigs (Huber, 1988).

(6) **Porcine balantidiosis:** A combination of acetarsol at 20mg/kg/day for 4 days and OTC at 15mg/kg/day twice daily for 4 days eliminated *Balantidium coli* infection in affected pigs (Huber, 1988).

(7) **Treatment of enteric infections of *E. coli* and *salmonella spp.*:** OTC is used for treatment of enteric infections caused by these two organisms (Schifferli *et al.*, 1982; Toutain and Raynaud, 1983). Unabsorbed OTC in the gut lumen act against *E.coli, salmonella* and other enteric infections. However resistance is now developing against OTC (Brander *et al.*, 1991).

(8) **Treatment of respiratory infections:** OTC is useful parenterally for the treatment of some respiratory system infections such as haemorrhagic septicaemia. All the tetracyclines possess some activity against some mycoplasms (Brander *et al.*, 1991). OTC is also used in the therapy of acute exacerbations of chronic bronchitis (Kamel and Brown, 2002).

(9) **Treatment of poultry diseases:** These include; collibacillosis (*E.coli*), infectious coryza (*Haemophililus gallinamum*), chronic respiratory disease (CRD) (*Mycoplasma gallisepticum*), air sacculitis (*M. melleagridis*), infectious synovitis (*M. synoviae*) clostridial dermatitis (*Clostridium septicium*), and necrotic enteritis (*Salmonella pullorum*). The oral dosage is mainly via water or feed (Brander *et al.*, 1991).

(10) **Mastitis:** OTC can be used for the treatment of mastitis but the formulation is slightly irritating to the mammary tissue (Brander *et al.*, 1991).

(11) **Foot rot and superficial infections:** OTC is used in the treatment of these conditions; it is applied as a purple coloured spray on fresh wounds of farm animals (Brander *et al.*, 1991).

(12) **Ehrlichiosis in dogs:** This tick-borne disease caused by *Ehrlichia canis* can be treated with OTC at high doses (Huber 1988, Brander *at al.*,1991; Riviere and Spoo 2001).

(13) Treatment of Urinogenital Tract Infections (UTI): OTC is used therapeutically for UTI infections whose etiology is *Rickettsia, Chlamydia, Mycoplasma* and *Vibrio* (Kamel and Brown, 2002).

(14) **Treatment of granuloma inguinale in man:** OTC and other tetracyclines are used in the treatment of granuloma inguinale (Huber, 1988; Kamel and Brown, 2002).

(15) **Treatment of Lyme disease in man:** OTC is used in the treatment of Lyme disease in man (Kamel and Brown, 2002).

(16) **Syphilis:** OTC can be used as an effective alternative therapy against syphilis in man (Kamel and Brown, 2002).

(17) **Prophylaxis against malaria in man:** Tetracyclines, especially doxycycline, are used chemoprophylactically against chloroquine resistant malaria caused by *Plasmodium falciparum* in man (Kamel and Brown, 2002). Currently doxyclycline is used in conjunction with artemenicin to treat chloroquine resistant malaria (personal observation, 2003).

(18) **Antidiuretic hormone (ADH) syndrome:** Demeclocycline is used in the treatment of the so-called inappropriate antidiuretic hormone (ADH) syndrome (Kamel and Brown, 2002) in man.

(19) **Treatment of river blindness in man.** The worm *Oncocerca volvulus* causes river blindness in man. Recently it has been reported (Abraham and Langworthy, 2000) that while still undergoing confirmatory tests it appears OTC might replace mectizan as the best treatment for river blindess. It does this by killing the bacterium *Wolbachia* which lives symbiotically in the worm *O.Volvulus* (Abraham and Langworthy, 2000). By killing the bacterium *Wolbachia*, the worm *O. volvulus* dies too curing the disease decisively. This is in contrast to mectizan which simply inhibits the worms reproduction rather than killing it, a treatment regime demanding repeated yearly therapies to put the reproduction of *O. volvulus* under control. Inspite of the creeping danger of resistance against OTC and other tetracyclines this discovery may provide a break through in the treatment of river blindness, a disease which affects approximately 18 million people world wide, 98% of whom live in Africa. While about 270 000 of these are totally blind, approximately 500 000 have partial blindness or impaired vision (Abraham and Langworthy, 2000).

2.3.8 Drug and chemical residues in edible tissues

The veterinarian administers various types of drugs and chemicals including OTC in food producing animals for diagnostic, prophylactic, therapeutic and feed additive purposes. The veterinarian must be fully conversant with the pharmacology and toxicological effects of the drugs used. He should be aware of the withdrawal times and

advise the farmer on the necessity to observe withdrawal period. This is very important from the public health stand point whereby residue levels in the human food supply chain must be controlled, not to exceed established tolerance levels or maximum residue levels (MRLS). Most violative drug residues in food producing animals are due to misuse of drugs or chemical preparations through non-adherence to label directions of the drug product (Taylor 1965, Booth 1988; Jones 1999). This misuse of drugs involves three causes:

(1) Non-observance of drug withdrawal periods: This has been reported by Taylor (1965) to account for about 75% of all the drug residues.

(2) Use of drugs to mask clinical signs during ante-mortem inspection prior to slaughter in the case of meat, or prolonging the shelf life of milk by using OTC and other antibiotics (Aboge *et al.*, 2000).

(3) Feeding of unapproved or unauthorized medicated feed to animals for example by administering a drug to a wrong species or administration by the wrong route (Paige *et al.*, 1997).

2.3.8.1 Antibiotics residues in edible tissues

Although antibiotics are used essentially in disease control and as feed additives for growth promotion, generally the use of antibiotics which may be deposited as residues in meat, milk and eggs must not be allowed to occur in food intended for human consumption (Booth and McDonald, 1988). However should their use be necessary as in therapeutic cases, then a withdrawal period (withholding period) must be observed until the residues are negligible i.e. reach concentration $\leq$ MRLS or are no longer detectable by

available methods (Booth, 1988). In the use of antibiotics for growth promotion, since they are only effective during the early growth phase of the animal (Table 2), their addition to feeds should generally be restricted, i.e. be used up to the indicated age limit (Booth, 1988).

Table 2: Recommended Age limits for animal feed antibiotic addition

	Animal species	Age limit
1	Poultry with exception of ducks and geese	8-10 weeks
2	Swine	4-6 weeks
3	Calves	3 months
4	Beef cattle	18 months
5	Lambs	2 months
6	Fur-bearing animals	2-3 months

Source: Booth (1988).

Feeding antibiotics OTC inclusive, beyond these age limits (Table 2) increases the probability of residue accumulation and is uneconomical. Of late the wisdom of feeding sub therapeutic levels of antibiotics as feed additives has become questionable. This is especially the case when the development of bacterial resistance and its subsequent transfer by the R factor is considered (Mercer, 1975; Booth, 1988).

Tetracyclines: The use of tetracylines as feed additives in animal feeds in low concentrations of 5-20 ppm does not produce residues in edible tissues (Booth, 1988). However the use of tetracyclines at this level in feeds may induce resistance to Enterobacteriaceae (Booth, 1988). Tetracycline residues have been detected in bones of pigs, calves and chicken when they were used in feeds at 5-80 ppm (Booth, 1988). While tetracyclines may not be toxic to man at a concentration of 1 ppm, at 5-7 ppm they may be

toxic (Booth, 1988). The various toxicities caused by OTC and other tetracyclines have already been reviewed.

The IV administration of tetracyclines at 4mg/kg to lactating cattle leads to tetracycline excretion in milk for about 36 hrs post injection. Again the IM administration of OTC at 5mg/kg produce detectable residues in the kidney 8 days post treatment (Pakkala *et al.*, 1980). Administration of OTC as intrauterine pessaries at 4mg/kg in lactating dairy cattle produce milk residues even up to 48 hrs past intra-uterine infusion (Booth, 1988). The intrammamary infusion of OTC at 426 mg total base at a 24 hr interval for three doses in the goat becomes undetectable in milk 108 hrs post treatment. This implies that the milk withholding period for goats is different and not enough for cows. Hence the milk withholding period for one species

cannot be extrapolated to another species (Hill *et al.*, 1982). In swine, a single vial dose of OTC at 50 mg/kg produce detectable OTC residues in edible tissues such as fat, heart, kidney, liver and muscle, 3 hrs post administration (Booth, 1988). Feeding of swine with OTC at 100g/900kg feed combined with penicillin at 50g/900kg feed and sulfamethazine 100g/900kg feed for 14 weeks produced OTC residues of less than 1 ppm on day 0 of withdrawal and could not be detected five days after withdrawal (Booth, 1988). Given the residue occurrence pattern in milk and meat and from the disposition kinetic of OTC it is imperative to strictly observe the OTC withdrawal times to make sure that the concentrations of OTC in these two vital livestock products are below the MRLs for OTC. The withdrawal periods of oxytetracycline and chlortetracycline in the various domestic species are shown in Table 3.

Table 3: Withdrawal Period of OTC and CTC in cattle, sheep, goats and swine

Species	Type of tetracycline	Withdrawal Period Days)	Limitations	Tolerance level ppm (mg/kg)
Cattle	Oxytetracycline HCl (injectable)	28	Not to be used in lactating cows.	Edible tissues 0.1
Swine	Oxytetracline (Injectable)	26-28	-	0.1
	Oxytetracline (Oral)	26	-	-

Source: Modified from Booth (1988)

In Tanzania, the use of supplementary feeding involving the use of feed additives to fatten animals prior to slaughter is virtually non-existent. Almost all the slaughtered animals are Tanzanian short-horn Zebu (TSZ) cattle which are mostly raised by grazing in communal land in all parts of the country, particularly by the Maasai, Sukuma and Gogo tribes.

However a lot of drugs especially oxytetracycline, trypanosides and antitheillerials are used abusively to treat and protect their cattle against various diseases. From this perspective the most likely source of drug residues especially OTC will be from the treatment and prophylaxis of diseases rather than from feed additives. OTC residues are likely to originate from the treatment of various diseases such as anaplasmosis, heartwater, ECF and CBPP. Much of the treatment is done by the livestock keepers themselves very abusively, without observing the right dosages, the right route of administration and non observance of withdrawal periods before sending the animals to the livestock markets. This study aims at establishing the presence of the OTC residues, their levels and main cause of their occurrence if they do indeed occur.

2.3.9 Methods of analysing tetracycline residues

Several methods may be available for antimicrobial detection but each has its own limitations. These include microbiological and physico-chemical methods as follows: The electrophoresis and bioautography (Staudhauders *et al.*, 1981; Lott *et al.*, 1985), mass spectrometry (Brumley and Sphon, 1981), radioimmunoassay method (Faraja and Ali, 1981; Blomquist and Hanngren, 1966; Ullber, 1977), and thin layer chromatography (Bossuyut *et al.*, 1976, Hammann *et al.*, 1979), have been used but have not been favoured.

The microbiological assay method using *Bacillus subtilis, Bacillus stearothermophilus* or *Bacillus cereus type* ATCC 11778 has the advantages of being the easiest, fastest and cheapest while handling many samples simultaneously. Analytical grade OTC is used to produce standard curves which are then used to give an estimate of the quantity of OTC present in a given sample by extrapolation from the size of its zone of inhibition. However together with the fluorimetric method (Wilson *et al.*, 1972), both are disadvantageous in being non-specific not only to individual antibiotics but also to groups of antibiotics such as tetracyclines. They also involve labourious sample preparations. Furthermore, the results of microbiological methods are highly prone to interference in their accuracy by other naturally occuring antimicrobial inhibitory substances such as lysozomes and lactoferin in milk (Carlsson and Bjorck, 1987). The microbiological assay methods detection limits for tetracylines has been reported to be about 0.1μg/g (Katz *et al.*, 1972), 0.27μg/g (Yoshida *et al.*, 1973), 0.2μg/g for egg yolk and 0.07μg/g for egg albumen (Rousdant *et al.*, 1987).

The gas chromatography/mass spectrometry method has been used for OTC (Trald *et al.*, 1985), CTC, TC, decleomycin, and 6 demethyltetracycline analysis (Kenion *et al.*, 1990). However it has been found that this method is not good for the quantitative analysis of tetracyclines (Voyksner *et al.*, 1990; Kijak *et al.*, 1991).

The high performance liquid chromatopraply method which has been used by several workers to quantify tetracyclines has been found to be highly sensitive, specific and possess a high precision or accuracy. Its main disadvantage is the involvement of time consuming and tedious solvent extraction. The method has been used for quantitative analysis of tetracyclines in honey (Sporns *et al.*, 1986; Oka *et al.*, 1987), with a detection limit of 1.2μg/g and 3.0μg/g for OTC and TC respectively. Also, it has been used for quantification of tetracyclines in human and dog sera (Nielsen - Ehle *et al.*, 1976). Furthermore, the HPLC method has been used to analyse tetracyclines in animal tissues (Dupont *et al.*, 1974), plasma urine and tissues (Sharma and Bevill, 1978; Ashworth, 1985), and liver (Oka *et al.*, 1985; Moats, 1986; Ikai *et al.*, 1987). Detection was up to a concentration of about 0.1 – 0.5 ppm.

When tetracyclines, were analysed using HPLC in bovine, chicken and porcine muscle the detection limit was 5-10μg/g and a recovery of 90% (Mulders *et al.*, 1989). HPLC determination of tetracycline residues in calves' tissues (Blanchflower *et al.*, 1989) and in meat (Yonida *et al.*, 1989) found the method to be a simple, accurate and reliable quantitative method. With the aid of a HPLC system, the method was used in the analysis of OTC and TC in milk (Fletouris *et al.*, 1990). Reversed phase HPLC was used in the quantitative analysis of tetracylines in eggs (Botsoglou *et al.*, 1984), in the simultaneous

analysis of six tetracyclines in bovine tissues, plasma and urine (Kondo *et al.*, 1988), determination of OTC and CTC residues in meat (Muriuki *et al.*, 2001), and determination of OTC, CTC and TC in edible animal tissues (Mac Neil, 2000).

A slightly modified version of Mac Neil's method (Mac Neil, 2000) was used in the analysis of OTC in beef samples in this research project using an ATIUNICAM HPLC system.

CHAPTER THREE

3.0 MATERIALS AND METHODS

Two types of studies were conducted. One involved a questionnaire to respondents and the other involved analysis of meat samples for presence of oxytetracycline residues.

3.1 Questionnaire study of the use pattern of OTC and other drugs by various stakeholders in Morogoro and Dodoma regions

3.1.1 Area of study

The area of study consisted of Morogoro and Dodoma regions' urban and rural districts. Villages were selected for administration of questionnaire and consisted of two types, some of the villages were located in catchment areas for cattle destined for slaughter at the municipal abattoirs. In Morogoro Urban district the areas included Kihonda, Bigwa, Kichangani and Kingolwira, and Melela, Wami Sokoine, Doma and Mangae villages in Morogoro Rural district. In Dodoma region the villages consisted of Ihumwa, Mtumba, Nzasa, Gawaye, Mpunguzi, Veyula and Ibihwa in Dodoma Urban and in Dodoma rural districts respectively. The number of questionnaire respondents for each category is as shown in Table 4.

Table 4: The number of questionnaire respondents for each category

Region	District	Category of respondent					
		Group L/stock keepers	Group II LFOs/ AFOs	Group III Meat traders	Group IV Vet. Surgeons	Group V Pharmac Dealers	Total
Morogoro	Morogoro (u)	16	12	5	7	9	49
	Morogoro (r)	49	8	-	-	-	57
Dodoma	Dodoma (u)	36	16	5	5	7	69
	Dodoma (r)	10	4	-	1	-	15
	Total	111	40	10	13	16	190

(u) = Urban, ® = Rural, Vet = Veterinary, Pharmac = Pharmaceutical

The number of liver, kidney and muscle samples collected and analyzed from the two municipal abattoirs are as shown on Table 5.

Table 5: Number of muscle, liver and kidney samples collected from Dodoma and Morogoro Municipal abattoirs

Abattoir	Tissue sample			Total number of samples analysed by H.P.L.C
	Liver	Kidney	Muscle	
Morogoro municipal abattoir	25	26	20	71
Dodoma municipal abattoir	20	25	15	61
Total	45	51	35	131

HPLC = High Performance Liquied Chromatography

3.1.2 Administration of questionnaire

A total of 190 randomly selected questionnaire respondents were interviewed in an attempt to establish the level of both use and abuse of OTC in the field. The respondents were from Morogoro Urban and Morogoro Rural districts in Morogoro region, and

Dodoma Urban and Dodoma Rural districts in Dodoma region. The questionnaire respondents consisted of 5 groups, that is Groups I, II, III, IV, and V (Table 4).

Group I respondents consisted of randomly selected farmers in some of the slaughter cattle catchment areas. The group consisted of 111 randomly selected livestock farmers (65 were from Morogoro and 46 were from Dodoma). A questionnaire consisting of 48 questions as shown in Appendix I was administered to the group.

Group II consisted of 40 randomly selected livestock personnel {Livestock Field Officers (LFOs) and Agricultural Field Officers (AFOs)} working with the farmers in the slaughter cattle catchment areas. Of the field personnel respondents, 31 were randomly selected Livestock Field Officers (LFOs); they had a livestock bias or had certificate or diploma level training in animal production, animal health, range management, meat inspection or other related courses. The other nine field personnel respondents were randomly selected Agricultural Field Officers (AFOs). The AFOs had an agronomy training bias at the certificate or diploma level in various agricultural courses such as crop production, horticulture, and nutrition plus a five months livestock production and health retraining course. A questionnaire consisting of 46 questions as shown in Appendix II was administered to the group.

Group III respondents consisted of randomly selected meat traders operating in the two municipal abattoirs. In each of the two municipal abattoirs five meat traders were randomly selected. The questionnaire administered to these respondents is as shown in Appendix III.

Group IV respondents consisted of Veterinary Pharmaceutical Dealers operating in Morogoro and Dodoma municipalities. This group consisted of 16 randomly selected respondents who, among other drugs, supply OTC to customers for management of livestock diseases. The questionnaire that was administered to the group is as shown in Appendix IV.

Group V respondents consisted of all veterinary surgeons working in the two regions under study, Dodoma and Morogoro regions, and some veterinary surgeons working in other parts of Tanzania. The questionnaire administered to this group is as shown in Appendix V.

3.2 Oxytetracycline residue studies in meat

3.2.1 Collection of meat samples

Meat (muscle, liver and kidney) samples used for OTC analysis were randomly collected from carcasses in two municipal abattoirs, one located in Morogoro Municipality and the other in Dodoma Municipality. Collection of the samples was done during the months of January and February 2002. About 100 g of liver, kidney and muscle tissue samples were collected from randomly selected carcasses. They were put in polythene bags and transported in cool boxes containing ice cubes at 4^0C. The samples were frozen at –20°C in the Department of Veterinary Physiology, Biochemistry, Pharmacology and Toxicology, Sokoine University of Agriculture, pending analysis.

3.3 Analysis of samples for oxytetracycline by high performance liquid chromatography

The principle applied in the high performance liquid chromatograph (HPLC) analysis of oxytetracycline (OTC) involved the extraction (separation) of OTC's from muscle, liver and kidney tissues with Mcllavaine buffer (pH 4.0), combined with disodium EDTA in the ratio of 26.8595:1 (V/W). The ultra filtered sample supernatant was cleaned by application or passage through a C-18 solid phase extraction column (cartridge). The OTC's were then separated by H.P.L.C using a reverse phase C8-10 column and read with a UV detector at 350nm wavelength.

3.4 Equipment

3.4.1 HPLC System

Analysis was performed with the aid of a high performance liquid chromatography system, ATIUNICAM, which comprised the following.

Detector: A Crystal 240 diode ray detector with variable wavelength UV detection. For OTC detection the wavelength was set at 350 nm, and at 0.001 absorbance full scale (AUFS), to detect the drug as it emerged from the column.

Delivery pump: A Crystal 200 Liquid Chromatograph with a built in pump was used. A solvent flow rate of 2 ml/minute was chosen, and 150 psi pressure.

Vacuum Degasser: A CSI6150 vacuum degasser (ATIUNICAM), was used.

Injection system: A Rhodyne 7125 sample injection system with a 20µl injection loop.

Printer: A Pantos-Raster type plotter printer, model LP-5200, set at a chart speed of 5mm/min was used to record detector signals to produce quantifiable peaks on a recorder chart.

The various components of the ATIUNICAM HPLC system are shown on Figures 4 and 5.

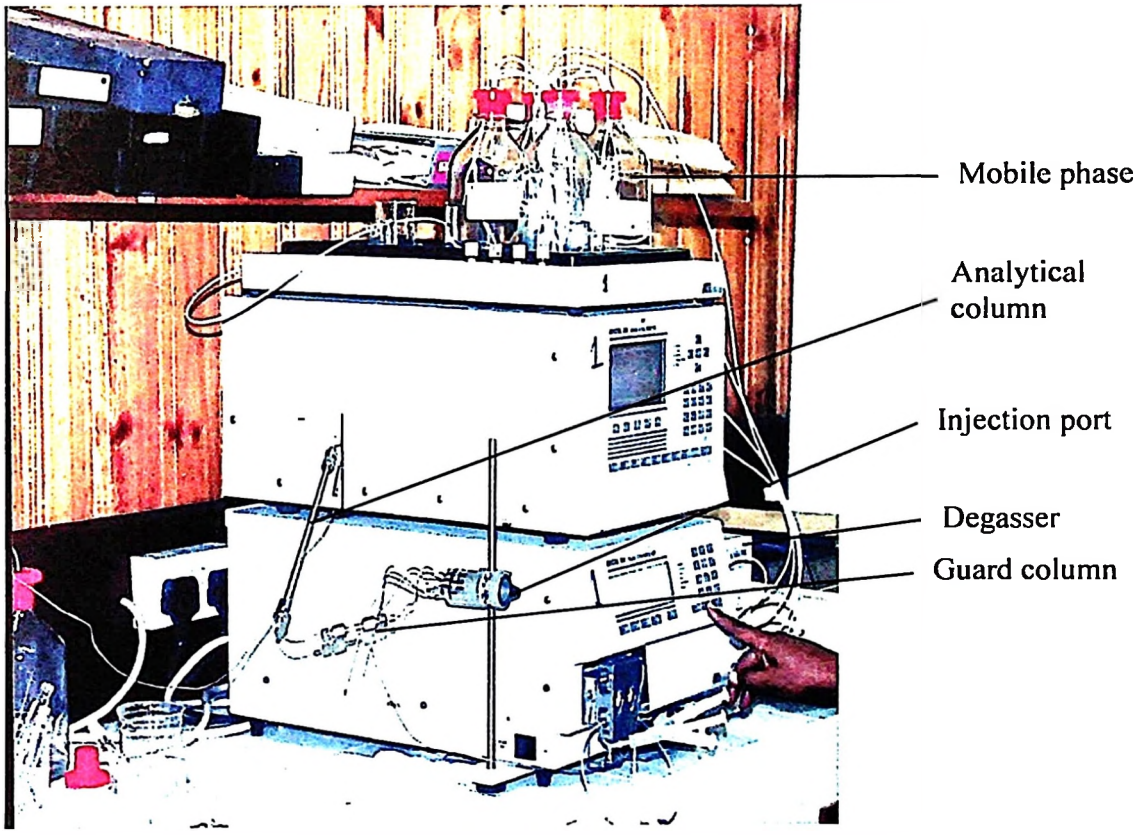


Figure 4: ATIUNICAM HPLC system showing mobile phase, analytical column, injection port, degasser and guard column

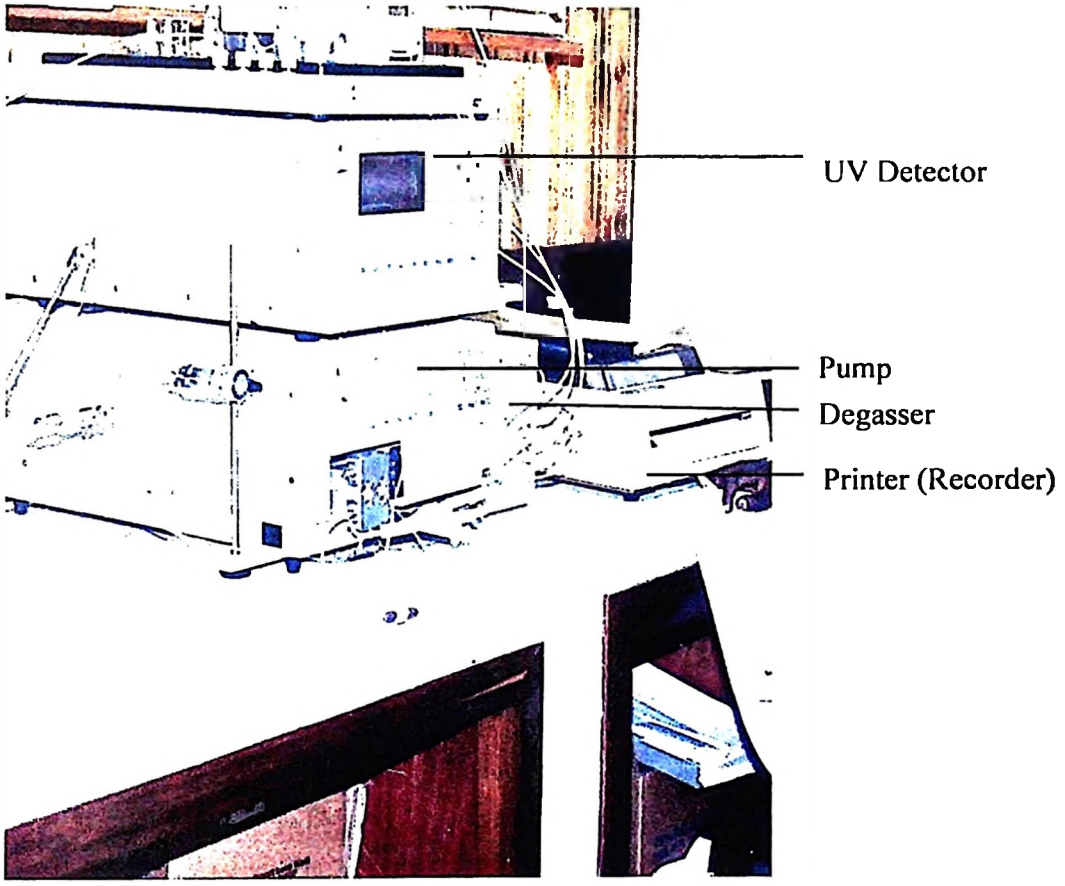


Figure 5: ATIUNICAM HPLC system showing a UV detector, pump, degasser and printer (recorder)

Liquid chromatograph (LC) column: A LiChrosorb RP8-10 (Li.R,P8-10-250A) column with a 250mm length x 4.6 mm internal diameter (id) and deactivated silica packing was used.

3.4.2 Other equipment

Cartridges: Supelclean LC-18, 6ml, (500mg) solid phase extraction cartridges were used (Figure 6).

Weighing balance: A Mettler PE 6000 balance weighing to ± 0.01g was used. Others included ae ADAM EQUIPMENT WA/80 balance weighing 0.000001g; a Sartorius BP 2105 balance weighing up to 0.00001 g.

PH meter: A CORNING pH meter model 7 was used. Others included a JENWAY 3305 pH meter measuring ± 0.05 units.

Sonator: An ultrasonic B&T Searle company sonator was used.

Stirrer: A Gallenhamp magnetic stirrer was used.

Centrifuge: A high speed centrifuge model Sigma 202MC with a maximum speed of 13500 rpm was used. Centrifuge tubes were Modified 15-ml Corning Falcon tubes trimmed to 12ml. The tubes were high strength centrifuge tubes able to withstand the high centrifugation speed of 10,000rpm.

Additional equipment included; 0.2, 0.45 μm porosity, 25mm diameter Whatman membrane filters; Millipore Swinnex 25 filter assembly or cartridge (i.d. = 25mm) (Figure 7) to mount the above membrane filters; Micropipette syringes of volumes 20 - 100μl, 40 - 200μl, and 1-5ml; disposable or non disposable tips; 10, 20ml plastic syringes; 1 ½" G 16 and G 18 needles; 6ml S.P.E tube adaptor; Small to medium sized glass funnels; 50, 250, 500 and 1,000 ml measuring glass cylinders; 5, 10, 25, 50, 500, 1000 and 2000ml volumetric flasks; vacuum pump to assist in the filtration of various solvents namely the mobile phase, HCl, distilled water for production of LC grade water (Distilled water was dionized and ultrafiltered with 0.2 μm porosity Whatman membrane filters); high purity de-ionizer and a Kenwood domestic blender with cutting blades to disintegrate

and homogenize meat samples. The blades of the homogenizer were low enough to homogenize tissue in a 30 - ml mixture.

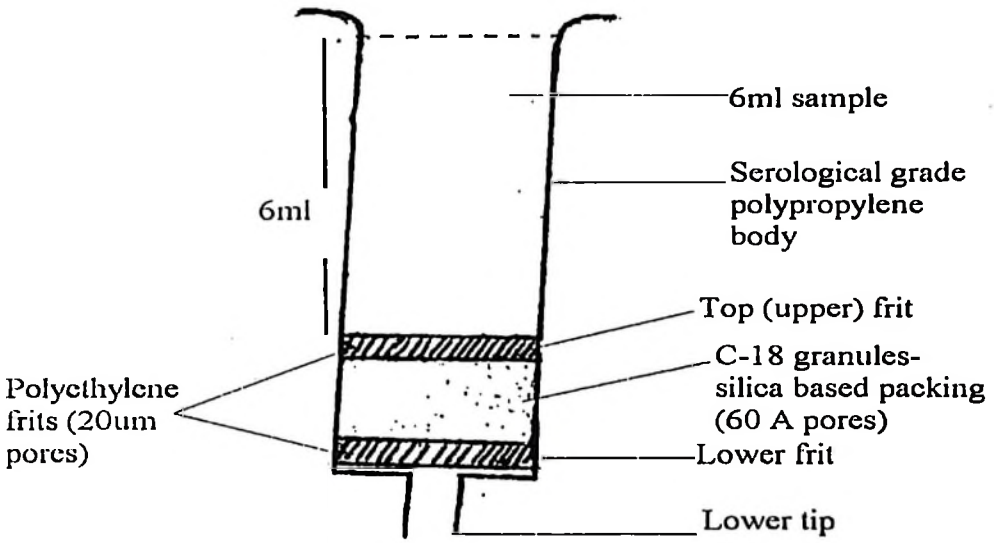


Figure 6: A Supelclean LC-18 S.P.E. Cartridge

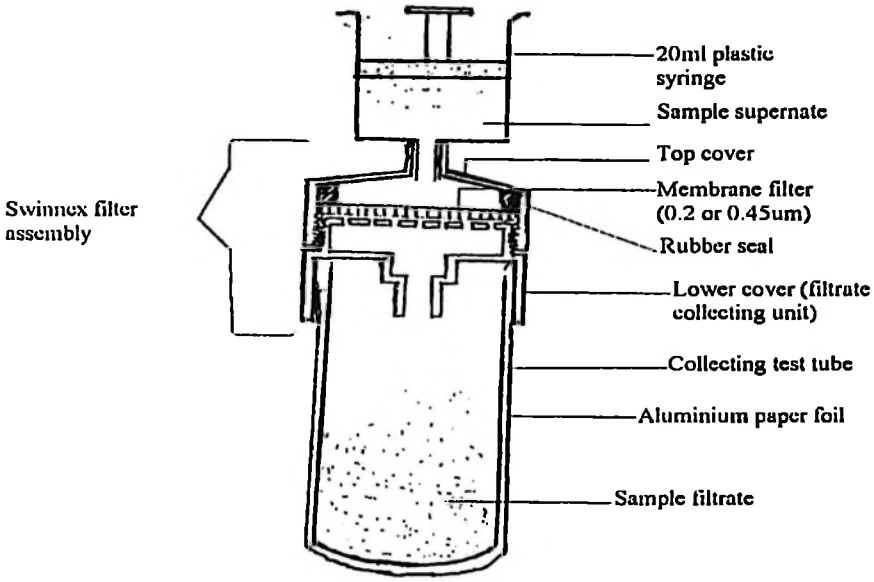


Figure 7: A Swinnex 25 Filtration assembly

3.4.3 Chemicals and solutions

OTC HPLC analysis involves the use of various chemicals and solutions, which had to be prepared prior to the analysis.

3.4.3.1 Chemicals

Monohydrate citric acid ($C_6H_8OH_20$) anallar (AR) or reagent grade, anhydrous disodium hydrogen phosphate ($Na_2 HPO_4$) AR grade, disodium ethylenediamine tetraacetic acid (i.e. disodium EDTA, AR grade), dihydrate oxallic acid (AR grade, Mol. wt = 126.04), oxytetracyline dihydrate (Sigma Co. Ltd, St. Louis, USA), methanol (L.C grade), acetonitrile (L.C grade), Hydrochloric acid, and ethanol (AR grade).

3.4.3.2 Solutions

LC grade water: This was prepared by de-ionizing distilled water which was ultra filtered using 0.2 μm pore size filters, and autoclaved. The water was used as solvent for the preparation of 0.01M aqueous oxallic acid used for mobile phase and O.E.S preparation. Ultrafiltration of water using 0.2 μm filters removed all bacteria and fungal spores. This inhibited bacterial and fungal growth, which might interfere with the pH of working solutions. Furthermore, bacterial and fungal metabolic products in the mobile phase, or in O.E.S may produce interfering peaks or may simply interfere with anionic/cationic exchange during the analysis of samples and hence interfere with column efficiency by clogging the column packing. Also bacterial and fungal contamination may clog the LC-18 S.P.E. cartridges, impairing the OTC cleaning/extraction process and recovery levels.

McIlavaine buffer (McB, pH 4.0± 0.05): This buffer was prepared by mixing 0.1M citric acid (CA) and 0.2M disodium hydrogen phosphate (DHP) in the ratio of 1: 0.625 and adjusting the pH of the mixture, with 0.1M HCL or 0.1M NaOH, to 4.0 ± 0.5.

0.1M citric acid solution: 21g of citric acid monohydrate (Mol. wt 210.04g) powder was placed into a 1000ml volumetric flask and de-ionized water added and allowed to dissolve by thoroughly mixing. More water was added to give a final volume of 1000 ml.

0.2M Anhydrous disodium hydrogen phosphate: 28.4g of anhydrous DHP (Mol wt 141.96g) was transferred into a 1000ml volumetric flask and dissolved thoroughly by vortexing in-de-ionized distilled water to give a final volume of 1000ml.

A volume of 1000ml of the 0.1M CA solution was mixed with 625ml of the 0.2M DHP solution in a 2000-ml volumetric flask to give 1625ml solution that was referred to as McIlavaine buffer (McB) with a pH 4.0.

When the weights of the CA and DHP and their subsequent dissolution has been precise in deionized water the pH for McB was 4.0+0.5 and needed no adjustment. Otherwise the McB was adjusted to pH4±0.05 using 0.1M HCl or 0.1M NaOH.

McIlavaine buffer was prepared on a weekly basis

Oxytetracycline separation (extraction) solution (O.S.S.): 60.5g disodium EDTA were dissolved in 1625ml of McIlavaine buffer (pH4.0). Vortexing enhanced thorough dissolution. The O.S.S. solution was prepared on a weekly basis.

O.01M oxalic acid (aqueous): A constituent of the mobile phase, and also the oxytetracycline elution solution (O.E.S), was prepared by dissolving 1.26g oxalic acid (Mol wt 126.04) in 1000ml of HPLC grade water. Shaking was done to enhance dissolution. The solution was always freshly prepared at the time of use.

3.4.3.3 Mobile phase

The mobile phase used for HPLC analysis of oxytetracycline consisted of a mixture of methanol (MeOH), acetonitrile (AC-N) and 0.01M oxalic acid (Ox-AC) (pH.2.0) in the ratio 1:2:7 V/V/V respectively. The mixture (i.e. mobile phase) was filtered in a clean sintered glass of size No. 3 with the aid of a vacuum pump. Before filtration process the

sintered glass was first cleaned by prior soaking for 48 hours in chromic acid, then rinsed in distilled de-ionized water. In the absence of a sintered glass filter, a 0.2 μm porosity Whatman filter membrane was used with the aid of a Swimmnex filter assembly and a 20 ml plastic syringe.

The filtered mobile phase was de-gassed using an ultrasonic B and T Searle Sonator until no more gas bubbles were visible in the mobile phase solution. Any remaining gasses were automatically removed by a CSI 6150 vacuum degasser attached to the HPLC system.

The mobile phase was freshly prepared on the day of use.

3.4.3.4 Oxytetracycline dihydrate ($C_{22}H_{24}N_2O_9$. $2H_2O$, Mol. wt = 496.5) standard solutions

The principle aim of the research work was to determine for the presence of violative OTC residues in meat of cattle slaughtered at the two municipal abattoirs. Thus the concentrations of the standard OTC solutions for the purpose was based on the JECFA committee OTC MRL's in meat i.e. in muscle (0.2mg/kq, or 0.2μg/g), liver (0.6mg/kg, or 0.6μg/g) and kidney (1.2 mg/kg, or 1.2μg/g). OTC (10.0 mg) was accurately weighed and transferred into a 10 ml volumetric flask and LC grade methanol added to produce a 1000ppm OTC stock solution. The solution was protected from light by wrapping the volumetric flask with aluminum paper foil, and stored at –20 °C. The solution was prepared fresh every three weeks.

This stock standard solution was used to prepare working OTC standards by serial dilutions. First a 10ppm standard solution of OTC in methanol was prepared from the stock solution. Then, 11 standard solutions, i.e. 5, 2.5, 1.25, etc to 0.01 and 0 ppm were prepared. Tubes containing the standard solutions were protected from light by wrapping them in aluminum foil. The standards were prepared on a weekly basis and stored at –20 °C.

3.4.3.4 Preparation of oxytetracycline spikes

Spikes were prepared for the purposes:

(i) To check for the viability of the extraction method.

(ii) To facilitate the calculation of the recovery factor of OTC's from the unknown samples.

(iii) These spikes were used for the calculation of the OTC concentrations from the areas of the unknown samples throughout the course of the analysis.

Preparation: Nine kidney samples each weighing 10g were separately ground into paste and placed in 25 ml labelled beakers; each sample paste was then mixed with 30ml of O.S.S. (OTC separation or extraction solution) in a 50 ml measuring cylinder and the weight and volume of mixture recorded.

Then 10ml of a 100ppm OTC solution was made from the 1000ppm OTC stock solution by mixing 0.1ml (100µl) of 1000ppm OTC stock solution with 9.9ml. of methanol in a 10ml. volumetric flask. The 100ppm OTC solution was subsequently used for the preparation of the spikes. From the combined volume of each kidney sample and O.S.S.

solution, volumes of the 100ppm OTC solution required to make spikes of the required concentration were calculated and transferred to the beakers to provide mixtures with the concentrations shown in Table 6.

Table 6: Spikes and their oxytetracycline concentrations used for HPLC analysis

Identification	Concentration of contents (ppm)
1	10
2	2.5
3	1.25
4	0.625
5	0.3125
6	0.16
7	0.08
8	0
9	0

Beakers No. 8 and 9 acted as blanks and contained no OTC. All the 9 spikes were then subjected through the extraction, clean up and elution process for OTC in the same manner as the unknown meat samples. With the aid of HPLC system (Section 3.4.1), they were then analyzed for OTC concentration. The spikes were run through the analytical process to check for the efficiency of the OTC extraction method. The OTC retention time of the spikes was compared with that of the OTC standards. Their areas under the curve were similarly compared with those of the standards, and provided a means of determining the fraction of OTC in test samples recovered during the extraction process. The fraction of OTC recovered during the extraction process was calculated from the spikes containing OTC at 1.25, 0.625, 0.3175, and 0 ppm. Injection of a sample was always followed by a known standard solution in methanol, and a spike. The blanks were used throughout the H.P.L.C analysis to check for the analytical column efficiency.

Before injection of an unknown sample, a blank kidney (BL-K) sample was first injected and run followed by a spike, and then an unknown sample. The 10 and the 2.5ppm spikes were not used routinely during analysis of unknown samples because of their delay in getting cleared from the analytical column and their potential to clog or overload the column with OTC.

3.5 Cleaning of glassware and other equipment

All the glassware and other equipment such as spatula, spoons, falcon tubes, and micropipette tips were thoroughly cleaned to ensure they had no contamination in order to prevent occurrence of interfering peaks during HPLC analysis. Glassware were first soaked in normal tap water mixed with a liquid 'foma' detergent for about 12 hours. The glassware was then rinsed thoroughly in plain tap water to remove the detergent, before soaking in 6N nitric acid. They were left to soak in the acid for about 24 hours for further cleanup of residual salts. They were then rinsed several times with de-ionized distilled water, transferred to an oven set at 80 – 100 ^{0}C, and left overnight to dry. Dry glassware and other equipment were kept in polythene bags to avoid recontamination.

In the case of plastic wares such as falcon tubes, and micropipette tips the cleaning procedure was the same as that for the glassware up to the stage of rinsing with dionized distilled water. They were then kept in racks and left to dry at room temperature or in an oven set at 40-50^{0}C for about 6-12 hours. They were stored in polythene paper bags to avoid recontamination.

3.6 Weighing, processing/cleanup and extraction of test samples

A sample of about 12-13 g of raw meat was cut into very small pieces using sharp pair of scissors or surgical blade. Then small amounts of this sliced meat sample were put on mortar and ground into a fine paste with the aid of a pestle, manually. The size of the sliced meat sample for grinding at a time was small (i.e. < 2g) to facilitate the grinding process into a fine paste. Then 10g of this ground sample paste was weighed on a balance (Mettler PE 6000). This procedure of grinding a sample and then weighing rather than weighing a 10g sample and then grinding was adopted to improve the accuracy of the weight of ground samples especially for liver samples. Losses due to left-overs on the pestle and mortar were minimized. After each successive grinding, the mortar and pestle were thoroughly washed with distilled water. The 10g-sample paste was transferred into a 50ml measuring cylinder containing 30ml of OTC separation solution (O.S.S). The measuring cylinder and its contents was weighed on the Mettler PE 6000 balance. The weight and volume of the meat sample and O.S.S. was recorded. The sample and O.S.S mixture was then transferred into a high speed Kenwood domestic blender and homogenized for 2 minutes. The sample homogenates were transferred into 12ml high strength centrifuge tubes.

3.7 Centrifugation of sample homogenate

The sample homogenates were centrifuged in a Sigma 202MC centrifuge at 10,000 rpm for 15 minutes and the supernatant transferred into test tubes and sealed to prevent evaporation of O.S.S- MeOH. The sediment was discarded. The sample tubes were kept in complete darkness in laboratory shelves to prevent OTCs degradation on exposure to light. The relatively high centrifugation speed and long duration of centrifugation was

intended to produce low sediment supernatant that facilitated subsequent filtration process of the samples. After centrifugation, the centrifuge tubes were thoroughly washed.

3.8 Sample filtration

The supernatant samples were filtered using 0.2 or 0.45 μm filter papers mounted in a Swinnex25mm filter assembly (Figure 6). A sterile 20ml syringe and G 16, 1½ inch needle were used to transfer the supernate filtrates. The test tubes with the sample filtrates were kept in the dark in laboratory shelves to protect them from light, to await OTC elution by LC-18 SPE cartridges. The filter assembly, syringe and needle were rinsed twice thoroughly with HPLC grade water before mounting a fresh filter paper for the next sample.

3.9 Sample extraction or elution

The sample filtrate (6ml) was passed on an activated Supelclean LC-18 SPE cartridge (Figure 7) mounted on a stand. The size of the LC-18 cartridge used for the OTC extraction was 6ml, 500 mg cartridges. The extraction or OTC recovery procedure on the Supelclean on LC-18 cartridge is essentially the same but variable depending on whether the extraction of the sample was done on a new or used cartridge. When a filtered sample was extracted using a brand new cartridge, the procedure involved 4 successive steps as follows. (1) activation of cartridge (2) addition of sample into the cartridge (3) rinsing or washing of contaminants from cartridge with LC grade water and finally (4) elution of the analyte i.e. OTC with OTC elusion solution (O.E.S), that was also the mobile phase.

Activation of Supelclean LC-18 cartridge: A volume, 6 ml of LC grade methanol, equal to the size of SPE tube, was added into the SPE tube in two 3ml aliquots semi-serially to allow it to soak by gravity without using an adaptor. Then 6.0 ml of LC grade water was added while the last 1mm height of the methanol was still above the top frit of the SPE tube i.e. semi-serially. An adaptor and 20ml syringe was used to facilitate the passage of the water through the S.P.E tube, but the flow rate was made to be very slowly i.e. drop wise at <5ml/min.

Addition of sample: Sample filtrate (6 ml) was added into the SPE tube by a micropipette syringe while 1 mm height of water was still above the top frit. An adaptor was used to facilitate the passage of the sample through the SPE tube but the rate of passage was maintained at < 5ml/min to ensure maximum retention of the OTC on the LC-18 tube packing. (However best results are obtained when the flow is by gravity).

Addition of water to wash off contaminants: 10ml of LC grade water was added and passed through the SPE. This was facilitated by an adaptor to remove sample contaminants retained on the C-18 cartridge granules.

Elution of OTC: A volume of 6ml of OES was then added into an SPE tube when only 1mm height of water remained above the upper frit of the tube. Just as the OES solution was being added to elute the OTCs retained on the LC-18 cartridge tube, a sterile plain vacutainer tube wrapped in an aluminium paper foil was mounted directly under the SPE tube to collect the eluted OTC sample. Once collected the tube was sealed with its rubber stopper and stored at –20 ^{0}C in a freezer ready for analysis of OTC by HPLC.

When the OTC extraction from the sample was perfomed using a used Supelclean C-18 cartridge then nine additional steps involving a superimposed or semi-serial addition of nine reagents was carried out before extraction of a subsequent sample. These steps were meant to carry out two functions. The first one involving steps (1) – (7) was meant to wash clean and remove traces of OTC and contaminants that might have remained after the elution of OTC by O.E.S. Without this, the recovery in subsequent samples would have been lower than the actual quantities present in the samples. The second function involving steps (8) and (9) were meant to reactivate the SPE cartridge and make it ready for extraction of the next sample. This was followed by the addition and subsequent extraction of the next sample.

(1) 6.0ml of LC grade water was added into the cartridge.

(2) Then 10ml of 1N HCl was added.

(3) 6.0ml of LC grade water was added.

(4) 6.0 ml of OTC elution solution was added.

(5) 20.0 ml of 1N HCl was added.

(6) 6.0ml of ethanol (AR grade) was added.

(7) 80.0ml of LC water was added.

(8) 10.0 ml of HPLC grade methanol was added. (The catridge is reactivated here).

(9) 6.0ml of LC grade water was added (forms water film around C-18 granules).

(10) 6ml of the next sample was added.

After adding the next sample (step number 10 above), elution and collection of the OTC sample extract was done in the same manner as with a brand new cartridge. All the samples in this experiment were eluted with new cartridges, except on rare occasions

especially while developing and modifying the protocol. Extreme care was taken when adding solutions into the C-18 cartridge. Successive solutions or reagents had to be added when about 1mm height or thickness of the preceeding solution was still above the upper frit to prevent the entry of air into the cartridge granules. If this happened the water film forming around the activated C-18 granules would dry up and the whole process would either have to be started afresh or the cartridge would have to be disposed off otherwise the recovery efficiency of the cartridge was impaired.

All solutions or reagents used in the elution process, for example hydrochloric acid, OES and OSS were ultra filtered in the same way as the sample to avoid clogging of the SPE catridges and the formation of unwanted chromatogram peaks during subsequent HPLC analysis of the samples. The whole extraction and cleanup procedure of OTC's from the meat sample is summarized on Figure 8.

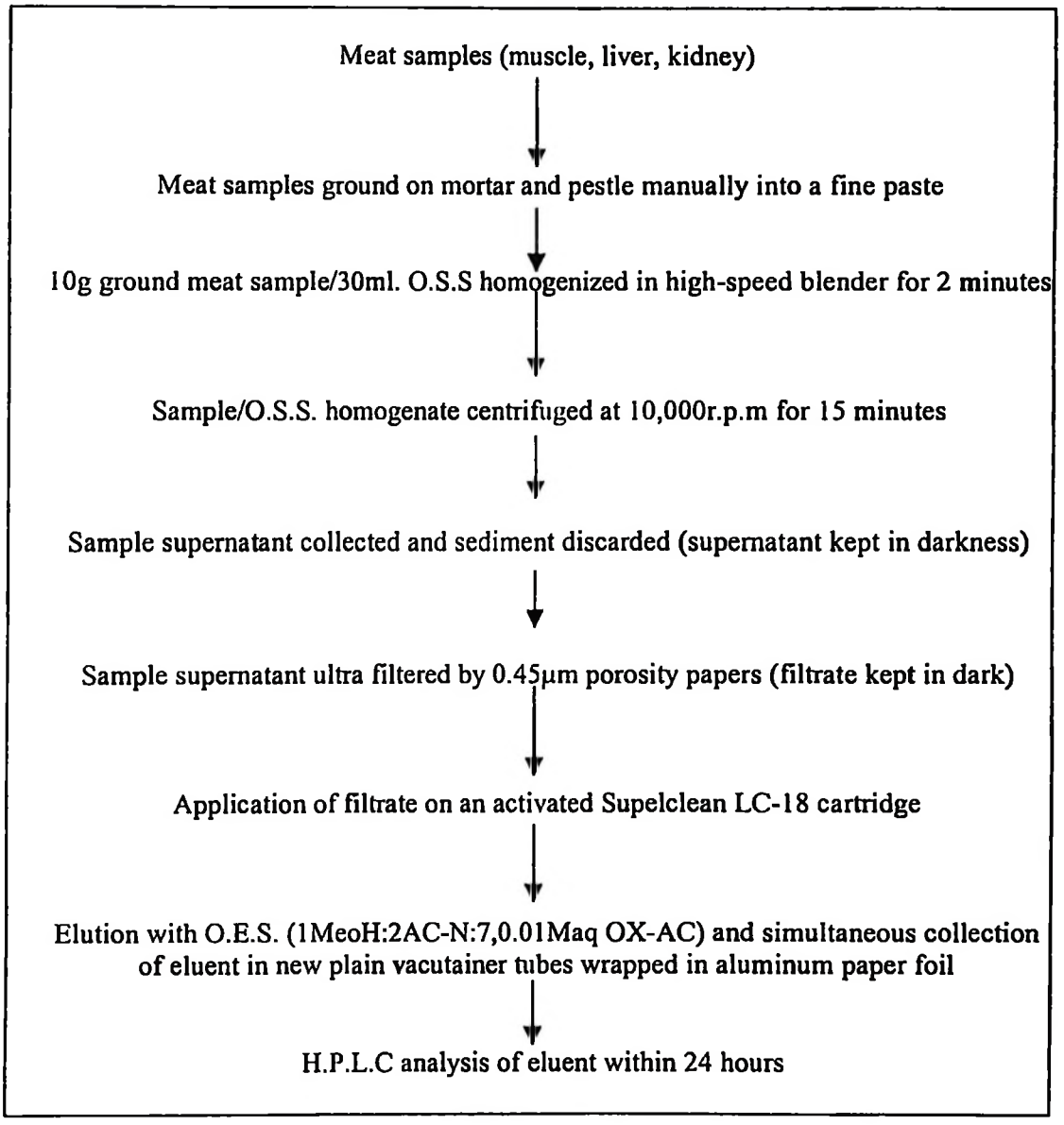


Figure 8: Extraction and clean up procedure of oxytetracycline from meat samples

3.10 Preliminary qualitative test for OTC (The sulfuric acid test)

Eluted samples were tested qualitatively for presence of OTC using the simple sulfuric acid test as follows: Concentrated sulfuric acid (2 ml) was added into approximately 0.5ml of sample eluent. In the presence of OTC the colour changed to grayish/silver (smocky)

depending on the amount of OTC present. A further addition of 2-4 mls of deionized water changed the colour of the contents into yellowish, the extent of which depended on amount of OTC present.

3.11 Analysis of oxytetracycline by HPLC system

Determination of OTC was done using a high performance or high pressure liquid chromatograph system equipped with an ATIUNICAM 240 constant flow pump working at a 150 psi pressure, and delivering a 2ml/min flow rate of the mobile phase, as described in Section 3.4. The separation of the OTC was done by a LiChrosorb RP 8-10 (250mm x 4.6 i.d) column with a methanol, acetonitrile and 0.01Maq. oxalic acid mobile phase in a 1:2:7 V/V/V ratio respectively at room temperature.

Fresh mobile phase was allowed to flow through the HPLC system for 1 hour to condition the column prior to OTC analysis. The pressure and flow rate were adjusted until the pressure stabilized at around 150psi and flow rate at the required 2 ml/min. Care was taken during the conditioning for the pressure not to rise above 350psi to avoid damage of the column and the HPLC system.

About 25-30µl methanol was drawn and injected into a 20µl volume loop. The system was allowed to run for 10minutes to allow enough time to produce a chromatogram (cgm), chromatographic report and the spectrum on the recorder, which had been set at a chart speed of 5 mm/min. There after, acetonitrile was injected and its cgm, cgm report and spectrum produced after an interval of another 10 minutes. This was followed by an injection of the mobile phase (MPH) and waited for 10 minutes to obtain its cgm, cgm

report and spectrum. Injection of methanol, acetonitrile and mobile phase was done routinely especially when a fresh mobile phase was used and the type of cgms, spectra and report content were noted to make sure that they conformed with manufacture's requirements. The mobile phase was injected from time to time as the samples were being run to check on column performance. Another reason was to take note of the retention time at which methanol and acetonitrile were eluted so that they did not cause confusion with those of the OTC, which was the target analyte. The UV detection wavelength had been set at 350 nm for OTC detection.

Oxytetracycline standard (25 – 30 µl) was injected and allowed to run for 15minutes to ensure complete clearance from the analytical column. The cgm, cgm report and spectrum were recorded and compared with those of MeOH, AC-N, and mobile phase. From this comparison the OTC peak was recorded from the cgm, as well as the retention time (tr) for OTC. All the OTC standards were run serially, and the retention times noted and were the same and similar to that of the 10ppm OTC standard. Methanol blanks were run in between to check for the analytical column condition. The areas under curve (AUC) for the standard were used to plot a standard curve. The column was then washed with methanol, then the mobile phase allowed to flow. Again 25-30µl of MeOH, AC-N and mobile phase were injected in series and their chromatograms, cgm reports and spectra recorded. Each injection was allowed 15 minutes to run for all constituents to be eluted before the next injection was made. This was followed by running the 9 tissue spikes consecutively, starting with the blanks, and recording their cgms, cgm reports and spectra. In between their runs OTC standards were run. The 10 ppm OTC tissue spike record were compared with those of 10ppm OTC standard. Records for the other spikes were

compared with their corresponding OTC standards and the proportion of OTC recovered was determined. The mean percent recovery of the spikes was used to correct for the final concentration of OTC in the unknown meat samples. A curve for the spikes of AUC versus concentration was plotted in order to read the concentrations of unknown directly from the graph.

After running all the tissue spikes and recording their cgms, cgm reports and their spectra the unknown samples were run. First a methanol blank sample was run then a tissue blank sample. This was aimed at determining the working efficiency of the column. A 25-30 µl sample of the 1.25ppm tissue spike was injected and run for 15 minutes and its cgm, cgm report and spectrum recorded. The retention time tr, height and area of the OTC peaks were noted by comparing with the earlier ones accumulated during the running of the tissue spike themselves. About 30µl of the first unknown sample was run for 15 minutes and its cgm, cgm report and spectrum recorded. Then the cgm was examined for peaks, and their retention times read on the cgm report. A peak with a similar retention time tr as that of the preceding 1.25 OTC tissue spike standard was taken as that of OTC in the unknown sample. Its tr, height and peak area were noted. Replicate injections of the meat sample were made, their cgm areas recorded and their mean areas calculated. The OTC concentration of this sample was calculated on the basis of the 1.25ppm spike area. Then an injection of the tissue blank was made followed by the injection of the spike containing OTC at 0.625ppm, and allowed to run for 15minutes. The OTC tr, the height and area were noted. If the peak area of these standards was half that of 1.25ppm spike this was an indication that the column efficiency was good for OTC analysis. The second unknown sample was injected; three runs were made. If there was no peak with retention time (tr)

similar to that of the 1.25ppm and 0.625 ppm OTC standards then this sample was considered negative for OTC. Another 30 µl of the next sample was injected and the same processes of reading the cgm records took place. If it was positive for OTC then its tr was compared to that of the 0.625 ppm tissue standard and its OTC concentration calculated on the basis of the 0.625ppm spike OTC mean cgm area. Then next an injection of the tissue spike blank was carried out. The 0.3175 ppm OTC tissue spike was injected, and its peak tr, height and area recorded. If its OTC peak area was 50% that the 0.625 ppm OTC tissue spike peak area then the column efficiency was considered good. Analysis of the next samples continued in the same manner, with intermittent injections of tissue spikes and blanks. The 2.5 ppm and 10.0 ppm standards were used during routine analytical process. Once they have been used to set the OTC retention time especially in fresh mobile phases they should no longer be used.

The area values for the standards and spikes were plotted against their OTC concentrations in ppm. The best line of fit was calculated using the equation $y = a + b \log x$ whereby "y" is the area (length) of peak mm^2, "a" is the y intercept, "b" is the slope and "x" is concentration of oxytetracyline in ppm. Concentrations of OTC in the unknown samples were also determined from the standard curve of the spikes.

Fresh mobile phase was allowed to run for 2-3 hours to allow column conditioning in order to avoid splitting of OTC peaks. Split peaks produce false peak areas and consequently false sample concentrations. From the peak area obtained from the OTC standards, the OTC concentration of an unknown sample (C_{su}) was calculated from the formula;

$$\text{Concentration of unknown sample } (C_{su}) \text{ ppm} = \frac{\text{Area } (A_{un}) \times \text{Conc } (C_{std}) \text{ppm}}{\text{Area } (A_{std})}$$

Where C_{std} is the concentration of the preceding known tissue spike in ppm

A_{std} is the peak area of the tissue spike

A_{un} is the peak area of the unknown OTC peak.

C_{su} is the OTC concentration in ppm of the unknown OTC positive sample.

The samples as well as the tissue spikes were run in triplicates during the HPLC analysis.

3.12 Statistical analysis

The questionnaire data were analysed by using Multiple Response and X^2 data analyses using an SPSS Computer Programme. The OTC residue data obtained from HPLC analysis of meat samples was analysed by use of t-test, X^2 test and one-way analysis of variance (ANOVA) with the aid of a computer.

CHAPTER FOUR

4.0 RESULTS

4.1 Questionnaire study results of the use pattern of OTC and other veterinary drugs by various stakeholders in Morogoro and Dodoma regions

4.1.1 The background information of livestock keepers

Livestock keepers located in Morogoro Urban and Morogoro Rural districts in Morogoro Region, and Dodoma Urban and Dodoma Rural districts in Dodoma Region were interviewed, with respect to sex, occupation, education, and formal livestock training, among other parameters. The background data from these interviwers is shown in Table 7. The total number of respondents was 46 in Dodoma Region and 65 in Morogoro Region, giving a total of 111.

4.1.1.1 Sex

The study revealed that out of 111 livestock keepers (respondents) interviwed, 90.1% were males and these were actually the livestock owners. Only 9.9% of the respondents were female livestock keepers. Female members of livestock keeping families did not want to be interviewed arguing that they were not the proprietors of the livestock. On the regional level there was no significant difference ($P>0.05$) in the percent age of livestock ownership between Morogoro (87.7% were male) and Dodoma (93.5% were male owners) regions. However on the district level there was a slight significant difference ($P<0.05$) whereby, the percent of respondent livestock ownership for males was 94% Dodoma urban, 90% Dodoma Rural, 50% Morogoro Urban, and 100% for Morogoro Rural.

4.1.1.2 Occupation

The main occupation of the livestock-keeping respondents was considered and it was observed that overall about 87.0% of the respondents were mixed livestock and crop farmers while only 9.0% were sole livestock keepers. There was a significant difference ($P<0.05$) on the main occupation of respondents regionally. While all 46 (100%) of Dodoma region respondents were mixed farmers only, About 76% of Morogoro respondents were mixed farmers and the remaining 15% were sole livestock keepers solely. At the district level there was a high significance of differences ($P<0.05$) with respect to the main occupation of the respondents. While all (100%) of the respondents in Dodoma Urban and Rural districts were mixed farmers, only 56% of Morogoro Urban and 84% of Morogoro respondents were mixed farmers. About 13% of Morogoro Urban respondents were sole livestock keepers and another 13% respondents from the same location were civil servants. About 16% of Morogoro Rural respondents were sole livestock keepers.

4.1.1.3 Education

Overall 55% of respondents in both regions never attended a classroom. They argued that they had an informal type of education; there was much skepticism on the ingenuity of this information especially for the pastoralists. About 39% of respondents had primary school level education while only about 5% had secondary school and advanced level education. At the regional level there was a significant difference ($P<0.05$) between the two regions where Dodoma respondents were more literate at 44% and 54% for informal and primary school level education respectively, compared with 63% and 28% for the same parameters in Morogoro Region respectively. The differences on these parameters were even more

significant at the district level (P<0.05). The values for the same parameters were 39% and 58% for Dodoma Urban, 60.0% and 40.0% for Dodoma Rural, 6.3% and 62.5% for Morogoro Urban, and 82% and 16% for Morogoro Rural districts respectively. About 4.6% of Morogoro Urban district respondents had secondary or advanced level education.

4.1.1.4 Formal livestock training

Overall only about 5.4% of livestock keepers had a formal training on modern livestock keeping methods. Of those who resided in Morogoro Urban district i.e. about 25% had formal livestock training whereas only 5.6% of Dodoma Urban respondents had some livestock training.

Table 7: Background information of livestock keepers in Dodoma and Morogoro regions

	Region				District									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
Variable	(n=46)	%	(n=65)	%	(n=36)	%	(n=10)	%	(n=16)	%	(n=49)	%	(n=111)	%
Sex														
Male	43	93.5	57	87.7	34	94	9	90	8	50	49	100	100	90.1
Female	3	6.5	8	12.3	2	5.6	1	10	8	50	0	0	11	9.9
	$X^2 = 1.01^{ns}$, df = 1						$X^2 = 34.96^{*}$, df = 3							
Occupation														
L/stock keeper	0	0	10	15.4	0	0	0	0	2	12.5	8	16	10	9
Mixed farmer	46	100	50	75.9	36	100	10	100	9	56.3	41	84	96	87
Civil servant	0	0	2	3.1	0	0	0	0	2	12.5	0	0	2	1.8
L/stock keeping and Business	0	0	1	1.1	0	0	0	0	1	6.3	0	0	1	0.9
L/stock keeping and civil servant	0	0	2	3.1	0	0	0	0	2	12.5	0	0	2	1.8
	$X^2 = 12.27^{*}$, df = 4						$X^2 = 39.67^{***}$, df = 12							
Education														
Informal	20	44	41	63.1	14	39	6	60	1	6.3	40	82	61	55
Primary	25	54	18	27.7	21	58	4	40	10	62.5	8	16	43	39
Secondary	1	2.2	2	3.1	1	2.8	0	0	1	6.3	1	2	3	2.7
Advanced Education	0	0	3	4.6	0	0	0	0	3	18.8	0	0	3	2.7
University	0	0	1	1.5	0	0	0	0	1	6.3	0	0	1	0.9
	$X^2 = 9.74^{*}$, df = 4						$X^2 = 52.09^{***}$, df = 12							
Formal L/stock training														
Yes	2	4.3	4	6.2	2	5.6	0	0	4	25	0	0	6	5.4
No	44	96	61	93.8	34	94	10	100	12	75	49	100	105	95
	$X^2 = 1.0$, df = 4						$X^2 = 15.39^{**}$, df = 3							

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 = Chi square; ns = non significance (X^2>0.5); * = Significance (0.01<X^2<0.05); ** = Significance (0.001<X^2<0.01); *** = Significance (X^2<0.001).

4.1.2 Experience in livestock keeping

Table 8 presents the results on parameters, which indicate the experience of the respondents in the livestock keeping business. The parameters include the number of years spent in livestock keeping, the farmers dependency on his cattle, and other means of livelihood for the livestock farmer.

4.1.2.1 Number of years logged in livestock keeping

Overall about 64% of the livestock keepers had an experience of up to 30 years in livestock keeping. About 34% had an experience of up to 20 years and 30% had an experience of between 21-30 years in livestock keeping, mainly as pastoralists. About 36% of the livestock keepers had an experience of > 30 years in livestock keeping. On a regional comparison there was a slight significant reference ($P<0.05$) in the livestock keeping experience between the two regions. While about 48% of Dodoma livestock keepers had $\leq$ 30 years experience in livestock keeping, 75.3% of Morogoro livestock keepers logged the same number of years; this number was significantly higher ($p<0.05$). The difference in livestock keeping experience on the district level was highly significant ($P<0.05$) with 56% of Dodoma Urban, 20% of Dodoma Rural, 87.6% of Morogoro Urban and 72% of Morogoro Rural district livestock keepers logging $\leq$ 30 years in livestock keeping. Morogoro Urban had the least experience whereby 81.3% of respondents had livestock keeping experience of $\leq$ 20 years.

4.1.2.2 Sole livestock dependency

Overall about 27% of respondents depended on livestock keeping as a sole source of family income as shown in Table 8. The rest (73%) had other parallel sources of income.

There was a highly significant difference ($P<0.05$) between the two regions in which all the Dodoma respondents had other sources of income, while 46.2% of Morogoro respondents depended on livestock income solely. The other 53.8% had an alternative source of income. The same trend of high significance of differences was echoed on the district level.

4.1.2.3 Other sources of dependence

Overall another major source of income for some of the livestock keepers was crop farming and livestock trading, which involved about 41% and 19% of the livestock keepers respectively. About 3% were civil servants while another 3% were petty traders. There was a highly significant difference ($P<0.05$) between the two regions and among the 4 districts on their livelihood dependencies. While in Dodoma region about 77% of the livestock keepers were also preoccupied with crop farming, only about 15.4% of the livestock keepers of Morogoro region were involved in crop farming. In Morogoro region, the other main preoccupation of livestock keepers was livestock trading. The same trend was observed in the districts.

Table 8: Experience in livestock keeping by Dodoma and Morogoro regions' livestock keepers

Variable	Region				District									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
	(n=46)	%	(n=65)	%	(n=36)	%	(n=10)	%	(n=16)	%	(n=49)	%	(n=111)	%
Number of years logged in livestock keeping														
< 20 years	11	24	27	41.5	11	31	0	0	13	81.3	14	29	38	34
21-30 years	11	24	22	33.8	9	25	2	20	1	6.3	21	43	33	30
> 30 years	24	52	16	24.6	16	44	8	80	2	12.5	14	29	40	36
	$X^2 = 9.02^{*}$, df =2						$X^2 = 30.04^{***}$, df = 6							
Livelihood dependency on livestock solely														
Yes	0	0	30	46.2	0		0	0	0	1	6.3	29	59	30	27
No	46	100	35	53.8	36		100	10	100	100	15	93.8	20	40.8	73
	$X^2 = 29.09^{***}$, df = 1						$X^2 = 46.23^{***}$, df = 3							
Others														
Civil servants	0	0	3	4.6	0		0	0	0	3	18.8	0	0	3	2.7
Livestock traders	2	4.3	19	29.2	2		5.6	0	0	0	0	19	39	21	19
Crop farmer	35	77	10	15.4	26		72	9	90	9	56.3	1	12	45	41
Traditional	0	0	1	1.5	0		0	0	0	0	0	1	2.9	1	0.9
Petty bussiness	2	4.3	1	1.5	2		5.6	0	0	1	6.3	0	0	3	2.7
Business man	0	0	2	3.2	0		0	0	0	2	12.5	0	0	2	1.8
Livestock traders/crop farmer	6	13	0	0.1	5		14	1	10	0	0	0	0	6	5.4
Civil servant/livestock trader and crop farmer	1	2.2	0	0	1		2.8	0	0	0	0	0	0	1	0.9
None	0	0	29	44.6	0		0	0	0	1	6.3	28	57	29	26
	$X^2 = 68.75^{***}$, df = 4						$X^2 = 130.0^{***}$, df = 24							

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 = Chi square; ns = non significance (X^2>0.5); * = Significance (0.01<X^2<0.05); ** = Significance (0.001<X^2<0.01); *** = Significance (X^2<0.001).

4.1.3 Background information of L.FO's/AFO's (Para veterinarians)

Table 9 presents a summary of the background information of the Livestock Field Officers (LFOs) and Agricultural Field Officer (AFOs) as revealed by the study. These two civil service technical cadres formed the largest group of Para-veterinarians, who have also been referred to as paravets.

4.1.3.1 Sex

Overall 60% and 40% of the LFOs/AFOs were male and female respectively and there was no significant difference ($P>0.05$) on the regional or district level on their distribution.

4.1.3.2 Professional qualifications

Overall 28% of the respondents had certificate level qualification only, 7.5% had a Diploma only and 65% had both a diploma and certificate in their respective disciplines. Again there was no significant difference ($P>0.05$) at the regional or district levels in their distribution in terms of distribution of the qualifications.

4.1.3.3 Professional or work experience

Overall 20% of the two technical cadres had a work experience of less than 15 years. About 40% had a work experience of 15-20 years while another 40% had a work experience of more than 20 years. Again on the regional and district levels there was no significant difference ($P>0.05$) on distribution of technical experience.

4.1.3.4 Nature of work

About 50% of the respondents were extension workers who were LFOs with some crop extension knowledge; 10% of the respondents were LFOs who were working predominantly as meat inspectors (Table 9). Actually these were the meat inspectors at the two municipal abattoirs. About 33% of respondents were AFOs who had some livestock extension knowledge i.e. retrained AFOs in livestock production. About 5% were respondents who had worked in Veterinary Clinics and Veterinary Investigation Centres (VICs).

Table 9: Background information of Livestock Field Officers/Agricultural Field Officers

Variable	Region				District									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
	(n=20)		(n=20)		(n=16)		(n=4)		(n=12)		(n=8)		(n=40)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Sex														
Male	14	70	10	50	10	63	4	100	4	33.3	6	75	24	60
Female	6	30	10	50	6	38	0	0	8	66.7	2	25	16	40
	X^2=1.67^{ns},df=1						X^2=7.01^{ns},df=3							
Professional Qualification														
Certifcate	6	30	5	25	4	25	2	50	2	16.7	3	38	11	28
Diploma	1	5	2	10	1	6.3	0	0	2	16.7	0	0	3	7.5
Certificate and Diploma	13	65	13	65	11	69	2	50	8	66.7	5	63	26	65
	X^2=0.42^{ns},df=2						X^2=4.04^{ns},df=6							
Professional Experience														
< 15 years	4	20	4	20	2	13	2	50	3	25	1	13	8	20
15-20 years	6	30	10	50	6	38	0	0	6	50	4	50	16	40
> 20 years	10	50	6	30	8	50	2	50	3	25	3	38	16	40
	X^2=2^{ns},df=2						X^2=5.94^{ns},df=6							
Nature of work														
Predominant L/stock, some crop extension and treatment	10	50	10	50	8	50	2	50	5	41.7	5	63	20	50
Predominant Meat inspection	4	20	0	0	4	25	0	0	0	0	0	0	4	10
Predominant crop, some L/stock extension and treatment	5	25	8	40	3	19	2	50	7	58.3	1	13	13	33
Extension, Treatment, vet clinic and VIC work	1	20	1	5	1	6.3	0	0	0	0	1	13	2	5
First and second option	0	0	1	5	0	0	0	0	0	0	1	13	1	2.5
	X^2=5.69^{ns},df=4						X^2=16.92^{ns},df=12							

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 =Chi square ;
ns = non significance (X^2>0.5); L/stock = livestock; < = less than; > = greater than; U = Urban; R = Rural;
VIC = Veterinary Investigation Centre; Vet = Veterinary.

4.1.4 Background information of pharmaceutical dealers of Dodoma and Morogoro municipalities

Background information profile of pharmaceutical dealers who were interviewed in this study is shown in Table 10.

4.1.4.1 Sex

Overall 75% of the veterinary drug shop dealers were of the male sex and 25% were females. A comparison of the two municipalities shows that there was no significant difference ($P>0.05$) on this parameter. About 71% of the dealers in Dodoma were male, and 78% in Morogoro were male also.

4.1.4.2 Qualifications of shop owner

Overall 62.5% of the drug shop owners (drug dealers) had a medical/ livestock/ pharmaceutical qualification. About 37.5% did not have any certificate level qualification. Again there was no significant difference ($P>0.05$) between the two municipalities.

4.1.4.3 Qualification of shop manager

Overall 68.8% of drug shop managers had pharmaceutical, medical or livestock certificate level qualification. About 31.3% did not have any qualification other than being family members of the shop owners. A comparison of the two regions showed a marked significance of differences ($P<0.05$) and this was due to the fact that while in Morogoro 100% of the shop managers had a qualification at certificate level, in Dodoma region only 28.6% of the shop managers had at least the minimum certificate level qualification to qualify to handle drugs.

4.1.4.4 Shop owner experience in the veterinary drug business

Table 10 shows that overall 56.3% of veterinary drug dealers had been in the business for less than 5 years while 31.3% had been in the business for more than 10 years. A comparison of the two municipalities or regions on this parameter showed no significant difference (P>0.05).

Table 10: Background information of pharmaceutical dealers in Dodoma and Morogoro Municipalities

Variable	Dodoma (n=7)		Morogoro (n=9)		Total (n=16)	
	No	%	No	%	No	%
Sex						
Male	5	71.4	7	77.8	12	75
Female	2	28.6	2	22.2	4	25
	$X2 = 0.09^{ns}$, df = 1					
Qualification of shop owner						
Medical/livestock/pharmaceutical qualification	3	45.9	7	77.8	10	62.5
Non-possession of above qualifications	4	57.1	2	22.2	6	37.5
	$X^2 = 2.05^{ns^{ns}}$, df = 1					
Qualification of shop manager						
Possession of medical/livestock/ pharmaceutical qualifications	2	28.6	9	100	11	68.8
Non-possession of the above	5	71.4	0	0	5	51.3
	$X^2 = 9.35^{**}$, df = 1					
Shop owner experience in the drug business						
< 5 years	3	42.9	6	66.7	9	56.3
5-10 years	3	42.9	2	22.2	31	31.3
> 10 years	1	14.3	1	11.1	2	12.5
	$X^2 = 0.97^{ns}$, df = 2					

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 =Chi square; ns = non significance (X^2>0.5); * = Significance (0.01<X^2<0.05); ** = Significance (0.001<X^2<0.01).

4.1.5 The background information profile of meat traders in Dodoma and Morogoro Municipalities

Table 11 shows the background information of meat traders who slaughtered cattle and other livestock at the municipal abattoirs.

4.1.5.1 Sex

Overall, in both municipalities, the meat trading business was 100% monopolized by the male sex.

4.1.5.2 Occupation

Various occupations were pursued by the meat traders. Overall 80% said they were mainly meat businessmen. About 10% said they were both meat traders and livestock keepers, while another 10% said they were meat traders, livestock keepers and crop farmers. A comparison of the two locations showed no significance of difference ($P > 0.05$).

4.1.5.3 Education

The level of education of the meat traders was also assessed. Overall only 60% had a primary school education, 10% had an informal education (e.g. adult education or on job training through experience), and only 30% had secondary school education. A comparison of this parameter between Dodoma and Morogoro showed no significant difference ($p > 0.05$).

4.1.5.4 Formal training in the meat business

Overall 100% of all meat traders in Dodoma and Morogoro municipalities had never undergone any meat trading course as shown in Table 11.

4.1.5.5 Number of years logged in the meat business

Overall about 20% of meat traders had been in the business for less than 10 years; 40 % had been in the business for 10-15 years; and another 40% had been in the business for more than 15 years.

4.1.5.6 Reasons for engaging in the butcher business

Table 11 shows the various reasons given by the meat traders as their basis of their decision to engage in the meat business. Overall 70% responded that they had a personal interest from childhood, 20% said they inherited their businesses from their father, uncles etc, while 10% said they were just practicing business because they had meat supply tenders to state institutions such as schools and prisons.

Table 11: Background information of meat traders in Dodoma and Morogoro Municipalities

Variable	Dodoma (n=5)		Morogoro (n=5)		Total (n=10)	
	No	%	No	%	No	%
Sex						
Male	5	100	5	100	10	100
Female	0	0	0	0	0	0
	X^2 = -,df = -					
Occupation						
Meat businessmen	4	80	4	800	8	80
Livestock keepers and meat business	1	20	0	0	1	10
Livestock keepers, meat business man crop farmer	0	0	1	20	1	10
	X^2 = 2.00ns,df = 2					
Education						
Informal	0	0	1	20	1	10
Primary	4	80	2	40	6	60
Secondary	1	20	2	40	3	30
	$X^2 = 2.00^{ns}$,df = 2					
Formal training in the meat business						
Yes	0	0	0	0	0	0
No	5	100	5	100	10	100
	No statistic No X^2					
Number of years logged in meat business						
< 10 years	1	20	1	20	2	20
10- 15 years	1	20	3	60	4	40
> 15 years	3	60	1	20	4	40
	$X^2 = 2.00^{ns}$,df = 2					
Reason for engaging in the butcher business						
Personal interest/initiative from childhood	3	60	4	80	7	70
Inherited from father, uncle etc	1	20	1	20	2	20
Acquisition of meat tender supply institutions	1	20	0	0	1	10
	$X^2 = 1.14^{NS}$, df = 2					

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 = Chi square; ns = non significance (X^2>0.5).

4.1.6 Problems encountered by livestock farmers

Problems encountered by livestock farmers are summarised in Table 12. About 95% of the farmers in the two regions cited livestock diseases as the leading problem in livestock keeping. Overall the other major problems were mentioned as pasture scarcity (28%) and

water availability (16%). The same trend was echoed at regional and district levels. Other major problems mentioned by the Morogoro Urban respondents included the scarcity of improved dairy bulls, cattle rustling and labour. When their animals fell sick they sought the assistance of LFOs/AFOs to assist in treatment, otherwise farmers treated animals themselves.

Table 12: Problems encountered in livestock keeping by farmers[1]

Variable	Region				District									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
	(n=46)	%	(n=65)	%	(n=36)	%	(n=10)	%	(n=10)	%	(n=10)	%	(n=111)	%
Disease	44	96	61	93.8	34	94	10	100	13	81.3	48	98	105	95
Pasture	13	28	18	27.7	13	36	0	0	7	43.8	11	22	31	28
Water	9	20	9	18.8	9	25	0	0	1	6.3	8	16	18	16
Scarcity of grazing land	0	0	2	3.1	0	0	0	0	0	0	2	4.1	2	1.8
Livestock theft	3	6.5	2	3.1	2	5.6	1	10	2	12.5	0	0	5	4.5
Labour	0	0	2	3.1	0	0	0	0	2	12.5	0	0	2	1.8
Improved bulls for dairy cattle	0	0	3	4.1	0	0	0	0	3	18.8	0	0	3	2.7
Low Productivity animals	1	2.2	1	1.5	0	0	1	10	1	6.3	0	0	2	1.8
Bush fires	1	2.2	1	1.5	1	2.8	0	0	0	0	1	2	2	1.8
Lack of drugs	0	0	1	1.5	0	0	0	0	0	0	1	2	1	0.9
Low market prices	3	6.5	3	4.6	3	8.3	0	0	2	12.5	1	2	6	5.4
Deaths (Mortalities)	4	8.7	0	0	4	11	0	0	0	0	0	0	4	3.6
Drug scarcity	2	4.3	0	0	2	5.6	0	0	0	0	0	0	2	1.8
No dipping facility	2	4.3	0	0	2	56	0	0	0	0	0	0	2	1.8

[1] Multiple response

n = number of respondents; U = Urban; R = Rural.

4.1.7 Management of sick animals by livestock farmers

Various options by which animals are managed by farmers when they fall sick are as shown in Table 13.

4.1.7.1 Options for sick animals

Overall 49% of the livestock farmers treated sick animals themselves, while 42% indicated that they consulted the paraveterinarians (LFOs/AFOs). About 16% merely kept watching the animals to recover spontaneously. In Dodoma region about 39% of farmers treated their animals by themselves while the same proportion of farmers, i.e. 40% depended on paraveterinarians to attend their animals. In contrast in Morogoro region 55% treated the sick animals themselves, 23% depended on paravets and another 23% did not attend their animals in anyway. At the district level, in Dodoma Urban district about 33% treated their animals themselves, 67% depend on LFOs and 8.4 waited for spontaneous recovery. In Dodoma Rural district, 60% treated themselves, 80% depended on i.e. LFOis/AFOs. In Morogoro Urban district, only 12.2% treated their animals by themselves. 93.8% depend on LFOs/AFOs to treat their animals. In Morogoro Rural district about 70% of the livestock farmers treated their animals by themselves while 30% depended on the spontaneous recovery of their animals. The emerging picture was that most of the sick animals in Morogoro Rural district were treated by their owners unlike the other three districts. Overall about 42% of the farmers said they called on LFOs/AFOs for treatment assistance if and when they were available. The same observation was echoed by the LFOs/AFOs themselves as shown on Table 13.

4.1.7.2 Options for the terminally ill animal

Tables 13 further shows the options taken by farmers when it became imminent that their treated animals wouldn't recover. Overall 68% of farmers sold and slaughtered their terminally ill animals when it became apparent they (animals) would not survive. About 3.6% waited for the animal to die and condemn; 11% of farmers consumed their treated animals upon death; 7% of the farmers euthanised and burried the terminally ill animals, while 2% sought advise from paraveterinarians. Some farmers comprising 2% of respondents provided no treatment to their terminally sick animals but upon noting death of the animals they consumed the meat. The last group of farmers, about 7% were not sure on what to do. While for Dodoma and Morogoro regions there was no significant difference ($P > 0.05$) between them, i.e. 76% of Dodoma region farmers and 61.5% Morogoro farmers sold and slaughtered their terminally sick animals, the differences between the districts were highly significant ($P<0.05$).

Table 13: Management of Sick animals by farmers

	Region				District									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
Variable	(n=46)	%	(n=65)	%	(n=36)	%	(n=10)	%	(n=16)	%	(n=49)	%	(n=111)	%
Options when Animals are sick[1]														
Self treatment	18	39	36	55.4	12	33	6	60	2	12.5	34	69	54	49
Treatment by L.F.O./A.F.O.	22	40	15	23.1	24	67	8	80	15	93.8	0	0	47	42
Self treatment or treatment by LFO/AFO	2	4.3	0	0	2	5.6	0	0	0	0	0	2	1.8	1.6
Spontaneous recovery	3	6.5	15	23.1	3	8.4	0	0	0	0	15	31	18	16
Paraveterinarians	1	2.2	0	0	0	0	1	10	0	0	0	0	1	0.9
None	0	0	1	1.5	0	0	0	0	1	6.3	0	0	1	0.9
Options terminally ill animal[1]														
Sell and slaughter	35	76	40	61.5	28	78	7	70	2	12.5	38	78	75	68
Spontaneous death and condemination	0	0	4	6.2	0	0	0	0	3	18.8	1	2	4	3.6
Treat and slaugther upon death	3	6.5	9	13.8	1	2.8	2	20	0	0	9	18	12	11
Euthanise and burry	3	6.5	5	7.7	3	8.3	0	0	4	25	1	2	8	7.2
Seek advise from L/stock personnel	1	2.2	1	1.5	1	2.8	0	0	1	6.3	0	0	2	1.8
No treatment wait for death, inspect and eat	2	4.3	0	0	1	2.8	1	10	0	0	0	0	2	1.8
DNK	2	4.3	6	9.2	2	5.6	0	0	6	37.5	0	0	8	7.2
	X^2= 8.84^{ns}, df = 6								X^2 = 70.38***, df = 18					

[1] Multiple response

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 =Chi square ;
ns = non significance (X^2>0.5); *** = Significance (X^2<0.001); DNK = Do Not Know.

4.1.8 Management of sick animals by Livestock Field Officers (LFOs)/ Agricultural Field Officers (AFOs)

Table 14 shows the response by LFOs/AFOs interviewed on the aspect of their provision of treatment to sick animals for livestock keepers. The options included whether they (LFOs/AFOs) personally attended the sick animals, advised farmers on post treatment issues such as withdrawal periods, and management of terminally sick animals.

4.1.8.1 Call by farmers to attend sick animals

Overall 98% of the LFOs/AFOs responded by saying that farmers called them from time to time to attend to their (farmers') sick animals. On this parameter there was no regional or district significance of differences ($P>0.05$).

4.1.8.2 Type of advise offered to farmers after recovery of their treated animals

Two main alternatives of advice were given on this parameter. Overall 68% of the LFOs/AFOs said they advised observation of a withdrawal period and then slaughter; 5.3% adviced immediate slaughter while 26% didn't have any advise to the farmer. On a regional and district comparison, there were no significant differences between the regions nor districts ($P>0.05$).

4.1.8.3 Type of advise offered to farmers for treated terminally sick animals

Overall 28% said they would advise the farmers to slaughter the animal, have it inspected and consumed by the people. Only 23% said they would advise sacrificing the animal and condemnation of the carcass. About 18% said they would continue to treat the animal to death and then have it condemned, while 7.5% said they would advise to have treatment to

the animal stopped, then let the animal die and condemn. About 13% said they would observe withdrawal period, i.e. stop treatment, observe withdrawal period and then have it slaughtered and consumed. But this group was hypocritical for it appeared that if the animals were about to die before the withdrawal period they would certainly slaughter and have them consumed. About 2.5% said their judgment and advice to the farmer on the fate of the terminally ill animal would depend on the type of drug that was being used for treatment. But if it was OTC then they would advice slaughter and consumption. Another 2.5% said they would observe the withdrawal period, which they didn't even know. Another 2.5% who were not sure of which advise to give said they would advise condemnation and disposal or withdrawal period and slaughter. The last 2.5% did not have any specific advise to the farmers.

A comparison of the responses between the two regions using the X^2 test, showed that there were no significant differences ($P>0.05$) of the responses between the two regions. While the right advice to the farmers in order to protect consumers would have been outright condemnation and disposal, in Dodoma region only 10%, and in Morogoro region only 35% of the LFOs/AFOs, mentioned this as their would be judgment and professional advice to the farmer. At the district level there were no significant differences ($P>0.05$) of the responses between the four districts respondents. Again at the district level, the proportion of those who responded positively to the condemnation and disposition verdict were, only 6.3% of Dodoma urban, 2.5% of Dodoma Rural, 41.7% of Morogoro Rural and 2.5% of Morogoro Rural LFOs/AFOs respectively. This means the professional people closest to the farmers were giving the wrong advise and hence letting the people to continue consuming drug-adulterated beef without any control.

Table 14: Management of sick animals by LFOs/AFOs

Variable	Region				District									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
	(n=20)		(n=20)		(n=16)		(n=4)		(n=12)		(n=8)		(n=40)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Call by farmers to attend sick animals														
Yes	20	100	19	95	16	100	4	100	12	100	7	88	39	98
No	0	0	1	5	0	0	0	0	0	0	1	13	1	2.5
	$X^2 = 1.03^{ns}$, df=1							$X^2 = 4.1^{ns}$, df=3						
Advice for treated recovered animals fute														
Observe W.P and slaughter	13	68	13	68.4	10	67	3	75	7	58.3	6	86	26	68
Advise immediate slaughter	2	11	2	5.3	1	6.7	1	25	0	0	0	0	2	5.3
DNK	4	21	6	31.6	4	27	0	0	5	41.7	1	14	10	26
	$X^2 = 12.4^{ns}$, df=2							$X^2 = 7.05^{ns}$, df=6						
Advise for treated non recovered animal														
Advise condemnation and bury	2	10	7	35	1	6.3	1	2.5	5	41.7	2	25	9	23
Let animal to die and condemn	0	0	3	15	0	0	0	0	0	0	3	38	3	7.5
Slaughter, inspect and consume depends on type of drug but with OTC	8	40	3	15	6	38	2	50	2	16.7	1	13	11	28
just slaughter and consume	1	5	0	0	1	6.3	0	0	0	0	0	0	1	2.5
Observe WP, if too long condemn	1	5	0	0	1	6.3	0	0	0	0	0	0	1	2.5
Observe WP and slaughter	5	25	0	0	4	25	1	25	0	0	0	0	5	13
Rx to death and bury	1	5	6	30	1	6.3	0	0	5	41.7	1	13	7	18
Advise condemnation and burry or observe WP and slaughter	0	0	1	5	0	0	0	0	0	0	1	13	1	2.5
DNK	0	0	1	5	0	0	0	0	0	0	1	13	1	2.5
	$X^2 = 12.4^{ns}$, df=8							$X^2 = 39.33,^{ns} = 24$						

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 = Chi square;
ns = non significance (X^2>0.5); DNK = Do Not Know; Rx= Treatment WP = Withdrawal Period.

4.1.9 Diseases encountered by livestock farmers

The farmers mentioned the most common diseases Table 15 affecting their animals, and which necessitated use of drugs for treatment and control. A list of the 20 main livestock diseases encountered by livestock keepers included bacterial, viral, protozoal, helminthosis, and non-infectious conditions. Overall 69% had mentioned contageous bovine pleuropneumonia (CBPP) as the leading disease problem in the two regions, followed by ECF at 64%, trypanosomosis 44%, babesiosis 36%, lumpy skin disease (LSD) 34%, anaplasmosis 25%, and contageous caprine pleuropneumonia (CCPP) 24%. The same trends were generally observed at the regional and district levels. The types of diseases affecting their animals were of paramount importance because they were the ones that dictated which type of drugs to use. In Dodoma the major disease problems were CBPP 63%, Blackquarter (BQ) 61%, CCPP 52%, footrot 46%, foot and mouth disease (FMD) 41% and anaplasmosis 37%. In Morogoro region the most important diseases were east coast fever (ECF) 76.9%, trypanosomosis 75.4%, CBPP 73.8%, LSD 56%, BQ 56%, FMD 51%, CCPP 50% and tick infestation 39%.

In Morogoro Urban the leading diseases were Anaplasmosis 31.3%, mastitis 25%, trypanosomosis 18.8%, ECF 18.8%, FMD 12.5%, CBPP 6.3%, LSD 6.3% and tick infestation 6.3%. In Morogoro Rural district the major diseases were CBPP 96%, ECF 96%, trypanosomosis 94%, LSD 74%, babesiosis 74%, FMD 63%, BQ 49% and heart water 45%.

Table 15: Specific diseases encountered by farmers [1]

Variable	Region				District									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
	(n=46)	%	(n=65)	%	(n=36)	%	(n=10)	%	(n=10)	%	(n=10)	%	(n=111)	%
Diseases encountered														
ECF	21	46	50	76.9	15	42	6	60	3	18.8	47	96	71	64
Trypanosomosis	0	0	49	75.4	0	0	0	0	3	18.8	46	94	49	44
CBPP	29	63	48	73.8	22	61	7	70	1	6.3	47	96	77	69
Heart water	2	4.3	22	33.8	2	5.6	0	0	0	0	22	45	24	22
LSD	1	2.2	37	56.9	1	2.8	0	0	1	6.3	36	74	38	34
FMD	19	41	33	50.8	18	51	1	10	2	12.5	31	63	52	47
BQ	28	61	24	36.9	20	56	8	80	0	0	24	49	52	47
Babesiosis	4	8.7	36	55.4	3	8.3	1	10	0	0	36	74	40	36
Anthrax	5	11	4	6.2	4	11	1	10	0	0	4	8.2	9	8.1
Worms	8	17	4	6.2	6	17	2	20	0	0	4	8.2	12	11
Anaplasmosis	17	37	11	16.9	12	33	5	50	5	31.3	6	12	28	25
Mastitis	0	0	4	6.2	0	0	0	0	4	25	0	0	4	3.6
Footroot	21	46	1	1.5	12	33	9	90	1	6.3	0	0	22	20
CCPP	24	52	3	4.6	18	50	6	60	1	6.3	2	4.1	27	24
Tick infestation	18	39	4	6.2	14	39	4	40	1	6.3	3	6.1	22	20
Brucelosis	3	6.5	0	0	3	8.3	0	0	0	0	0	0	3	2.7
Flies	4	8.7	0	0	2	5.6	2	20	0	0	0	0	4	3.6
Diarrhoea/ Dysentry	4	8.7	0	0	3	8.3	1	10	0	0	0	0	4	3.6
Plastic Bags	10	22	0	0	8	22	2	20	0	0	0	0	10	9
None	0	0	5	7.5	0	0	0	0	5	31.3	0	0	5	4.5

[1] Multiple response

ECF = East Coast Fever; CBPP = Contagious Bovine Pleuropneumonia; CCPP = Contagious Caprine Pleuropneumonia; LSD = Lumpy skin disease; FMD = Foot and mouth disease; BQ = Black quarter; U = Urban; R = Rural; n = number of respondents.

4.1.10 Occurrence of diseases as experienced by Livestock Field Officers (LFOs)/Agricultural Field Officers (AFOs)

Table 16 shows the occurrence of various diseases as experienced by the LFOs/AFOs. Overall about 27 major diseases were reported to occur in the two regions. The top 15 diseases reported to occur in terms of number (%) of respondents were ECF (80%), anaplasmosis (80%), worm infestation (53%), milk fever (50%), CBPP (45%), babesiosis (45%) and trypanosomosis (33%). Others were LSD (30%), mastitis (30%), CCPP (28%), FMD (25%), black quarter (25%), heart water (23%), mange (23%) and diarrhoea/dysentery (18%). On the regional and district levels there was a significant difference of occurrence of the diseases as indicated by the percent of the respondents. In Dodoma region ECF was mentioned by 100% of the respondents while in Morogoro region 60% did so. About 100% did so in Dodoma Urban and Rural districts while only 42% and 88% did so in Morogoro Urban and Rural districts respectively.

While only 15% mentioned trypanosomosis in Dodoma region, 50% mentioned it in Morogoro region, 19% in Dodoma Urban, none in Dodoma Rural, 33% in Morogoro Urban, and 75% in Morogoro Rural. LFOs/AFOs mentioned trypanosomosis as one of the major disease problems. CBPP was mentioned as a major disease problem by 55% of Dodoma region LFOs/AFOs, while only 35% in Morogoro region did so. At the district level only 44% of Dodoma Urban, 100% of Dodoma Rural, 8.3% of Morogoro Urban and 75% of Morogoro Rural LFOs/AFOs mentioned CBPP as a major disease problem.

There was some agreement on the occurrence of anaplasmosis whereby 90% of Dodoma region and 70% of Morogoro region LFOs/AFOs pointed out anaplasmosis as a problem.

At the district level 88%, 100%, 58% and 88% of respondents of Dodoma Urban, Dodoma Rural, Morogoro Urban, and Morogoro Rural districts respectively mentioned anaplasmosis as the major disease problem. Milk fever, which was pointed out by all respondents (100%) of Dodoma region as a major problem, was not mentioned at all in Morogoro region; 13% of Dodoma Urban respondents mentioned it as a problem. In the other districts no mention was made.

Another area of significant difference between the two regions and between the four districts of study was the occurrence of CCPP. While 50% of Dodoma region respondents indicated CCPP as a major disease problem only 5% of Morogoro LFOs/AFOs did so. And while 44% of Dodoma Urban, and 75% of Dodoma Rural respondents mentioned CCPP as a major problem, only 8.3% and 11% of Morogoro Urban and Morogoro Rural LFOs/AFOs pointed to CCPP as a major disease problem.

Table 16: Occurrence of diseases as experienced by livestock Field Officers/Agricultural Field Officers [1]

Variable	Region				District									
	Dodoma (n=20)		Morogoro (n=20)		Dodoma (U) (n=16)		Dodoma (R) (n=4)		Morogoro (U) (n=12)		Morogoro (R) (n=8)		Total (n=40)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Disease Occurrence														
ECF	20	100	12	60	16	100	4	100	5	41.7	7	88	32	80
Trypanosomosis	3	15	10	50	13	19	0	0	4	33	6	75	13	33
CBPP	11	55	7	35	7	44	4	100	1	8.3	6	75	18	45
Heart water	6	30	3	15	4	25	2	50	1	8.3	2	25	9	23
Lumpy skin disease (LSD) ,	3	15	9	45	2	13	1	25	3	25	6	75	12	30
Foot and Mouth disease (FMD)	5	25	5	25	4	25	1	25	0	0	5	63	10	25
Black quarter	10	50	0	0	7	44	3	75	0	0	0	0	10	25
Babesiosis	11	55	7	35	9	56	2	50	2	16.7	5	63	18	45
Anthrax	5	25	0	0	3	19	2	50	0	0	0	0	5	13
Worms	9	45	12	60	7	44	2	50	5	41.7	7	88	21	53
Anaplasmosis	18	90	14	70	14	88	4	100	7	58.3	7	88	32	80
Mastitis	8	40	4	20	6	38	2	50	3	25	1	13	12	30
Foot root	4	20	1	5	4	25	0	0	0	0	1	13	12	13
Mange	3	15	6	30	2	25	1	25	4	33.3	2	25	9	23
CCPP	10	50	1	5	7	44	3	75	1	8.3	0	0	11	28
Milk fever	2	100	0	0	2	13	0	0	0	0	0	0	2	50
Rinderpest	1	5	0	0	1	6.3	0	0	0	0	0	0	1	2.5
Wounds	0	0	2	10	0	0	0	0	2	16.7	0	0	2	5
Diarhoea/ Dysentery	4	20	3	15	2	13	2	50	1	8.3	2	25	7	18
Liver flukes	1	5	0	0	0	0	1	25	0	0	0	0	1	2.5
Retained placenta	1	5	0	0	1	6.3	0	0	0	0	0	0	1	2.5
Bloat/Tympany	1	5	0	0	1	6.3	0	0	0	0	0	0	1	2.5
Abscess	1	5	0	0	1	6.3	0	0	0	0	0	0	1	2.5
Pneumonia	2	10	0	0	2	13	0	0	0	0	0	0	2	5
Dystocia	3	15	0	0	3	19	0	0	0	0	0	0	3	7.5
Tuberculosis	1	5	0	0	1	6.3	0	0	0	0	0	0	1	2.5
Haemorrhagic septicaemia	1	5	0	0	0	0	1	25	0	0	0	0	1	2.5
DNK	0	0	5	25	0	0	0	0	4	33.3	1	13	5	13

[1] Multiple response

ECF = East Coast Fever; CBPP = Contagious Bovine Pleuropneumonia; CCPP = Contagious Caprine Pleuropneumonia; LSD = Lumpy skin disease; FMD = Foot and mouth disease; BQ = Black quarter; U = Urban; R = Rural; n = number of respondents; DNK = Do not know.

4.1.11 Livestock diseases for which livestock keepers purchase drugs

Table 17 shows a list of most important diseases according to drug dealers' responses. Overall 93.8% of the drug dealers said ECF was the most frequent disease often complained about by the farmers. Others included anaplasmosis 69%, trypanosomosis 56%, CBPP 56%, black quarter 44%, mastitis 44%, babesiosis 38%, Footrot 38%, Heart water 38%, worms infestation 31% and FMD 25%. Other important diseases mentioned included LSD 18%, anthrax 18% and Diarrhoea/dysentery 18%.

Table 17: Diseases mentioned by veterinary pharmaceutical dealers against which drugs are purchased by livestock keepers in Dodoma and Morogoro municipalities

Variable	Dodoma (n=7)		Morogoro (n=9)		Total (n=16)	
	No	%	No	%	No	%
Disease[1]						
ECF	7	100	8	88.9	15	93.8
Trypanosomosis	2	28.6	7	77.8	9	56.3
CBPP	6	85.7	3	33.3	9	56.3
Heart water	1	14.3	4	44.4	5	31.3
LSD	0	0	3	33.3	3	18.3
FMD	2	28.6	2	22.2	4	25
Black quarter	5	71.4	2	22.2	7	43.8
Babesiosis	3	42.9	3	33.3	6	37.5
Anthrax	3	42.9	0	0	3	18.8
Worms	3	42.9	2	22.2	5	31.3
Anaplasmosis	4	57.1	7	77.8	11	68.8
Mastitis	2	28.6	5	55.6	7	43.8
Footrot	6	85.7	0	0	6	37.5
Mange	1	14.3	0	0	1	6.3
CCPP	2	28.6	0	0	2	12.5
Milk fever	1	14.3	0	0	1	6.3
Diarrhoea/Dysentery	0	0	3	3.3	3	18.8
Keratoconjuctivitis	1	14.3	0	0	1	6.3

[1] Multiple response

ECF = East Coast Fever; CBPP = Contagious Bovine Pleuropneumonia; CCPP = Contagious Caprine Pleuropneumonia; LSD = Lumpy skin disease; FMD = Foot and mouth disease; n = number of respondents.

4.1.12 Drugs used by livestock farmers to treat their sick animals

Farmers made mention of the various single and combinations of drug, which they used for treatment against various diseases of livestock, as shown in Tables 18 and 19. Overall about 81% of the livestock farmers used one of the four formulations of tetracyclines to treat their animals. The results showed that OTC was the most highly used drug by farmers in the two regions. The four OTC formulations were OTC 10%, OTC 20%, OTC-DNK, and OTC spray. Many of the livestock keepers who administered OTC to their sick animals could not differentiate between the 10% and 20% formulations. This group had been designated to use OTC-DNK (i.e. OTC do not know, or non defined OTC). Within the group of farmers using OTC, 35% used OTC 20% (i.e. LA), 27% used OTC-DNK, 16% used OTC 10%, and 3.6% used OTC spray. Hence overall the total percentage of farmers using injectable OTC was 78%. Other leading drugs used by farmers and their percentages were: 42% used Berenil® (diminazine aceturate), 36% used Samorin® (isometamidium chloride), 29% used Novidium® (homidium chloride), 11% used Butalex® (buparvoquine), 9.8% used Stelladone® (Chlorfenviphos), 9.1% used traditional medicine, 8.1% used Streptopen® (steptomycin-penicillin combination) and 6.9% used Parvexon® (parvaquone). Between the regions, there was the same trend on the use of OTC. It was the most highly used drug in both regions and districts, as exemplified by the percentage of its use as follows: 41.72% Dodoma region, 76.8% Morogoro region, 36.8% Dodoma Urban district, 60% Dodoma Rural district, 40.1% Morogoro Urban and 123% cumulative percentage Morogoro Rural district. The main formulation of OTC used was OTC 20% or LA formulation.

In spite of the common trend in the use of OTC in the regions and district there were significant differences in the use of other important drugs as exemplified by the percentage responses. In Dodoma region other important drugs were: traditional medicine 20%, tylosin 4%, Stelladone 4%, albendazole 4.3%, Streptopen 2% and penicillin 2%. In Morogoro region other leading drugs were Berenil 72%, Samorin 63%, Novidium 50%, Streptopen 47%, Butalex 19%, Clexon 8% and Tylosin 6%.

Table 18: Single drugs used by farmers [1]

Variable	Region				District									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
	(n=46)	%	(n=65)	%	(n=36)	%	(n=10)	%	(n=10)	%	(n=10)	%	(n=111)	%
OTC - 10%	4	8.7	17	15	1.1	2.8	3	30	1	6.7	12	25	17	16
OTC - 20%	8	17	38	34.5	6	17	2	20	1	6.7	29	59	38	35
OTC - DNK	7	16	30	27.3	6	17	1	10	4	26.7	19	39	30	27
OTC - Spray	1	2.2	4	3.6	1	2.8	0	0	2	13.3	1	2	4	3.6
Penicillin	1	2.2	5	7.8	1	2.8	0	0	1	6.7	3	6.1	5	4.4
Streptopen®	1	2.2	30	46.9	0	0	1	10	0	0	30	18	31	8.1
Imical®	0	0	1	1.6	0	0	0	0	0	0	1	2	1	0.9
Tylosin	2	4.3	4	6.3	2	5.6	0	0	1	6.7	3	6	6	5.5
Butalex®	0	0	12	18.8	0	0	0	0	2	13.3	10	2.4	12	11
Clexon®	0	0	5	7.8	0	0	0	0	0	0	5	10	5	4.5
Parvexon®	0	0	1	1.6	0	0	0	0	0	0	1	2	1	6.9
Berenil®	0	0	46	71.9	0	0	0	0	0	0	46	94	46	42
Novidium®	0	0	32	50.1	0	0	0	0	0	0	32	65	32	29
Samorin®	0	0	40	62.5	0	0	0	0	2	13.3	38	78	40	36
Diminasan®	0	0	1	1.6	0	0	0	0	0	0	1	2	1	0.9
Milsan®	0	0	1	1.6	0	0	0	0	0	0	1	2	1	0.9
Albendazole	2	4.3	2	3.1	2	5.6	0	0	0	0	2	4.1	4	3.6
Traditional medicine	9	20	1	1.6	8	22	1	10	0	0	1	2	10	9.1
Stelladone®	2	4.3	0	0	2	5.6	0	0	0	0	0	0	2	9.8

[1] Multiple response

n = number of respondents; U = Urban; R = Rural; DNK = Do Not Know; OTC = Oxytetracycline ;Streptopen = Streptomycin- penicillin; Imical = Imizol; Clexon® = parvaquone; Butalex® = buparvoquine; Diminasan® = diminezine aceturate ; Milsan® = , Levamisole hydrochloride; Samorin® = isometamidium chloride; Parvexon® = pavaquone Clexon® = parvaquone; Novidium® = homidium chloride and Stelladone® = chlorfeviphos.

4.1.13 Combination drugs used for treatment by livestock farmers

Table 19 shows a list of combination drugs used by livestock farmers in the treatment of their animals. Overall the most intensively used drug combinations was reported by farmers to involve OTC. Drug combinations involving OTC accounted for 105% cumulative percentage of all responses and these included OTC – Butalex®, 40%; OTC – Berenil®, 30%; OTC – Clexon®, 22%; OTC – Parvexon®, 10%; OTC – Crystaline penicillin, 2.6%; and OTC – deep wash – spray, 2.6%. The only major non-OTC combination used was intramammary formulation, mostly penicillin- Penstrep®, 58%. Other major drug combinations used were OTC- Penstrep®, 6.4%; and Parvexon® - Berennil® 2.6%. In Dodoma Region farmers did not use combination drugs at all. In Morogoro Region combination drugs used included OTC – Streptopen®, 9.8%; dipping – Streptopen®, 5.9%; OTC- dipping (spray), 3.9%; Parvexon® - Berenil®, 3.9%; Butalex®-Berenil®, 2%; salt water – ashes, 2%; Streptopen® - fortified procaine penicillin, 2%; OTC - Clexon® – Butalex®, 2%; and OTC – Novidium®-Berenil®, 2%.

Table 19: Combination drugs used by farmers for animal treatment

	Region				District									
	Dodoma		Morogoro		Dodoma (u)		Dodoma (R)		Morogoro(U)		Morogoro (R)		Total	
Variable	(n=46)	%	(n=65)	%	(n=36)	%	(n=10)	%	(n=16)	%	(n=49)	%	(n=111)	%
Combination Drugs [1]														
OTC and Parvexon ®	0	0	8	15.7	0	0	0	0	0	0	8	18	8	10
OTC and Butalex ®	0	0	31	60.8	0	0	0	0	2	3.3	29	64	31	40
OTC and Berenil ®	0	0	23	45.1	0	0	0	0	1	16.7	22	49	23	30
OTC and Clexon ®	0	0	17	33.3	0	0	0	0	0	0	17	38	17	22
OTC and Human Crystapen ®	0	0	2	3.9	0	0	0	0	0	0	2	4.4	2	2.6
OTC and Streptopen ®	0	0	5	9.8	0	0	0	0	0	0	5	11	5	6.4
OTC and Dip wash spray	0	0	2	3.9	0	0	0	0	0	0	2	4.4	2	2.6
Butalex ® and Berenil ®	0	0	1	2	0	0	0	0	0	0	1	2.2	1	1.3
Salt water and Ashes	0	0	1	2	0	0	0	0	0	0	1	2.2	1	1.3
Streptopen ® and Human PPF ®	0	0	1	2	0	0	0	0	0	0	1	2.2	1	1.3
Dipping and streptopen ®	0	0	3	5.9	0	0	0	0	0	0	3	6.7	3	3.8
Parvexon ® and Berenil ®	0	0	2	3.9	0	0	0	0	1	16.7	1	2.2	2	2.6
OTC and Clexon ® and Butalex ®	0	0	1	2	0	0	0	0	0	0	1	2.2	1	1.3
OTC and Novidium ® and Berenil ®	0	0	1	2	0	0	0	0	0	0	1	2.2	1	1.3
Human PPF ® and Dipping/Spraying	0	0	1	2	0	0	0	0	0	0	1	2.2	1	1.3
Dipping and salt water I/Mamaries and Penstrep ®	0	0	1	2	0	0	0	0	0	0	1	2.2	1	1.3
	0	0	1	2	0	0	0	0	3	50	15	33	45	58
DNK	27	100	18	35.3	21	100	6	100	1	16.7	0	0	1	1.3

[1] Multiple response

n = number of respondents; U = Urban; R = Rural; DNK = Do Not Know; OTC = Oxytetracycline; Parvexon ® = pavaquone; Butalex ® = buparvoquine; Berenil ® = diminazine aceturate; Clexon ® = parvaquone; Crystapen ® = crystalline penicillin; Streptopen ® = streptomycin-penicillin; Novidium ® = homodium chloride; Penstrep ® = penicillin - streptomycin.

4.1.14 Drugs used by Livestock Field Officers (LFO)/Agricultural Field Officers (AFOs) for treatment of animals in the field

LFOs/AFOs were the closest, and most readily available livestock technical professionals to the livestock keepers in the field. The LFOs/AFOs mentioned the various drugs and drug combinations that they used for therapy against diseases when called upon by farmers; the list of drugs is as presented in Tables 20 and 21.

The top 10 most used drugs (with % respondents in brackets) were: OTC-20% (68%), OTC-10% (63%), vaccines (38%), Berenil ® (diminazine aceturate) 35%, Ivomec ® (Ivermectin) 33%, Milsan ® (Levamisole hydrochloride), 2.5%, Samorin ® isometamidium chloride; (23%), Streptopen ® streptomycin-penicillin 20%, Butalex ® buparvoquine 18%, intra-mammary preparations (18%). Other most important drugs used in the field were OTC Spray (15%), Tylosin (13%), and Terit ® Halofuginone (13%). Overall OTC was the most widely used drug by LFOs/AFOs in the field, for treatment and control of livestock diseases.

Table 20: Single drugs used for treatment by Livestock Field Officers (LFO/ Agricultural Field Officers (AFOs) in the field

Variable	Region				District									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
	(n=46)	%	(n=65)	%	(n=36)	%	(n=10)	%	(n=10)	%	(n=10)	%	(n=111)	%
Single drugs used [1]														
OTC - 10%	14	70	11	55	10	63	4	100	5	41.7	6	75	25	63
OTC - 20%	17	85	10	50	14	88	3	75	3	25	7	88	27	68
OTC - Spray	3	15	3	15	3	19	0	0	1	8.3	2	25	6	15
Streptopen®	7	35	1	5	4	25	3	75	0	0	1	13	8	20
Tylosin	4	20	1	5	3	19	1	25	0	0	1	13	5	13
Butalex®	3	15	4	20	3	19	0	0	3	25.3	1	13	7	18
Clexon®	1	50	1	5	1	6.3	0	0	1	8.3	0	0	2	5
Parvexon®	6	30	2	10	5	31	1	25	2	16.7	0	0	8	20
Berenil®	5	25	9	40	4	35	1	25	3	25	6	75	14	35
Novidium®	1	5	3	15	1	6.3	0	0	1	8.3	2	25	4	10
Samorin®	1	5	8	40	1	6.3	0	0	2	16.7	6	75	9	23
Diminasan®	1	5	1	5	1	6.3	0	0	1	8.3	0	0	2	5
Milzan®	2	40	6	30	3	19	1	25	2	16.7	4	50	10	25
Imizol	0	0	2	10	0	0	0	0	0	0	2	25	2	5
Albendazole	2	10	2	10	1	6.3	1	25	0	0	2	25	4	10
Terit®	5	25	0	0	4	25	1	25	0	0	0	0	5	13
IMammaries	5	25	2	10	3	19	2	50	2	16.7	0	0	7	18
Ivomec®	4	20	9	45	3	19	1	25	5	41.7	4	50	13	33
Vaccines	8	40	7	35	5	31	3	75	1	8.3	6	75	15	38
Ca-Borogluconate	1	5	0	0	1	6.3	0	0	0	0	0	0	1	2.5
Sulfonamide Injection	2	10	0	0	1	6.3	1	25	0	0	0	0	2	5
Tramazole®	0	0	0	0	0	0	0	0	1	8.3	0	0	1	2.5
OTC DNK	2	10	2	10	2	13	0	0	2	16.7	0	0	4	10
Dipping	3	15	0	0	3	19	0	0	0	0	0	0	3	7.5
Trodax®	1	50	0	0	0	0	1	25	0	0	0	0	1	2.5
Lasix	0	0	1	5	0	0	0	0	1	8.3	0	0	1	2.5
Milvem®	0	0	1	5	0	0	0	0	0	0	1	13	1	2.5

[1] multiple response

n = number of respondents; U = Urban; R = Rural; DNK = Do Not Know; OTC = Oxytetracycline; Streptopen® = streptomycin-penicillin; Butalex® = buparvoquine; Clexon® = parvaquone; Parvexon® = paivaquone; Berenil® = diminazine aceturate; Novidium® = homodium chloride; Samorin® = isometamidium chloride; Diminasan® = diminazine aceturate; Milzan® = Levamisole hydrochloride; Ivomec® = Ivermection; Trodax® = Nitroxynil injection; Milvem® = Levamisole hydrochloride.

4.1.15 Combination drugs used by Livestock Field Officers (LFO)/Agricultural Field Officers (AFOs) in the field

Table 21 shows a list of various drug combinations and their percentage of use by the LFOs/AFOs Their consideration in terms of their therapeutic values and drug interactions which might occur are important. About 11 drug combinations which involve OTC have been mentioned. Their intensity of use on the basis of percent responses (data in brackets) were: OTC 20% - parvexon® (44%), OTC 10%- parvexon® (33%), OTC-10% - sulfadimidine (33%), OTC-20% - Butalex® (22%), and the rest of the other combinations have the same level of use at 11% each.

Table 21: Combination drugs used for treatment by LFOs and AFOs

Variable	Region				District								Total	
	Dodoma (n=20)		Morogoro (n=20)		Dodoma (U) (n=16)		Dodoma (R) (n=4)		Morogoro (U) (n=12)		Morogoro (R) (n=8)		(n=40)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Combination drugs used for treatment [1]														
OTC 10% and Parvexon®	2	40	1	25	2	5	0	0	0	0	1	33	3	33
OTC 20% and Parvexon®	3	60	1	25	3	75	0	0	0	0	1	33	4	44
OTC 10% and Butalex®	0	0	1	25	0	0	0	0	0	0	1	33	1	11
OTC 20% and Butalex®	1	20	1	25	0	0	1	100	0	0	1	33	2	22
OTC 10% and Berennil®	1	20	0	0	1	25	0	0	0	0	0	0	1	11
OTC 20% and Berennil®	1	20	0	0	1	25	0	0	0	0	0	0	1	11
OTC 10% and Sulfadiminazine	2	40	1	25	1	25	1	100	0	0	1	33	3	33
OTC 10% and Butalex® and Lasix (Frusemide)	0	0	1	25	0	0	0	0	0	0	1	33	1	11
OTC DNK and Butalex® and Lasix	0	0	1	25	0	0	0	0	1	100	0	0	1	11
OTC 20% and Clexon®	0	0	1	25	0	0	0	0	0	0	1	33	1	11
OTC 20% and Butalex® and Diminasan®	1	20	0	0	1	25	0	0	0	0	0	0	1	11

[1] Multiple response

n = number of respondents; U = Urban; R = Rural; DNK = Do Not Know; OTC = Oxytetracycline; Butalex® = buparvoquine; Clexon® = parvaquone; Parvexon® = paivaquone; Berenil® = diminazine aceturate; Diminasan® = diminazine aceturate.

4.1.16 Experience with OTC therapy by Livestock Field Officers (LFOs)/Agricultural Field Officers (AFOs) in the field

Table 22 shows the LFOs/AFOs experience with the use of OTC in the field under 5 parameters namely OTC intensity of use, its use for all diseases, whether other drugs are used, reasons for the use of the other drugs, and whether there has been any resistance development.

4.1.16.1 OTC use intensity in the field

With respect to LFOs'/AFOs' opinion on their experience on the use of OTC as a drug of choice in the field, overall about 85% of all the respondents suggested OTC as the most widely or most intensely used drug for treatment in the field. A regional comparison revealed a significant difference ($P<0.05$) between the two regions and a slight significant difference ($P<0.05$) between the districts. This was due to the AFO especially in Morogoro Urban who were not only not aware of OTC's intensity of use but do not know the use of OTC in veterinary medicine as well.

4.1.16.2 Use of OTC only to treat all livestock diseases

Analysis of the responses of LFOs/AFOs on use of OTC to treat all diseases indicated that overall 98% agreed that it could not treat all diseases. Further they indicated that some diseases required other drugs; only 2.5% said OTC could treat all diseases. A comparison of the responses at the regional and district level showed that there were no significant differences ($P>0.05$) at the two levels.

4.1.16.3 The need for use of drugs other than OTC

Table 22 shows that overall 75% of respondents agreed that under certain circumstances drugs other than OTC were used to treat livestock diseases. 10% of respondents said there was no need for the use of other drugs, while 15% simply didn't know whether there was need for other drugs or not. A comparison at the regional level showed a significant difference ($P<0.05$) in the responses between the two regions and this was caused by the AFO's who constituted most of the 30% of those who didn't know in Morogoro region. A comparison at the district level showed no significant difference ($P>0.05$) of the district

responses. On need to use drugs other than OTC for disease treatment at other times, overall 53% of LFOs/AFOs mentioned that the choice of drug depended on the type of disease, while 48% said they didn't know. A comparison at the regional level showed no significant differences ($P>0.05$) while at the district level there was a high significant differences ($P<0.05$) due to the high number of AFOs in Morogoro Urban district where 92% of the respondents said they did not know why there could be a need for the use of other drugs.

4.1.16.4 LFOs' opinion on the development of resistance against OTC

On development of resistance to OTC therapy, overall 65% of LFOs/AFOs said they had not experienced resistance so far and OTC was still working well; only 13% said there could be some resistance though they could not substantiate it. About 23% didn't know because they were not using it any way. A comparison on the regional and district levels showed no significant differences ($P>0.05$) at both levels of comparison. The use of the various drugs mentioned by the livestock farmers and the pharmaceutical dealers who supplied the drugs (Table 23) again reiterated the responses by LFOs/AFOs.

Table 22: LFOs/AFOs experience with oxytetracycline (OTC) in therapy of livestock diseases in the field

Variable	Region				District									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
	(n=20)		(n=20)		(n=16)		(n=4)		(n=12)		(n=8)		(n=40)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
OTC use intensity														
Most highly used	20	100	14	70	16	100	4	100	7	58.3	7	88	34	85
DNK	0	0	6	30	0	0	0	0	5	41.7	1	13	6	15
	X^2 =7.06**, df = 1						X^2 =10.26*, df = 3							
Use of single/same drug for all disease treatment														
Yes	1	5	0	0	1	6.3	0	0	0	0	0	0	1	2.5
No	19	95	20	100	15	94	4	100	12	100	8	100	39	98
	X^2 = 1.03^{ns}, df =1						X^2 = 1.54^{ns}, df =3							
Other drug used														
Yes	16	80	14	70	12	75	4	100	7	58.3	7	88	30	75
N0	4	20	0	0	4	25	0	0	0	0	0	0	4	10
DNK	0	0	6	30	0	0	0	0	5	41.7	1	13	6	15
	X^2 =10.13**, df = 2						X2 =4.66ns, df = 26							
Why use other drugs														
Varieity or different disease RX	13	65	8	40	9	56	4	100	1	8.3	7	88	21	53
DNK	7	35	12	60	7	44	0	0	11	91.7	1	13	19	48
	X^2 =2.51^{ns}, df =1						X^2 =17.03***, df = 3							
Resistance dev.														
Yes	3	15	2	10	2	13	1	25	1	8.3	1	13	5	13
No	14	70	12	60	12	75	2	50	6	50	6	75	26	65
DNK	3	15	6	30	2	13	1	25	5	41.7	1	13	9	23
	X^2 =1.35^{ns}, df =2						X^2 =4.63^{ns}, df =6							

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 = Chi square; NS = Non significance (X^2>0.5); * = Significance (0.01<X^2<0.05); ** = Significance (0.001<X^2<0.01); *** = Significance (X^2<0.001) DNK = Do Not Know; Rx= Treatment.

4.1.17 Drugs purchased by livestock keepers at veterinary pharmacies

Table 23 shows a list of veterinary drugs purchased mainly by the livestock keepers from veterinary drug dealers in Dodoma and Morogoro for management of livestock diseases. Overall the top 15 drugs mentioned by the drug dealers regularly purchased (% responses in brackets) were: OTC-20% (94.0%), Parvexon® (94%), OTC-10% (88%), Berennil® (69%) Samorin® (56%), Butalex® (56%), Penstrep®/Streptopen® (44%), Tylosin (44%), intra-mammary formulations (44%), Diminasan® (38%), Vaccines (31%), OTC Spray (31%), Novidum® (31%), Imizol (31%), and albendazole (25%). The three most important drugs on the basis of sales, according to the pharmaceutical dealers, are shown in Table 23.

Table 23: Drugs purchased by livestock keepers at pharmacies in Dodoma and Morogoro Municipalities

Variable	Dodoma (n=7)		Morogoro (n=9)		Total (n=16)	
	No	%	No	%	No	%
Name of drug [1]						
OTC -10%	7	100	7	77.8	14	87.5
OTC - 20%	7	100	8	88.9	15	93.8
OTC eye/wound powder	1	14.3	0	0	1	6.3
OTC spray	5	71.4	0	0	5	31.3
Penstrep/Streptopen®	5	71.4	2	22.2	7	43.8
Tylosin	5	71.4	4	22.2	7	43.8
Butalex®	3	42.9	6	66.7	9	56.3
Parvexon®	7	100	8	88.9	15	93.8
Berenil®	3	42.9	8	88.9	11	68.8
Novidium®	1	14.3	4	44.4	5	31.3
Samorin®	0	0	9	100	9	56.3
Diminasan®	1	14.3	5	55.6	6	37.5
Milzan®	3	42.9	1	11.1	4	25
Imizol®	0	0	5	55.6	5	31.3
Albendazole	3	42.9	1	11.1	4	25
Stelladone®	3	42.9	2	22.2	5	31.3
Tick-fix®	1	14.3	1	11.1	2	12.5
I/Mammaries	2	24.6	5	55.6	7	43.6
Ivomec®	1	14.3	1	11.1	2	12.5
Penicillin	1	14.3	0	0	1	6.3
Grenade®	1	14.3	2	22.2	3	18.8
Buparvoquine	1	14.3	0	0	1	6.3
Tacktick®	3	42.9	1	11.1	4	25
Bayticol®	2	24.6	0	0	2	12.5
Vaccine	1	14.3	4	44.4	5	31.3
Ca-borogluconate	1	14.3	0	0	1	6.3
Sulfonamide injectable	0	0	2	22.2	2	12.5
Kaoline	0	0	1	11.1	1	6.3
Veriben®	0	0	1	11.1	1	6.3
Tramazol®	1	14.3	0	0	1	6.3
Veridium®	0	0	1	11.1	1	6.3
Dominex®	1	14.3	1	11.1	2	12.5
Zymofax®	3	42.9	0	0	3	18.8

n = number of respondents; U = Urban; R = Rural; DNK = Do Not Know; OTC = Oxytetracycline Butalex® = buparvoquine; Clexon® = paivaquone; Parvexon® = parvaquone; Berenil® = diminazine aceturate; Novidium® = homodium chloride; Samorin® = isometamidium chloride; Diminasan® = diminazine aceturate; Milzan® = Levamisole hydrochloride; Ivomec® = Ivermection; Trodax® = Nitroxynil injection; Milvem® = Levamisole hydrochloride; Stelladone® = Chlofenviphos; Tick-fix® = Amitraz – 12.5%; Grenade® = Pyrethroid cyhalothrin; Tacktick® - Amitraz – 12.5%; Bayticol® = ; Veriben® = Dibenxamidine diaceturate; Tramazol® = Albendazole 10%; Veridium® = Isometamidium Chloride hydrochloride; Dominex® = Alphacypermethrin; Zymofax® = Levamisole oxychozanade.

4.1.18 The most important drugs in the field on basis of sales

Table 24 shows the percentage responses on the rate of use of various drug formulations. About 94% of the pharmaceutical dealers said OTC-20% was the most commonly used drug formulation. Overall the second most important drug formulation was OTC-10%, which scored overall 88%. In Dodoma it scored 100% while for Morogoro the score was 78%. Hence, though OTC 10% was overall second also in Dodoma, in Morogoro it was third at 78% after Samorin®, which was second at 89%. The third most important drug overall was Samorin® which scored 50% overall. While in Dodoma it scored 0%, in Morogoro it scored 89% as the second most important drug used in the field.

Table 24: The most important drugs used in the field on the basis of sales[1]

Variable	Dodoma (U) (n=7)		Morogoro(U) (n=9)		Total (n=16)	
	No	%	No	%	No	%
Name of drug						
OTC -10%	7	100	7	77.8	14	87.5
OTC - 20%	7	100	8	88.9	15	93.8
OTC spray	1	14.3	0	0	1	6.3
Penstrep®/streptopen®	4	57.1	0	0	4	25
Tylosin	3	42.9	0	0	3	18.8
Butalex®	0	0	1	11.1	1	6.3
Parvexon®	3	42.9	1	11.1	4	25
Berenil®	1	14.3	6	66.7	7	43.8
Novidium®	1	14.3	4	44.4	5	31.3
Samorin®	0	0	8	88.9	8	50
Diminasan®	1	14.3	4	44.4	5	31.3
Milsan®	2	28.6	0	0	2	12.5
Albendazole	2	28.6	0	0	2	12.5
Stelladone®	2	28.6	1	11.1	3	18.8
Tick-fix®	0	0	1	11.1	1	6.3
I/Mammaries	1	14.3	1	11.1	2	12.5
Ivomec®	0	0	1	11.1	1	6.3
Grenade®	0	0	1	11.1	1	6.3
Tacktick®	2	28.6	0	0	2	12.5
Veridium®	0	0	1	11.1	1	6.3
Dominex®	1	14.3	0	0	1	6.3
Zymofax®	1	14.3	0	0	1	6.3
Tramazol®	1	14.3	0	0	1	6.3

1 multiple response

n = number of respondents; U = Urban; OTC = OxytctracyclineButalex® = buparvoquine; Parvexon® = parvaquone; Berenil® = diminazine aceturate; Novidium® = homodium chloride; Samorin® = isometamidium chloride; Diminasan® = diminazine aceturate; Milsan® = Levamisole hydrochloride; Ivomec® = Ivermection; Milvem® = Levamisole hydrochloride; Stelladone® = Chlofenviphos; Tick-fix® = Amitraz – 12.5%; Grenade® = Pyrethroid cyhalothrin; Tacktick® - Amitraz – 12.5%; Tramazol® = Albendazole 10%; Veridium® = Isometamidium Chloride hydrochloride; Dominex® = Alphacypermethrin; Zymofax® = Levamisole oxychozanade.

4.1.19 Pharmaceutical dealers' opinion on use of drugs purchased by livestock keepers

Table 25 shows the pharmaceutical dealers opinion on various aspects involving livestock keepers and drugs purchased by them as per their experience.

4.1.19.1 Intensity of OTC use in the field on the basis of sales

On the intensity of use of OTC formulations in the field, overall 81% agreed that it was the most intensely used drug in the field, 13% said it was moderately used, while only 6% said it was only lightly used. A comparison of the two locations, i.e. Dodoma and Morogoro, showed no significant difference (P>0.05).

4.1.19.2 Knowledgeability of livestock keepers on drug use

Overall in Dodoma and Morogoro, all (100%) of the pharmaceutical dealers agreed that the knowledge of farmers on the handling and administration of drugs was inadequate.

4.1.19.3 Who administers drugs purchased by livestock keepers

Overall 69% of the pharmaceutical dealers agreed that the drugs were administered by the livestock keepers themselves, whereas the remaining 31% were of the opinion that either the livestock keepers or the LFOs/AFOs administered the drugs. A comparison of the two locations shows a slight significance of differences ($P < 0.05$) and this can be clearly discerned from the proportions. While in Dodoma only 43% of drugs are administered by livestock keepers, in Morogoro a hefty 89% thought livestock keepers administered the drugs.

4.1.19.4 Knowledge of farmers of the dangers of drug misuse to public health

The pharmaceutical dealers said that the farmers knowledge on dangers which could ensue as a result of drug misuse was inadequate by 100%. From the responses of the above three groups of OTC stakeholders it was obvious that OTC in its various formulations was the most widely used drug in the treatment of livestock diseases in Dodoma and Morogoro regions.

Table 25: Pharmaceutical dealers opinion on use of drugs purchased by livestock keepers

Variable	Dodoma (U) (n=7)		Morogoro (U) (n=9)		Total (n=16)	
	No	%	No	%	No	%
Intensity of OTC use in the field on basis of sales						
Very high to most used vet drug	5	71.4	8	88.9	13	81.3
Moderately used	2	28.6	0	0	2	12.5
Low	0	0	1	11.1	1	6.3
	$X^2 = 3.5^{ns}$, df = 2					
Knowledgeability of livestock keepers on drug use						
Inadequate	7	100	9	100	16	100
	No statistic No X^2, No df					
Administration of drugs purchased by livestock keepers						
By livestock keepers themselves.	3	42.9	8	88.9	11	68.8
by both livestock keepers and paravets (LFO's) etc	4	57.1	1	11.1	5	31.3
	$X^2 = 3.88^*$, df =1					
Knowledgeablility of farmers on the dangers of drug misuse to public health						
Adequate	0	0	0	0	0	0
Inadequate	7	100	9	100	16	100
	X2 = -, df = - since there is no statistical difference					

n = number of respondents; U = Urban; df = degree of freedom; X^2 = Chi square; ns = non significance (X^2>0.5); * = Significance (0.01<X^2<0.05); OTC = Oxytetracyline.

4.1.20 Dose regimen and handling of OTC by livestock farmers

Criteria for OTC dose determination, awareness of the drug expiry date, awareness of OTC withdrawal period, OTC duration of treatment, the dosage rate, and stocking of OTC, route of administration, and use of drug combinations both of which constitute the mode of application, are presented in Table 26.

4.1.20.1 Dose determination criteria

The basis of estimation of doses for drugs used by the livestock keepers who treat their sick animals on their own is shown in Table 26. Overall about 76% of the farmer respondents indicated that their dose estimation was based on the body weight or size of the animal. About 4.8% said they based their dose estimation on the age of the animal which may be difficult to come by while 15% said it was the veterinarian or paravet who did the estimation when they consulted them for treatment. About 65% did not know or did not have any basis of dose estimation but merely administered an amount of drug they pleased. Another 2% had no basis also and hence chose either to treat or not to treat their animals. If they did treat, then they used any amount of drug they pleased.

4.1.20.2 Observance of WP after treatments

Table 26 shows the drug withdrawal period (WP) duration basis used by livestock farmers. Overall about 1% observed one day withdrawal period, 22% waited for the animal to gain weight, 57% waited for financial needs to arise, 2.7% observed 2-7 days WP, 1% observed 28 days WP and 20% did not have any particular waiting period. On a regional comparative basis there was not difference between the two regions; because while 26% of Dodoma farmers did not have any particular waiting period, 15% of Morogoro region

Livestock farmers did the same. Again while 59% of Dodoma farmers waited for domestic financial needs to arise to sell their animals, 55% of Morogoro region farmers did the same. Further more while 15% of Dodoma region farmers waited for the recovered animals to gain weight, 26% of Morogoro farmers did the same.

On the district level while farmers in Dodoma Urban district sold their recovered animal only when animal has gained weight (19%) and a domestic financial need arose (47%), Dodoma Rural farmers sold slaughter animals only when a financial need has arose (100%). Farmers in Morogoro Rural district sold animals when they had gained weight (31%) and there was a domestic financial stress (69%). On the other hand while 6% Morogoro Urban farmers observed a one day WP, 13% waited for the animal to gain weight, 13% waited for financial stress, 19% observed a 2 –7 days WP, 6% observed a 28 day WP and 56% did not have any particular basis for WP.

Table 26: Drug use regime by livestock farmers [1]

Variable	Region				District									
	Dodoma (n=22)		Morogoro (n=61)		Dodoma (U) (n=17)		Dodoma (R) (n=5)		Morogoro (U) (n=14)		Morogoro (R) (n=47)		Total (n=83)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Dose determination criteria														
Bwt	14	64	49	80..3	10	59	4	80	2	14.3	47	100	63	76
Age	3	14	1	1.6	1	5.9	2	40	0	0	1	2.1	4	4.8
Vet/paravet determines	1	4	5	11	18	1	5.9	0	0	11	0	0	12	15
DNK	5	23	0	0	5	29	0	0	0	0	0	0	0	6
none	0	0	2	3.3	0	0	0	0	2	14.2	14.2	0	2	2.4
Observance of WP after treatment Rx and recovery														
One day WP observance	0	0	1	1.5	0	0	0	0	1	6.3	0	0	1	0.9
Wait to gain weight	7	15	17	26.2	7	19	0	0	2	12.5	15	31	24	22
Wait until financial need arises	27	59	36	55.4	17	47	10	100	2	12.5	34	69	63	57
2-7days WP observance	0	0	3	4.6	0	0	0	0	3	18.8	0	0	3	2.7
> 28 days WP observance	0	0	1	1.5	0	0	0	0	1	6.3	0	0	1	0.9
DNK	12	26	10	15.4	12	33	0	0	9	56.3	1	2	22	20

1 multiple response

n = number of respondents; U = Urban; R = Rural; DNK = Do Not Know; OTC = Oxytetracycline; WP = withdrawal period; Rx = Treatement; Bwt = Body weight; Vet. = Veterinary.

4.1.21 Storage and handling of drugs by livestock farmers

4.1.21.1 Drugs OTC inclusive stocking by farmers

Table 27 shows that 68% of farmers did stock drugs. On the regional, level 44 % of Dodoma farmers did stock drugs while in Morogoro about twice as many farmers i.e. 85% did stock giving rise to a high significant difference ($P< 0.05$). Also while only 40% of Dodoma Urban and 60% of Dodoma Rural district farmers stock drugs, all 100% of all Morogoro Rural farmers stock drugs in Morogoro Urban districts only 38% its farmers stock drugs for use to their animal.

4.1.21.2 Criteria for time limit of drugs use

4.1.21.2.1 Knowledge of disadvantages of expired drugs by farmers

Table 27 shows that overall 69% of farmers knew that they were not supposed to purchase or use expired drugs. On the regional level the significance of differences was high ($P< 0.05$); while only 37% of Dodoma farmers were aware of the prohibition of use of expired drugs, in Morogoro 91% knew well about the prohibition of use of expired drugs. At the district level the differences were highly significant ($P< 0.05$).

4.1.21.2.2 Knowledge of the particular disadvantages of expired drugs

Table 27 shows the level of awareness of livestock farmers in Dodoma and Morogoro regions of the specific dangers of using expired drugs. Overall about 48% were aware of the possible loss of potency, 54% mentioned toxicity due to degradation, 2.7% mentioned loss of animal condition, 1% mentioned reduced milk production, while 9% were concerned of the possible occurrence of non specific economic losses. Further more 33% were not aware of any specific dangers from using expired veterinary drugs for animal treatement.

Table 27: Storage and handling of drugs by farmer

	Region				District									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
Variable	(n=46)	%	(n=65)	%	(n=36)	%	(n=10)	%	(n=16)	%	(n=49)	%	(n=111)	%
Stocking (stocking of drugs)														
Yes	20	43.5	55	84.6	14	38.9	6	60	6	37.5	49	100	75	68
No	26	56.5	10	15.4	22	61.1	4	40	10	62.5	0	0	36	32
	$X^2 = 20.80^{***}$, df = 1						$X^2 = 4389^{***}$, df = 3							
Use of non-expired drugs e.g.OTC														
Yes	17	37	59	90.8	14	38.9	3	30	14	87.5	45	91.8	76	69
No	29	63	6	9.2	22	61.1	7	70	2	12.5	4	8.2	35	32
	$X^2 = 36.13^{***}$, df = 1						$X^2 = 36.52^{***}$, df = 3							
Knowledge of disadvantages of expered drug use [1]														
Loss of potency	14	30.4	39	60	12	33.3	2	20	12	75	27	55.1	53	48
Toxicity due to degradation	13	28.3	47	72.3	9	25	4	40	12	75	35	71.4	60	54
Animal loss of condition	0	0	3	4.6	0	0	0	0	0	0	3	6.1	3	2.7
Reduced milk production	0	0	1	1.5	0	0	0	0	0	0	1	2	1	0.9
Economic loss	4	8.7	6	9.2	4	11.1	0	0	5	31.3	1	2	10	9
DNK	28	60.9	9	13.8	22	61.1	6	60	2	12.5	7	14.3	37	33

[1] Multiple response

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 = Chi square; ns = non significance (X^2>0.5); *** = Significance (X^2<0.001) DNK = Do Not Know; OTC = Oxytetracyline.

4.1.22 OTC regime used by LFOS/AFOS

Table 28 shows the OTC regime used by LFOs/AFOs under the five parameters of; the basis of dose determination, duration of treatment, duration of WP, whether or not they did stock drugs e.g. OTC used for treatment, and time period of use beyond the expiry date.

4.1.22.1 Dose determination basis by LFOs/AFOs

Table 28 shows the basis of dose determination by multiple responses of the LFOs/AFOs interviewed. Overall 97% said they estimated the dose on the basis of the body weight or body size of the animal. However about 9% indicated they estimated the dose on the basis of age of the animal.

4.1.22.2 Duration of treatment

Table 28 shows the various alternatives used by the respondents on the duration of treatment period. Overall 28% said they carried out the treatment for 6-7 days i.e. a week before calling it off or changing the drug, 18% treated for 4-5 days, 8% treated for 2-5 days, 15% treated for 11 – 21 days, 13% treated for 8-10days and 20% did not know for how long to continue treating the animal. On this parameter there were no significant differences ($p > 0.05$) on the responses between the two regions. However between the districts there was a significant difference between the districts ($p < 0.05$).

4.1.22.3 Duration of withdrawal period (WP)

Table 28 shows the summary of the OTC withdrawal times as practiced by the LFO/AFOs. While overall, a highly significant proportion (63% respondents) said they

didn't know for how long they should wait for OTC to get cleared from the animals body, 13% mentioned a WP of 4 –7 days, 13% mentioned 8-14 days, while 5% mentioned 15 –21 days for OTC WP. Furthermore 2.5% mentioned a 22 – 28 days WP, 2.5% mentioned 2 –3 days, and 2.5% mentioned correctly that they would look for the WP on the drugs data sheet as directed by the manufacturer.

A comparison of the regions and districts on the responses showed there was a slight significant difference of the responses both at the regional and district levels. While overall, about 63% of all repondents did not know the WP for OTC, in Dodoma region about of 40% LFOs/AFOs did not know what to advise the farther on OTC WP, in Morogoro region about 85% did not know either. At the district level the proportions of the respondents were; 44% Dodoma urban, 25% Dodoma rural, 83.3% Morogoro urban, and 88% of Morogoro rural district LFOs/AFOs respectively.

4.1.22.4 Stocking of Drugs for treatment

Table 28 shows the extent of drug stocking by the LFO's AFO's for field treatment purposes. Overall 68% of respondents said they stocked drugs for treatment while 32% did not. A regional and district comparison revealed a slight significance of difference ($P < 0.05$) on this parameter at both levels which was due to the significantly higher number of retrained AFO's in Morogoro region. All of them belonged to the 32% Overall proportion of those respondents who said they did not stock drugs.

4.1.22.5 Time beyond the OTC expiry date

Table 28 shows the time beyond the OTC expiry date mentioned by the respondents during which they continued to use the expired drug. While 18% said they didn't know, 38% said they disposed the drug as soon as it expired i.e. zero days tolerance. About 20% said they tolerated its use for 4-6 more months beyond the expiry date, 15% mentioned 3 months beyond, 7.5% mentioned 1 month beyond and 2.5% mentioned 2 months beyond. A comparison between the two regions showed no significant differences ($P>0.05$). However a comparison at the district level showed that there was a slight significance of differences ($P<0.05$) which probably was due to the AFO's from Morogoro Urban district where 50% of the respondents said they didn't know for how much longer they could use the expired OTC.

Table 28: OTC regime practised by LFOs/AFOs

Variable	Region				District									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
	(n=20)		(n=20)		(n=16)		(n=4)		(n=12)		(n=8)		(n=40)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Dose determination basis [1]														
Body weight	19	100	13	92.9	15	100	4	100	6	85.7	7	100	32	97
Age	1	53	2	14.3	0	0	0	0	1	14.3	1	14	3	9.1
Duration of treatment														
2-5 days	2	10	1	5	1	6.3	1	25	0	0	1	13	3	7.5
4-5 days	4	20	3	15	4	25	0	0	2	16.7	1	13	7	18
6-7 days	6	30	5	25	5	31	1	25	3	25	2	25	11	28
8-10 days	5	25	0	0	4	25	1	25	0	0	0	0	5	13
11-21 days	1	5	5	25	1	6.3	0	0	1	8.3	4	50	6	15
DNK	2	10	6	30	1	6.3	1	25	6	50	0	0	8	20
	$X^2 = 10.23^{ns}$, df = 5						X2 = 26.17*, df = 5							
Duration of WP														
See W.P. on data sheet	0	0	1	5	0	0	0	0	0	0	1	13	1	2.5
2-3 days	1	50	0	0	1	6.3	0	0	0	0	0	0	1	2.5
4-7 days	3	15	2	10	2	13	1	25	2	16.7	0	0	5	13
8-14 days	5	25	0	0	4	25	1	25	0	0	0	0	5	13
15-21 days	2	10	0	0	1	6.3	1	25	0	0	0	0	2	5
22-28 days	1	5	0	0	1	6.3	0	0	0	0	0	0	1	2.5
DNK	8	40	17	85	7	44	1	25	10	83.3	7	88	25	63
	$X^2 = 13.44^*$,df = 6						$X^2 = 21.35^*$,df=18							
Stocking of drug e.g OTC														
Yes	17	85	10	50	9	100	8	73	4	33.3	6	75	27	68
No	3	15	10	50	0	0	3	27	8	66.7	2	25	13	33
	$X^2 = 5.58^*$,df = 1						$X^2 = 11.06$*,df=3							
Time beyond expiry data														
Direct disposition i.e. 0 days	8	40	7	35	5	31	3	75	4	33.3	3	38	15	38
1 month	2	10	1	5	2	13	0	0	0	0	1	13	3	7.5
2 months	1	5	0	0	1	6.3	0	0	0	0	0	0	1	2.5
3 months	2	10	4	20	1	6.3	1	25	0	0	4	51	6	15
4-6 months	6	30	2	10	6	38	0	0	2	16.7	0	0	8	20
DNK	1	5	6	30	1	6.3	0	0	6	50	0	0	7	18
	$X^2 = 7.64^{ns}$,df = 5						X2 = 30.6*,df = 15							

[1] Multiple response

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 = Chi square; ns = non significance (X^2>0.5); * = Significance (0.01<X^2<0.05); DNK = Do Not Know; WP = Withdrawal Period; OTC = Oxytetracyline.

4.1.23 Dosage rates used by livestock keepers

The OTC dosage rates used by the two main user groups of users i.e. the livestock farmers and LFOs/AFOs are as follows.

4.1.23.1 Dosage rates used by livestock keepers in the treatment of calves

Table 29 shows the dosage rate used by livestock keepers in calves for three types of formulations i.e. OTC 10%, 20%, and OTC-DNK. Considering the use of the 10% formulation in calves, overall 82.9% of farmers did not know which dose to use for calves while 10% used between 1-5ml, 2% used 6-10 ml, 3% used 11-20ml, and another 3% used 21-30ml of OTC per day (i.e. every 24hours). Between the regions there was not any significant difference ($P>0.05$) on the 10% dosage rate in calves. However a comparison of the districts exemplified a slight significance of difference ($P<0.05$) because of the use by specific dosage of 30% of Morogoro Rural farmers versus the other districts, which in some cases did not use OTC in calves at all. With respect to the 20% formulation, while overall about 67% of farmers were not sure of which dose to use, the most popular dosage rate in calves was 11-20ml (12%),1-2mls (6.3%) followed by 21-30ml and 6-10ml each with a 5.4% propotion.

Comparing the regions there was a slight significance of differences ($P<0.05$). This was because while Dodoma farmers favoured the dose bracket of 1-5ml (3.5%), the Morogoro farmers favoured the higher doses of 6-10ml (9.2%), 11-20ml (20%) and 21-30ml (9.2%). While 85% of Dodoma farmers estimated the dose by guessing, 54% of Morogoro farmers did so. A comparison of the districts showed a high significance of difference ($P<0.05$) on the calf dosage levels of OTC 20% (LA). While about 13% of Dodoma Urban farmers

administered 1-5ml, 20% of Dodoma Rural farmers used 1-2ml. All 100% of Morogoro Urban farmers did not use any dose at all i.e. the 6-30ml range was not used at all. A look at Morogoro Rural districts showed that only 2% used 1-2ml, 8% used 3-5ml, 12% used 6-10mls, 27% used 11-20ml, 12% used 21-30ml while 39% did not have a specific dose to use. Overall, about 39% of the Morogoro Rural farmers used the higher bracket dosage rate of 11-30ml of OTC per day (i.e every 24hours). The percentage of farmers who did not have a specific dosage were 86%, 80%, 100%, and 39% for Dodoma Urban, Dodoma Rural, Morogoro Urban and Morogoro Rural districts respectively.

Some farmers did not differentiate between the 10% and 20% formulation and hence were designated as using OTC-DNK. Their representation and relationship with those specifically using the 10% and 20% formulations is shown in Figure 9. Overall 82% of farmers did not know which dose of OTC to use. About 6.3% of farmers used the 6-10ml doses, 4.5% used the 11-20ml dose and 3.6% used the 3-5ml dose. Comparing the regions there was a moderate significance of difference ($P<0.05$) in dosage rates whereby Dodoma farmers used the lower dosage range of 1-5mls (11%) and the Morogoro farmers used 6-20ml (18%) and 21-30ml (1.5%) dosage range. About 89% of Dodoma farmers and 77% of Morogoro farmers dose their animals by guessing. A comparison of the districts revealed no significant difference ($P>0.05$) in this formulation.

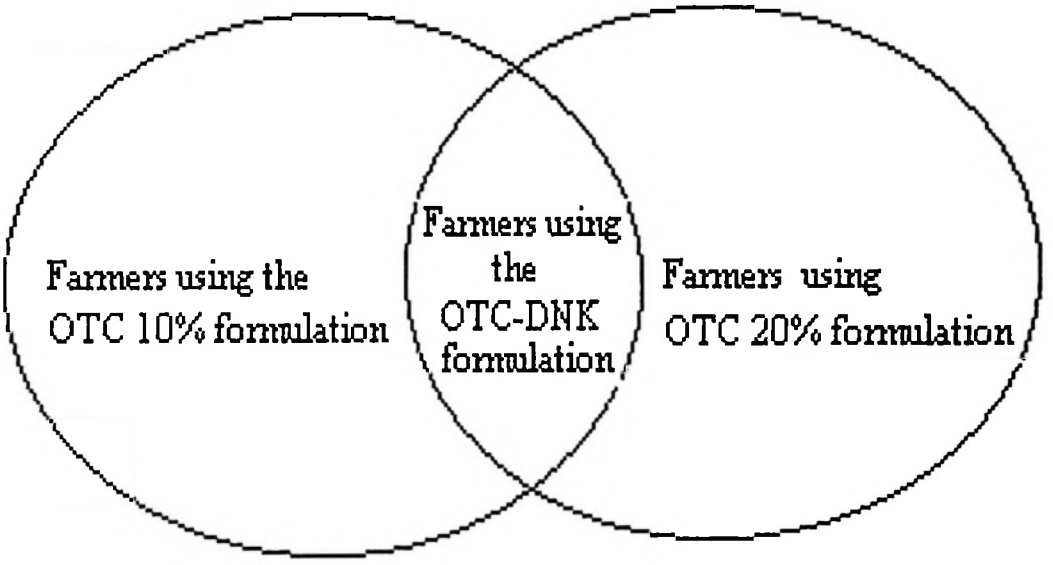


Figure 9: The relationship between farmers using OTC 10%, OTC 20% and OTC -"DNK" formulations.

Table 29: Oxytetracycline dosage rates used by livestock keepers in calves per day

Variable	Region				District									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
	(n=46)		(n=65)		(n=36)		(n=10)		(n=16)		(n=49)		(n=111)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
OTC 10% calf dose (ml/animal)														
1-2ml	3	6.5	1	1.5	1	2.8	2	20	0	0	1	2	4	3.6
3-5ml	1	2.2	6	9.2	0	0	1	10	0	0	6	12.2	7	6.3
6-10ml	0	0	2	3.1	0	0	0	0	0	0	2	4.1	2	1.8
11-20ml	0	0	3	4.6	0	0	0	0	0	0	3	6.1	3	2.7
21-30ml	0	0	3	4.6	0	0	0	0	0	0	3	6.1	3	2.7
dnk	42	91.3	50	76.9	35	97.2	7	70	16	100	34	69.4	92	82.9
	$X^2 = 10.32^{ns}$, df = 5						$X^2 = 27.53^{*}$, df =15							
OTC 20% calf dose (ml/animal)														
1-2ml	6	13	1	1.5	4	11.1	2	20	0	0	1	2	7	6.3
3-5ml	1	2.2	4	6.2	1	2.8	0	0	0	0	4	8.2	5	4.5
6-10ml	0	0	6	9.2	0	0	0	0	0	0	6	12.2	6	5.4
11-20ml	0	0	13	20	0	0	0	0	0	0	13	26.5	13	11.7
21-30ml	0	0	6	9.2	0	0	0	0	0	0	6	12.2	6	5.4
dnk	39	84.8	35	53.8	31	86.1	8	80	16	100	19	38.8	74	66.7
	$X^2 = 28.16^{*}$, df=5						$X^2 = 51.91^{***}$, df = 15							
OTC DNK calf dose (ml/animal)														
1-2ml	3	6.5	0	0	2	5.6	1	100	0	0	0	0	3	2.7
3-5ml	2	4.3	2	3.1	2	5.6	0	0	0	0	2	4.1	4	3.6
6-10ml	0	0	7	10.8	0	0	0	0	1	6.3	6	12.2	7	6.3
11-20ml	0	0	5	7.7	0	0	0	0	0	0	5	10.2	5	4.5
21-30ml	0	0	1	1.5	0	0	0	0	0	0	1	2	1	0.9
dnk	41	89.1	50	76.9	32	88.9	9	90	15	93.8	35	71.4	91	82
	$X^2 = 14.05^{**}$, df=5						$X^2 = 20.62^{ns}$, df = 15							

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 = Chi square; ns = non significance (X^2>0.5); * = Significance (0.01<X^2<0.05); ** = Significance (0.001<X^2<0.01); *** = Significance (X^2<0.001) ; OTC-DNK = Non specified OTC formulation; dose –dnk = non specified dosage range; OTC = Oxytetracyline.

4.1.24 Dosage rates used by livestock keepers in the treatment of adult cows

Table 30 shows the dosage rates used by livestock keepers in adult cows for the three types of OTC formulations. Considering the 10% OTC formulation, overall, 83% of the farmers did not know which type of dose to use, 6% used 5-10 ml, 4% used 11-20ml,

2.7% used 21-49ml and 4% used 50-120ml. Comparing the two regions, there was no significant difference ($p>0.05$) with respect to this formulation. At the district level there was slight significant difference ($P<0.05$). Considering the dosage rate of the 20% formulation, overall 65% of all the respondents in the two regions did not know which dose to use i.e. they estimated dose rates by guessing. Again overall 5% of farmers used a dosage of 5-10ml, 8.1% used a dose of 11-20ml, 9% used a dose of 21-49ml, and 11.7% used a dose range of 50-120ml per day (i.e. at 24 hour intervals).

A comparison between the two regions indicates that there was high significance of difference ($P< 0.05$) in the dosage rates of this OTC formulation. Dodoma region farmers used the low dosage rates of 5-10ml (6.5%) and 11-20ml (8.3%) only and none of the higher dose ranges. Morogoro farmers used all dosage ranges but with a higher preference for the higher dose range i.e. while only 10% of Morogoro region farmers used 5-20ml, 15% used 21-49ml, and 20% used 50-120mls at 24hour interval. In Dodoma region 85% of farmers did not have a specific dosing rate for this formulation, while in Morogoro region they constituted 52%.

A comparison of the districts on the dosage rates of this formulation showed a significant difference ($P< 0.05$). While the percent of farmers without a specific dosage regime was 86% in Dodoma Urban, 80% in Dodoma Rural and 93% in Morogoro Urban districts, only 39% of farmers in Morogoro Rural district did the same. Furthermore, farmers of those three districts used the lower dosage range where 14% Dodoma Urban, 20% of Dodoma Rural and only 6% of Morogoro Urban district used the 5-20ml dose range. This contrasted with the Morogoro Rural district farmers where, while only 14% of the farmers

used the 5-20ml dose range, 20% used the 21-49ml dosage and 27% used the 50-120ml per day (i.e 24 hour intervals) dosage range.

Considering the adult cow dosage rate for the OTC-DNK formulation, overall it was found that 80% of farmers did not have specific dosage rates. About 14% of farmers used 5-20ml, while 45% used 21- 49ml and about 1% used 50-120ml. A comparison of the two regions showed no significant difference ($P > 0.05$) and even at the district level there were no significant difference ($P > 0.05$).

Table 30: Oxytetracycline dosage rates used by Livestock keepers in adult cows per day

Variable	Region				District									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
	(n=46)		(n=65)		(n=36)		(n=10)		(n=16)		(n=37)		(n=111)	
	n	%		%		%		%		%		%		%
OTC 10% adult cow dose (ml/animal)														
5-10ml	2	4.3	5	7.7	1	2.8	1	10	0	0	5	10.2	7	6.3
11-20ml	2	4.3	2	1.3	0	0	2	20	0	0	2	4.1	4	3.6
21-49ml	0	0	3	4.6	0	0	0	0	0	0	3	6.1	3	2.7
50-120ml	0	0	4	6.2	0	0	0	0	0	0	4	8.2	4	3.6
dnk	42	91.3	51	78.5	35	97.2	7	70	16	100	35	1.4	93	83.4
	X^2 = 6.08ns, df = 4						X^2 = 23.74*, df =12							
OTC 20% adult cow dose (ml/animal)														
5-10ml	3	6.5	3	2.6	3	8.3	0	0	0	0	3	6.1	6	5.4
11-20ml	4	8.3	5	7.7	2	5.6	2	20	1	6.3	4	8.2	9	8.1
21-49ml	0	0	10	15.4	0	0	0	0	0	0	10	20.4	10	9
50-120ml	0	0	13	20	0	0	0	0	0	0	13	26.5	13	11.7
dnk	39	84.8	34	52.3	31	86.1	8	80	15	93	19	38.8	73	65.8
	X^2 = 20.81***, df = 4						X^2 = 43.13 ***, df = 12							
OTC -DNK adult cow dose (ml/animal)														
5-10ml	4	8.7	8	12.3	3	8.3	1	10	0	0	8	16.3	12	10.8
11-20ml	1	2.2	3	4.6	1	2.8	0	0	0	0	3	6.1	4	3.6
21-49ml	0	0	5	7.7	0	0	0	0	1	6.3	4	8.2	5	4.5
50-120ml	0	0	1	1.5	0	0	0	0	0	0	1	2	1	0.9
dnk	41	89.1	48	73.8	32	88.9	9	90	15	93.8	33	67.3	89	80.2
	X^2= 5.80ns, df =4						X^2 = 11.92^{ns}, df = 12							

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 =Chi square; ns = non significance (X^2>0.5); * = Significance (0.01<X^2<0.05); *** = Significance (X^2<0.001) ; OTC-DNK = Non specified OTC formulation; dose -dnk = non specified dosage range; OTC = Oxytetracyline.

4.1.25 Dosage rates used by livestock keepers in the treatment of bulls

Table 31 shows the dosage rates used by livestock farmers in the treatment of bulls for the 3 types of OTC formulations. Considering the 10% OTC formulation, overall 86% of farmers did not have a specific dosage rate. About 3% of farmers used the 5-10ml dose range, 5% used 11-20ml, 4% used 21-49ml and 4% used 50-120ml for the bulls.

A comparison of the regions shows no significant differences i.e. ($P > 0.05$). In Dodoma region 9% of farmers used the 5-20ml-dosage range while 91% did not even have a dose rate. In Morogoro region only 17% of the farmers used the drug while 82% did not have a dosage regime. A comparison of the districts showed that there was significant difference ($P < 0.05$). Considering the dosage rates for the 20% (LA) formulation, overall 67.6% of livestock farmers in both regions did not know which dose to use, 2.7% used 5-10ml, 7.2% used 11-20ml, 10.8% used 21-49ml and 11.7% used 50-120ml per day.

A regional comparison showed that there were significant differences ($P < 0.05$) between the two regions where 83% of Dodoma farmers and 57% of Morogoro farmers did not have a specific dosage regime. While approximately 17% of Dodoma farmers used the 5-20 ml range, and none of the higher dose ranges, only 14% of Morogoro region farmers used the 5-20ml dosage range, 19% used 21-49ml, and another 20% used the 50-120ml dose range per day. At the district level there was a high significant difference ($P < 0.05$) whereby, while none of the farmers in Dodoma Urban, Dodoma Rural and Morogoro Urban districts used the 21-49ml, and 50-120ml dose ranges, 25% and 27% of Morogoro Rural district farmers used the two dose ranges respectively. Considering the dosage rate of OTC-DNK in the treatment of bulls, overall 81% of farmers did not have a specific

dose. About 8% used the 5-20ml range, 7% used 21-49ml and 2% used the 50-120ml dose ranges.

A comparison of the regions showed no significant differences ($P< 0.05$). About 89% of Dodoma farmers and 75% of Morogoro farmers did not have a specific dosage for this formulation. While 10% of Dodoma farmers used the 5-20ml dose only 6% of Morogoro farmers used the same dosage range. Furthermore, only about 15% of Morogoro farmers used the higher 21-120ml range and hence the no significant difference ($P>0.05$) between the two region. At the district level, no significant differences ($P> 0.05$) were observed.

4.1.26 Dosage rates used by LFOs/AFOs (paraveterinarians or Paravets) for treatment of cattle in the field

The LFOs/AFOs who were the key extension field staff working most closely with and for the livestock keepers totaled about 6,000 only (MOAFS and MOWLD, 2002) countrywide. The LFOs/AFOs were called by livestock keepers to assist them in the treatment of their animals. In the process they administered various drugs for therapy and, as the data has already shown the most commonly used drug was OTC in its two formulations. The various OTC doses they administered to three main groups of body weights are provided in Tables 32 to 34.

Table 31: Oxytetracyline dosage rates used by livestock keepers in bulls per day

Variable	Region				District									
	Dodoma (n=46)		Morogoro (n=65)		Dodoma (U) (n=36)		Dodoma (R) (n=10)		Morogoro (U) (n=16)		Morogoro (R) (n=37)		Total (n=111)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
OTC 10% bull dose (ml/animal)														
5-10ml	2	4.3	1	1.5	1	2.8	1	10	0	0	1	2	3	2.7
11-20ml	2	4.3	3	4.6	0	0	2	20	0	0	3	6.1	5	4.5
21-49ml	0	0	4	6.2	0	0	0	0	0	0	4	8.2	4	3.6
50-120ml	0	0	4	6.2	0	0	0	0	0	0	4	8.2	4	3.6
dnk ml	42	91.3	53	81.5	35	97.2	7	70	16	100	37	75.5	95	85.6
	$X^2 = 6.75^{ns}$, df =4						$X^2 = 22.39^{*}$, df =12							
OTC 20% bull dose (ml/animal)														
5-10ml	2	4.3	1	1.5	2	5.6	0	0	0	0	1	2	3	2.7
11-20ml	6	13	2	13	4	11.1	2	20	0	0	2	4.1	8	7.2
21-49ml	0	0	12	18.5	0	0	0	0	0	0	12	24.5	12	10.8
50-120ml	0	0	13	20	0	0	0	0	0	0	13	26.5	13	11.7
dnk ml	38	82.6	37	56.9	30	88.3	8	80	16	100	21	42.9	75	67.6
	$X^2 = 24.82^{***}$, df =4						$X^2 = 46.82^{***}$, df = 12							
OTC-DNK bull dose (ml/animal)														
5-10ml	2	4.3	4	6.2	2	5.6	0	0	0	0	4	8.2	6	5.4
11-20ml	3	6.5	2	3.1	2	5.6	1	10	1	6.3	1	2	5	2.5
21-49ml	0	0	8	12.3	0	0	0	0	0	0	8	16.3	8	7.2
50-120ml	0	0	2	3.1	0	0	0	0	0	0	2	4.1	2	1.8
dnk ml	41	89.1	49	75.4	32	88.9	9	90	15	93.8	38	69.4	90	81.1
	$X^2 = 8.58$ns, df = 4						$X^2 = 17.89^{ns}$, df. =12							

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 Chi square = ; ns = non significance (X^2>0.5); * = Significance (0.01<X^2<0.05); *** = Significance (X^2<0.001) OTC-DNK = Non specified OTC formulation; dose –dnk = non specified dosage range; OTC = Oxytetracyline.

4.1.26.1 OTC dosage rates used by LFOs/AFOs in the treatment of calves

Table 32 shows the dosage rate used by LFOs/AFOs in the treatment of calves for the three types of OTC formulations i.e. OTC 10% OTC 20%, and OTC-DNK. As with the livestock farmers, some LFOs/AFOs did not differentiate between OTC 10% and OTC 20% (LA). Considering the use of the 10% OTC by LFOs overall 45% of the LFOs/AFOs did not know which dose to use, while 5% of them used the 3-5 ml range, 42.5% used 6-10ml, and 7.5% used 11-20 ml. A comparison at the regional level showed no significant difference ($P>0.05$) while at the district level there was significant difference ($P<0.05$) probably due to the high number of drug non-conversant AFOs in Morogoro Urban district. From the percentage it appeared the most popular dosage used by the LFOs/AFOs was the 6-10ml dose range; this was used by about 43% of the respondents. With the OTC 20% formulation, overall 16% of respondents again did not know which dose to use. About 7.5% used the 1-2ml dose range, 15% used 3-5ml, 20% used 6-10ml and 17.5% used 11-20mls. A regional comparison showed a significant difference ($P<0.05$) and the same trend was observed at the district level.

The LFOs/AFOs who could not differentiate between the OTC 10% and OTC 20% formulations, were designated to have used OTC-DNK. Overall 95% of these OTC-DNK users used DNK doses i.e. did not know the doses to use either. About 2.5% used 3-5ml while another 2.5% used 6-10ml. A comparison at the regional and district levels showed a non-significant differences ($P>0.05$) at both levels.

Table 32: OTC dosage rates used by LFOs/AFOs in the treatment of calves

Variable	REGION				DISTRICT									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
	(n=20)		(n=20)		(n=16)		(n=4)		(n=12)		(n=8)		(n=4)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
OTC 10% calf dose (ml/animal)														
3-5ml	2	10.0	0	0.0	2	12.5	0	0.0	0	0.0	0	0.0	2	5.0
6-10ml	6	30.0	11	55.0	4	25.0	2	50.0	5	41.7	6	75.0	17	42.5
11-20ml	3	15.0	0	0.0	1	6.3	2	50.0	0	0.0	0	0.0	3	7.5
dnkml	9	45.0	9	45.0	9	56.3	0	0.0	7	58.3	2	25.0	18	45.0
	X^2= 6.47^{ns}. df = 3								X^2= 20.80*. df = 9					
OTC 20% calf dose (ml/animal)														
1-2ml	0	0.0	3	15.0	0	0.0	0	0.0	1	8.3	2	25.0	3	7.5
3-5ml	2	10.0	4	20.0	1	6.3	1	25.0	1	8.3	3	37.5	6	15.0
6-10ml	4	20.0	4	20.0	3	18.8	1	25.0	2	16.7	2	25.0	8	20.0
11-20ml	7	35.0	0	0.0	5	31.3	2	50.0	0	0.0	0	0.0	7	17.5
dnkml	7	35.0	9	45.0	7	43.8	0	0.0	8	66.7	1	12.5	16	40.0
	X^2= 10.92*. df = 4								X^2= 22.09*. df = 12					
OTC – DNK calf dose (ml/animal)														
3-5mls	1	5.0	0	0.0	1	6.3	0	0.0	0	0.0	0	0.0	1	2.5
6-10mls	0	0.0	1	5.0	0	0.0	0	0.0	1	8.3	0	0.0	1	2.5
dnkml	19	95.0	19	95.0	15	93.8	4	100.0	11	91.7	8	100.0	38	95.0
	X^2= 2.00^{ns}. df = 2								X^2= 3.88^{ns}. df = 6					

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 =Chi square; ns = non significance (X^2>0.5); * = Significance (0.01<X^2<0.05); OTC-DNK = Non specified OTC formulation; dose –dnkml = non specified dosage range; OTC = Oxytetracyline.

4.1.26.2 OTC dosage rates used by LFOs/AFOs in adult cows/day

Table 33 shows the dosage rates used by LFOs/AFOs in the treatment of adult cows for the three types of OTC formulations. Considering the OTC 10% formulation, overall 45.0% (i.e. ≈ ½) of the respondents did not know which dose of the 10% OTC formulation to use when treating their animals. About 10% of the respondents used 5-10ml., 25% used 11-20ml, 10% used 21-30ml, 7.5% used 31-40ml; and 2.5% used the 41-50ml dose range. A comparison of the responses at the regional and district levels showed a non-

significance of differences (P>0.05). With the 20% OTC formulation, overall, 45% or about half of the respondents did not know which dose of this formulation to use in an adult Tanzania Short Horn Zebu (TSZ) cow. Out of this 45% dnk dose user group, the majority came from Morogoro region where 65% of the regional respondents belonged to group. The corresponding percent for Dodoma region was 25% only. Considering the OTC-DNK formulation, overall 92.5% of all used dnk dose rates i.e. did not know the dose of the OTC-DNK 20% formulation to use in an adult TSZ cow. Another 7.5% used 11-20ml. A comparison of the responses showed no significant differences (P>0.05) both at the regional and district levels.

Table 33: Oxytetracycline dosage rates used by LFOs/AFOs in adult cows

Variable	REGION				DISTRICT									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
	(n=20)		(n=20)		(n=16)		(n=4)		(n=12)		(n=8)		(n=4)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
OTC 10% adult cow dose (ml/animal)														
5-10ml	3	15.0	1	5.0	3	18.8	0	0.0	0	0.0	1	12.5	4	10.0
11-20ml	5	25.0	5	25.0	3	18.8	2	50.0	2	16.7	3	37.5	10	25.0
21-30ml	1	5.0	3	15.0	0	0.0	1	25.0	1	8.3	2	25.0	4	10.0
31-40ml	2	10.0	1	5.0	2	12.5	0	0.0	1	8.3	0	0.0	3	7.5
41-50ml	1	5.0	0	0.0	1	6.3	0	0.0	0	0.0	0	0.0	1	2.5
dnkml	8	40.0	10	50.0	7	43.8	1	25.0	8	66.7	2	25.0	18	45.0
	X^2=3.56^{ns}, df=5								X^2=14.56^{ns}, df=15					
OTC-20% adult cow dose (ml/animal)														
5-10ml	5	25.0	4	20.0	4	25.0	1	25.0	0	0.0	4	50.0	9	22.5
11-20ml	8	40.0	3	15.0	7	43.8	1	25.0	0	0.0	3	37.5	11	27.5
21-30ml	2	10.0	0	0.0	1	6.3	1	25.0	0	0.0	0	0.0	2	5.0
dnkml	5	25.0	13	65.0	4	25.0	1	25.0	12	100.0	1	12.5	18	45.0
	X^2=7.94^{ns}, df=3								X^2=26.55^{ns}, df=9					
OTC-DNK adult cow dose (ml/animal)														
11-20ml	2	10.0	1	5.0	2	12.5	0	0.0	1	8.3	0	0.0	3	7.5
dnkml	18	90.0	19	95.0	14	87.5	4	100.0	11	91.7	8	100.	37	92.5
	X^2=0.36^{ns}, df=1								X^2=1.56^{ns}, df=3					

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 =Chi square; ns = non significance (X^2>0.5); OTC-DNK = Non specified OTC formulation; dose –dnkml = non specified dosage range; OTC = Oxytetracyline.

4.1.26.3 OTC dose rates used by LFOs/AFOs in the treatment of bulls

Table 34 shows the dosage rates used by LFOs/AFOs to treat TSZ bulls for the three formulations of OTC. Considering the 10% OTC formulation overall 42.5% of the LFOs/AFOs interviewed did not know the dose for OTC to use to treat a bull. Another 2.5% used 5-10ml, 22.5% used 11-20ml, 25% used 21-30ml and 7.5% used 31-40ml. A comparison at the regional and district levels showed no significant differences (P>0.05) at both levels. Cosidering the OTC 20% formulation 45% of the respondents did not know which dose to use. About 7.5% used the 5-10 ml dose range, 30% used 11-20ml,

12.5% used 21-30ml and 5.0% used 31-40ml. A comparison of the responses at the regional and district levels showed no significant difference (P>0.05). For the OTC-DNK formulation or non-defined OTC overall 90% of the respondents said they did not know the dose to use in a bull. The 90% level of dnk dose range respondents is reflected in both regions. About 7.5% said they used 11-20mls while the remaining 2.5% said they used 21-30ml. A regional and district comparison showed no significant differences (P>0.05).

Table 34: Oxytetracycline dose rates used by LFOs/AFOs in the treatment of bulls

Variable	REGION				DISTRICT									
	Dodoma		Morogoro		Dodoma (U)		Dodoma (R)		Morogoro (U)		Morogoro (R)		Total	
	(n=20)		(n=20)		(n=16)		(n=4)		(n=12)		(n=8)		(n=4)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
OTC – 10% bull dose (ml/animal)														
5-10ml	1	5.0	0	0.0	1	6.3	0	0.0	0	0.0	0	0.0	1	2.5
11-20ml	5	25.0	4	20.0	4	25.0	1	25.0	3	25.0	1	12.5	9	22.0
21-30ml	5	25.0	5	25.0	3	18.8	3	50.0	1	8.3	4	50.0	10	25.0
31-40ml	1	5.0	2	10.0	0	0.0	1	25.0	1	8.3	1	12.5	3	7.5
dnkml	8	40.0	9	45.0	8	50.0	0	0.0	7	58.3	2	25.0	17	42.5
	$X^2=1.50^{ns}$, df=4						$X^2=12.84^{ns}$, df=12							
OTC 20% bull dose (ml/animal)														
5-10ml	2	10.0	1	5.0	1	6.3	1	25.0	0	0.0	1	12.5	3	7.5
11-20ml	6	30.0	6	30.0	6	37.5	0	0.0	2	16.7	4	50.0	12	30.0
21-30ml	3	15.0	2	10.0	2	12.5	1	25.0	0	0.0	2	25.0	5	12.0
31-40ml	1	5.0	1	5.0	0	0.0	1	25.0	1	8.3	0	0.0	2	5.0
dnk	8	40.0	10	50.0	7	43.8	1	25.0	9	77.0	1	12.5	18	45.0
	$X^2=0.76^{ns}$, df=4						$X^2=18.42^{ns}$, df=12							
OTC – DNK bull dose (ml/animal)														
11-20ml	2	10.0	1	5.0	2	12.5	0	0.0	1	8.3	0	0.0	3	7.5
21-30ml	0	0.0	1	5.0	0	0.0	0	0.0	1	8.3	0	0.0	1	2.5
dnkml	18	90.0	18	90.0	14	87.5	4	100	10	83.3	8	100	36	90.0
	$X^2=1.33^{ns}$, df=2						$X^2=3.98^{ns}$, df=6							

n = number of respondents; U = Urban; R = Rural; df = degree of freedom; X^2 =Chi square; ns = non significance (X^2>0.5); OTC-DNK = Non specified OTC formulation; dose –dnkml = non specified dosage range; OTC = Oxytetracyline.

4.1.27 Routes of administration used by livestock farmers

4.1.27.1 Routes of administration for OTC 10%

Routes of administration used by livestock keepers for various drugs in Dodoma and Morogoro Regions are as shown in Tables 35 and 36, respectively. Tables 37, 38, and 39 show the various routes of administration for various drugs at the district level. In Dodoma Region (Table 35) all (100%) livestock farmers administered OTC 10% via the IM route. On the other hand in Morogoro region (Table 36) about 61% of the farmers administered 10% OTC formulation by the I/V route, 29% administered it by the IM route, 6.5% administered it by the intrathoracic route, and 3.2% administered it orally.

Table 37 shows that 100% of Dodoma Urban district farmers administered of OTC 10% by the intramuscular (IM) route, while 100% of Morogoro Urban farmers used the IM route for administration of the OTC 10% formulation (Table 38). Table 39 shows that in Morogoro Rural district, administration of OTC 10% was done through the IV route by 66% of the livestock farmers; 24% of the farmers used the IM route, 7% of the farmers used intrathoracic route, and 3.4% of the farmers used the oral route.

4.1.27.2 Routes of administration for OTC 20%

Table 35 shows that 84% of Dodoma Region farmers administered OTC 20% by the IM route, while 16% of the farmers did not know which specific route of administration to use. In Morogoro region (Table 36) 60.5% of the farmers used the IV route, 26.3% of the farmers used the IM route, 9.2% of the farmers used the intrathoracic route, and 3.9% used the intraperitoneal route.

Table 37 shows that 7% of Dodoma Urban district farmers administratered OTC 20% by IM route. In this district, 24% of the farmers did not know which route of administration to use. Table 38 shows that 100% of Morogoro urban districts farmers administered OTC 20% by the IM route. About 62% of the Morogoro rural district farmers used the IV route to administer OTC 20% (Table 39), 24.3% of the farmers used the IM route, 10% used intrathoracic route, and about 4% used the intraperitoneal route.

4.1.27.2.1 OTC - DNK routes of administration by livestock farmers

At regional level (Table 35) 85% of the Dodoma Region livestock farmers administered OTC-DNK by the IM route. Table 36 shows that in Morogoro region 58% of the farmers administered OTC-DNK by the I/V route, 23.7% by the IM route, 13.2 % by the intrathoracic route and 2.6% by the intraperitoneal route. Table 37 shows that 82% of Dodoma Urban district farmers administered OTC-DNK by IM route. Table 37 shows that 82% of Dodoma urban farmers used the IM route to administer OTC-DNK. Table 38 shows that 75% of Morogoro Urban farmers administered OTC DNK by the IM route, while the other 25% was administered by the paravets (LFOs) by a route known to themselves. Table 39 shows the various routes of administration for Morogoro Rural district farmers. About 65% of its livestock keepers administered OTC-DNK via the IV route, 17.6% via the IM route, 15 % via the intrathoracic route, and 3% via the intraperitoneal route.

4.1.27.2.2 OTC spray route of administration by livestock keepers

In Dodoma region (Table 35) 100% of the farmers administered OTC spray topically, and in Morogoro Region (Table 36) 66.7% of the farmers applied it topically as well.

Table 35: Routes of drug administration by Dodoma region livestock keepers[1]

Variable	Dodoma region (n=46)																			
	Route of administration																			
	IM		Topical		Dipping spraying		Warm water wash		P/Oral		Salt water		Pouron		DNK		Admin by paravet		Total	
	n=73		n=1		n=6		n=4		n=54		n=1		n=12		n=118		n=5		n=274	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Drug																				
OTC - 10%	20	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	7.3
OTC - 20%	26	84	0	0	0	0	0	0	0	0	0	0	0	0	5	16	0	0	31	11
OTC - DNK	23	85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	15	27	9.9
OTC - Spray	0	0	1	100	1	100	0	0	0	0	0	0	0	0	0	0	0	0	1	0.4
Penicilin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Himan PPF®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Penstrep ®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptope n®	1	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.4
Tylosin	3	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1.1
Sulfonami le Injection ®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butalex®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clexon®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parvexon ®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Berenil®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Novidium ®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Samorin®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diminasa n®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Milsan®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Albendaz ole	0	0	0	0	0	0	0	0	2	100	0	0	0	0	0	0	0	0	2	0.7
Iodine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

1 multiple response

n = number of respondents; U = Urban; Admin. = Administered; Paravet = Paraveterinarian; IM = intramuscular; OTC = OxytetracyclineButalcx® = buparvoquine; Parvexon® = parvaquone; Berenil® = diminazine aceturate; Novidium® = homodium chloride; Samorin® = isometamidium chloride; Diminasan® = diminazine aceturate; Milsan® = Levamisole hydrochloride; Streptopen = Steptomycin – Penicilline; Penstrep® = Penicilline-Steptomycin; Human PPF® = Penicillin; Clexon® = Parvaquone.

Table 36: Route of drug administration by Morogoro region livestock keepers [1]

	Morogoro region (n=65)																											
	Route of administration																											
	IV		IM		IT		IM on hump		Topical tincture		Dipping/ spray		Mouth wash		I/PL		Warm water wash		P/oral		Salt water wash		I mammaries		I/uterine pessaries		Dnk		Total	
	n=247		n=104		n=19		n=1		n=10		n=9		n=2		n=13		n=1		n=4		n=1		n=1		n=2		n=25		n=452	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Drug																														
OTC - 10%	19	61	9	29	2	6.5	0	0	0	0	0	0	0	0	0	0	0	0	1	3.2	0	0	0	0	0	0	0	0	31	6.9
OTC - 20%	46	61	20	26	7	9.2	0	0	0	0	0	0	0	0	3	3.9	0	0	0	0	0	0	0	0	0	0	0	0	76	17
OTC -dnk	22	58	9	24	5	13	0	0	0	0	0	0	0	0	1	2.6	0	0	0	0	0	0	0	0	0	0	0	0	38	8.4
OTC - Spray	0	0	0	0	0	0	0	0	2	67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	33	0	0	3	0.7
Penicillin	3	60	2	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	1.1
Human PPF®	2	40	2	40	0	0	0	0	0	0	0	0	0	0	2	40	0	0	0	0	0	0	0	0	0	0	0	0	5	1.1
Penstrep®	4	67	4	67	1	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1.3
Streptopen®	16	49	16	49	2	6.1	1	3	0	0	0	0	0	0	7	12	0	0	0	0	0	0	0	0	0	0	0	0	33	7.3
Tylosin	1	25	1	25	2	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0.9
Sulfonamide Injection	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.2
Butalex®	2	13	14	88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	3.5
Clexon®	1	20	4	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	1.1
Parvexon®	2	67	1	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0.7
Berennil®	56	81	13	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	69	15
Novidium®	33	81	8	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	41	9.1
Sanwrin®	39	77	11	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	51	11
Diminasan®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.2
Milsan®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.2
Albendazole	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.4
Iodine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.2

[1] Multiple response

n = number of respondents; U = Urban; OTC = Oxytetracycline; Butalex® = buparvoquine; Parvexon® = paivaquone; Berenil® = diminazine aceturate; Novidium® = homodium chloride; Samorin® = isometamidium chloride; Diminasan® = diminazine aceturate; Milsan® = Levamisole hydrochloride; Streptopen = Steptomycin – Penicilline; Penstrep® = Penicilline-Steptomycin; Human PPF® = Penicillin; Clexon® = Parvaquone.

Table 37: Routes of drug administration of drugs by Dodoma Urban district livestock keepers[1]

Variable	Dodoma Urban district(n=36)																	
	Route of administration																	
	IM		Topical		Dipping spraying		Warm water wash		POral		Salt water		Pouron		dnk		Admin by paravet		Total	
	n=39		n=1		n=6		n=4		n=54		n=1		n=12		n=91		n=5		n=213	
	n		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Drug																				
OTC - 10%		100	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	2	0.9
OTC - 20%	16	76	0	0	0	0	0	0	0	0	0	0	0	0	5	24	0	0	21	9.9
OTC -dnk	18	82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	18	22	10
OTC - Spray	0	0	0	0	1	100	0	0	0	0	0	0	0	0	0	0	0	0	1	0.5
Tylosin	3	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1.4
Albendazole	0	0	0	0	0	0	0	0	2	100	0	0	0	0	0	0	0	0	2	0.9
Dipping	0	0	0	0	2	100	0	0	0	0	0	0	0	0	0	0	0	0	2	0.9
Traditional medicine	0	0	0	0	0	0	0	0	22	32	0	0	0	0	5	19	0	0	27	13
DNK	0	0	0	0	0	0	0	0	0	0	0	0	0	0	81	99	1	1.2	82	39
Salt water solution	0	0	0	0	0	0	0	0	9	100	0	0	0	0	0	0	0	0	9	4.2
Warm water	0	0	0	0	0	0	4	20	5	25	0	0	11	55	0	0	0	0	20	9.4
Paracetamol (human tablets)	0	0	0	0	0	0	0	0	5	100	0	0	0	0	0	0	0	0	5	2.3
OTC Human capsule	0	0	0	0	0	0	0	0	9	90	0	0	1	10	0	0	0	0	10	4.7
Stelladone®	0	0	1	50	1	50	0	0	0	0	0	0	0	0	0	0	0	0	2	0.9
Tick-fix®	0	0	0	0	1	100	0	0	0	0	0	0	0	0	0	0	0	0	1	0.5
Salt water wash	0	0	0	0	0	0	0	0	0	0	1	100	0	0	0	0	0	0	1	0.5
Ngao®	0	0	0	0	1	100	0	0	0	0	0	0	0	0	0	0	0	0	1	0.5
Ashes	0	0	0	0	0	0	0	0	1	100	0	0	0	0	0	0	0	0	1	0.5
Tobacco juice	0	0	0	0	0	0	0	0	1	100	0	0	0	0	0	0	0	0	1	0.5

1 multiple response

n = number of respondents; OTC = Oxytetracycline Stelladone® = Chlorfenviphos; Tick-fix® = Amitraz – 12.5%; P oral = Per oral IM = intramuscular; Route dnk = non specific route of drug administration; Admin= Administration; Paravet = Paraveterinarian; Ngao = 25% deltamethrin..

Table 38 : Routes of drug administration used by Morogoro Urban Livestock keepers[1]

Variable	Morogoro Urban district (n=16) Route of administration																	
	IV		IM		Tropical tincture		Dipping/ Spraying		IMammary		IUterine		dnk		Admin by paravet		Total	
	n=1		n=12		n=2		n=2		n=1		n=2		n=5		n=13		n=38	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Drug																		
OTC - 10%	0	0	2	100	0	0	0	0	0	0	0	0	0	0	0	0	2	3.5
OTC - 20%	0	0	2	100	0	0	0	0	0	0	0	0	0	0	0	0	2	5.3
OTC -dnk	0	0	3	75	0	0	0	0	0	0	0	0	0	0	1	25	2	11
OTC - Spray	0	0	0	0	1	50	0	0	0	0	1	50	0	0	0	0	2	5.3
Penicillin	1	50	1	50	0	0	0	0	0	0	0	0	0	0	0	0	2	5.3
Tylosin	0	0	1	100	0	0	0	0	0	0	0	0	0	0	0	0	1	2.6
Butalex®	0	0	2	100	0	0	0	0	0	0	0	0	0	0	0	0	2	5.3
Samorin®	0	0	1	50	0	0	0	0	0	0	0	0	0	0	1	50	2	5.3
Dipping	0	0	0	0	0	0	2	100	0	0	0	0	0	0	0	0	2	5.3
Salt mouth wash	0	0	0	0	1	100	0	0	0	0	0	0	0	0	0	0	1	2.6
DNK	0	0	0	0	0	0	0	0	0	0	0	0	5	100	0	0	5	13
Vitamins	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2.6
Minerals	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2.6
Admin. By paravet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	24
I/mammaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2.6
I/Uterine pessaries	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2.6

[1] Multiple response

OTC = Oxytetracycline Butalex® = buparvoquine; Samorin® = isometamidium chloride; OTC-DNK = Non specified OTC formulation; drug DNK = Non Specified Drug: Route dnk = non specific route of drug administration; Admin= Administration; Paravet = Paraveterinarian; IV = Intravenous; IM = Intramuscular; I/Mammary = Intra mammary; IUterine = Intra uterine.

Table 39: Routes of drug administration used by Morogoro Rural livestock keepers[1]

Variable	Rural district (n=49)																							
	Route of drug administration																							
	IV		IM		IT		IMon hump		Tropical tuncture		Dipping spraying		Mouth wash		IPL		Warm water wash		P Oral		Salt water		dnk		Total	
	n=246		n=92		n=19		n=1		n=8		n=7		n=2		n=13		n=1		n=4		n=1		n=20		n=414	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Drug																										
OTC - 10%	19	66	7	24.1	2	7	0	0	0	0	0	0	0	0	1	0	0	0	1	3.4	0	0	0	0	29	7
OTC - 20%	46	62	18	24.3	7	10	0	0	0	0	0	0	0	0	3	4	0	0	0	0	0	0	0	0	74	18
OTC -dnk	22	65	6	17.6	5	15	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	34	8
OTC - Spray	0	0	0	0	0	0	0	0	1	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Penicilin	2	67	1	33.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1
Human PPF	2	40	1	20	0	0	0	0	0	0	0	0	0	0	2	40	0	0	0	0	0	0	0	0	5	1
Penstrep®	4	67	1	16.7	1	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1
Streptopen®	16	49	7	21.2	2	6	1	3	0	0	0	0	0	0	7	21	0	0	0	0	0	0	0	0	33	8
Tylosin	1	33	0	0	2	67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1
Sulfonamide injection	0	0	1	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Butalex®	2	14	2	14.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	3
Clexon®	1	20	1	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	1
Parvexon®	2	67	2	66.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1
Berenil®	56	81	56	81.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	69	17
Novidium®	33	81	33	80.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	41	10
Samorin®	39	80	10	20.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	49	12
Diminasan®	0	0	0	0	0	0	0	0	1	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Milsan®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100	0	0	0	0	1	0
Albendazone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	100	0	0	0	0	2	1
Iodine	0	0	0	0	0	0	0	0	1	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

[1] Multiple response

n = number of respondents; OTC = OxytetracyclineButalex® = buparvoquine; Parvexon® = parvaquone; Berenil® = diminazine aceturate; Novidium® = homodium chloride; Samorin® = isometamidium chloride; Diminasan® = diminazine aceturate; Milsan® = Levamisole hydrochloride; Clexon® = parvaquone; Streptopen® = Streptomycin - penicillin; Penstrep® = Penicillin-Streptomycin; Route dnk = non specific route of drug administration; IV =Intravenous; IM = intramuscular; = ; IT =Intrathoracic; IMon on hump = intramuscular on hump; IPL = Intraperitoneal; P Oral = per oral.

4.1.28 Routes of administration of drugs used by LFOs/AFOs

Tables 40 and 41 shows the routes of administration of various drugs, OTC inclusive, used by LFOs/AFOs in the two regions of study. The IM route was the most commonly used route of administration for most injectable formulations.

Table 40: Drug routes of administration by LFOs/AFOs in Dodoma region[1]

Single drug	IV		IM		SC		Dipping sprying pour on		Mouth wash		Poral		IMmaries		Totals	
	n=9		n=83		n=27		n=8		n=2		n=5		n=6		n=108	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Single drugs administered																
OTC - 10%	9	25	34	94.4	9	25	2	5.6	1	2.8	0	0	1	2.8	36	33.3
OTC - 20%	0	0	17	100	0	0	1	5.9	0	0	1	5.9	1	5.9	17	15.7
Streptopen®/ penstrep®	0	0	4	100	0	0	0	0	0	0	0	0	0	0	4	3.7
Tylosin	0	0	3	75	0	0	1	25	0	0	0	0	0	0	4	3.7
Butalex®	0	0	1	50	0	0	0	0	1	50	0	0	0	0	2	1.9
Parvexon®	0	0	3	100	1	33	0	0	0	0	0	0	0	0	3	2.8
Berenil®	0	0	6	100	0	0	0	0	0	0	0	0	0	0	6	5.6
Novidium®	0	0	1	100	1	100	0	0	0	0	0	0	0	0	1	0.9
Diminasan®	0	0	1	100	0	0	0	0	0	0	0	0	0	0	1	0.9
Milsan®	0	0	1	50	0	0	0	0	0	0	1	50	0	0	2	1.9
Albendazole	0	0	0	0	1	100	0	0	0	0	2	20	0	0	1	0.9
IMamaries	0	0	1	25	0	0	0	0	0	0	0	0	4	100	4	3.7
Ivomec®	0	0	0	0	2	100	1	50	0	0	1	50	0	0	2	1.9
Vaccine	0	0	0	0	13	100	0	0	0	0	0	0	0	0	13	12
Sulfornamide Injectable	0	0	1	100	0	0	0	0	0	0	0	0	0	0	1	0.9
OTC -DNK	0	0	9	100	0	0	2	22.2	0	0	0	0	0	0	9	8.3
Dipping	0	0	0	0	0	0	1	100	0	0	0	0	0	0	1	0.9
Trodax®	0	0	1	100	0	0	0	0	0	0	0	0	0	0	1	0.9
OTC spray	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Samorin®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lasix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[1] Multiple response

n = number of respondents; OTC = Oxytetracycline; Butalex® = buparvoquine; Parvexon® = parvaquone; Berenil® = diminazine aceturate; Novidium® = homodium chloride; Samorin® = isometamidium chloride; Diminasan® = diminazine aceturate; Milsan® = Levamisole hydrochloride; Streptopen® = Streptomycine - penicillin; Penstrep® = Penicillin-Streptomycin; Route dnk = non specific route of drug administration; Trodax® = Nitroxynil injection ; IV =Intravenous; IM = intramuscular; = ; P Oral = per oral.; SC= Subcutaneous; Immaries = Intramammaries; Ivomec® = Ivermection.

Table 41 : Drug routes of administration by LFO/AFO - Morogoro region[1]

Single drug	IV		IM		SC		Dipping, sprying pouron		Mouth wash		Poral		I/mmaries		dnk		Totals	
	n=		n=43		n=24		n=3		n=		n=9		n=2		n=1		n=75	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Single drugs administered																		
OTC - 10%	0	0	20	100	1	5	1	5	0	0	0	0	0	0	0	0	20	26.7
OTC - 20%	0	0	1	100	0	0	0	0	0	0	0	0	0	0	0	0	1	1.3
Streptopen®/penstrep®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tylosin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butalex®	0	0	4	100	0	0	0	0	0	0	0	0	0	0	0	0	4	5.3
Parvexon®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Berenil®	0	0	14	100	0	0	0	0	0	0	0	0	0	0	0	0	14	18.7
Novidium®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diminasan®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Milsan®	0	0	0	0	4	57.1	0	0	0	0	7	100	0	0	0	0	0	0
Albendazole	0	0	0	0	0	0	0	0	0	0	2	100	0	0	0	0	0	0
I/Mamaries	0	0	0	0	0	0	0	0	0	0	0	0	2	100	0	0	0	0
Ivomec®	0	0	0	0	6	85.7	0	0	0	0	0	0	0	0	1	14.3	0	0
Vaccine	0	0	0	0	12	100	0	0	0	0	0	0	0	0	0	0	0	0
SulfonamideInjectable	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTC - DNK	0	0	2	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dipping	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trodax®	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTC spray	0	0	0	0	1	50	2	100	0	0	0	0	0	0	0	0	2	2.7
Samorin®	0	0	1	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lasix	0	0	1	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[1] Multiple response

n = number of respondents; IV = intravenous; IM = intramuscular; IT= Intrathorax, IM on hump = intramusclle on hump; I = Intra; OTC = Oxytetracycline; Butalex® = buparvoguine; Parvexon® = paivaquone; Berenil® = diminazine aceturate; Novidium® = homodium chloride; Samorin® = isometamidium chloride; Diminasan® = diminazine aceturate; Milsan® = Levamisole hydrochloride; Streptopen® = Streptomycine - penicillin; Penstrep® = Penicillin-Streptomycin; Route dnk = non specific route of drug administration; Trodax® = Nitroxynil injection ; IV =Intravenous; IM = intramuscular; = ; P Oral = per oral.; SC= Subcutaneous; Immaries = Intramammaries; Ivomec® = Ivermection.

4.1.29 Marketing of animals by livestock farmers

Table 42 shows the selection criteria of animals to be sent for sale by farmers. Overall 71% of farmers said they sent only healthy animals to livestock market for sale, in order to fetch a good market price. About 17% said they sent a mixture of both healthy and unhealthy animals for sale. About 2% sold only unhealthy animals, while 47% sent healthy animals but only those (cows) which were infertile, and low milk producers. Regionally 61% of Dodoma farmers sold only healthy animals, and 79% of farmers in Morogoro Region also did the same. At the district level 69% of Dodoma Urban, 30% of Dodoma Rural, 19% Morogoro Urban and 98% of Morogoro Rural districts sold healthy animals only.

Table 42: Marketing of animals by farmers [1]

Variable	Region				District									
	Dodoma (n=46)		Morogoro (n=65)		Dodoma (U) (n=36)		Dodoma (R) (n=10)		Morogoro (U) (n=16)		Morogoro (R) (n=49)		Total (n=111)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Selection criteria of animals for sale														
Healthy animals only	28	61	51	78.5	25	69	3	30	3	18.9	48	98	79	71
Unhealthy animals only	0	0	2	3.1	0	0	0	0	0	0	2	4.1	2	1.8
A mixture of both healthy and unhealthy	17	37	2	3.1	10	28	7	70	0	0	2	4.1	19	17
Infertile cows, low milk producers, excess bulls	4	8.7	48	73.8	3	8.3	1	10	10	62.5	38	78	52	47
DNK	1	2.2	5	7.7	1	2.8	0	0	5	31.3	0	0	6	5.4

[1] Multiple response

n = number of respondents; DNK = Do Not Know; R =Rural; U= Urban.

4.2 HPLC analysis for oxytetracycline residues in meat samples

4.2.1 The standard curves for OTC chromatographic and tissue spike standards

Triplicate values of the OTC chromatographic standards were obtained from HPLC recordings and two standard curves were determined. One for the chromatographic standards and another for the kidney tissue spike standards. The correlation between OTC concentration and area under curve (AUC): OTC chromatographic standards (stds) of 0.0ppm, 0.32 (0.3175)ppm, 0.625ppm, 1.25ppm 2.5ppm were made by dissolving OTC powder in mobile phase. 20µl aliquots of the stds were injected in turns successively into the HPLC analytical column Lichrosorb RP8-10 (4.6mm id.x25.0cm) and the retention time tr, corresponding peak heights and AUC were recorded. The concentration of the standard were plotted against AUC the results of which are shown in Figure 10.

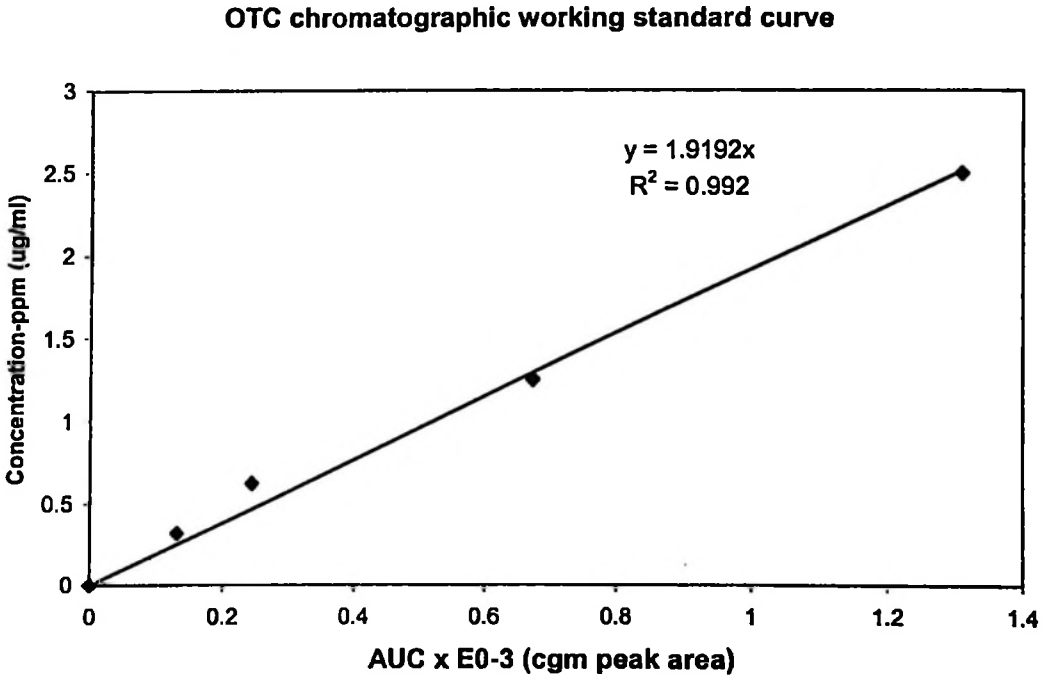


Figure 10: The correlation between OTC concentration and AUC

The correlation coefficient R^2 between OTC concentration was 0.992. This high value indicates a very high correlation between the two parameters.

OTC Spike standards (stds) of 0.0ppm, 0.32 (0.3175) ppm 0.625ppm, 1.25ppm, 2.5ppm were prepared by dissolving OTC in the mobile phase (MeoH:AC-N:OX-AC) in the ratio 1:2:7 respectively. 20µl aliquots of the standards were injected into the HPLC and the retention time tr, peak heights and AUC recorded. Approximately 10g of kidney tissue were ground into a fine paste mixed with 30 mls of 0.S.S (OTC extraction or separation solution), its weight and volume recorded and the volume of the kidney sample and O.S.S. was spiked with a calculated volume of a 100ppm OTC solution suitable to achieve the required spike concentration with respect to the volume of the mixture after which the spiked mixture was homogenized for 2 minutes, centrifuged at 10,000 r.pm for 15 minutes ultrafiltared by a 0.2-0.4 µm membrane filter, and then applied on a C-18 catridge for OTC elution. The eluted OTC samples were then quantified on un ATIUNICAM HPLC system. The concentration of the spike standard were plotted against AUC (Figure 11).

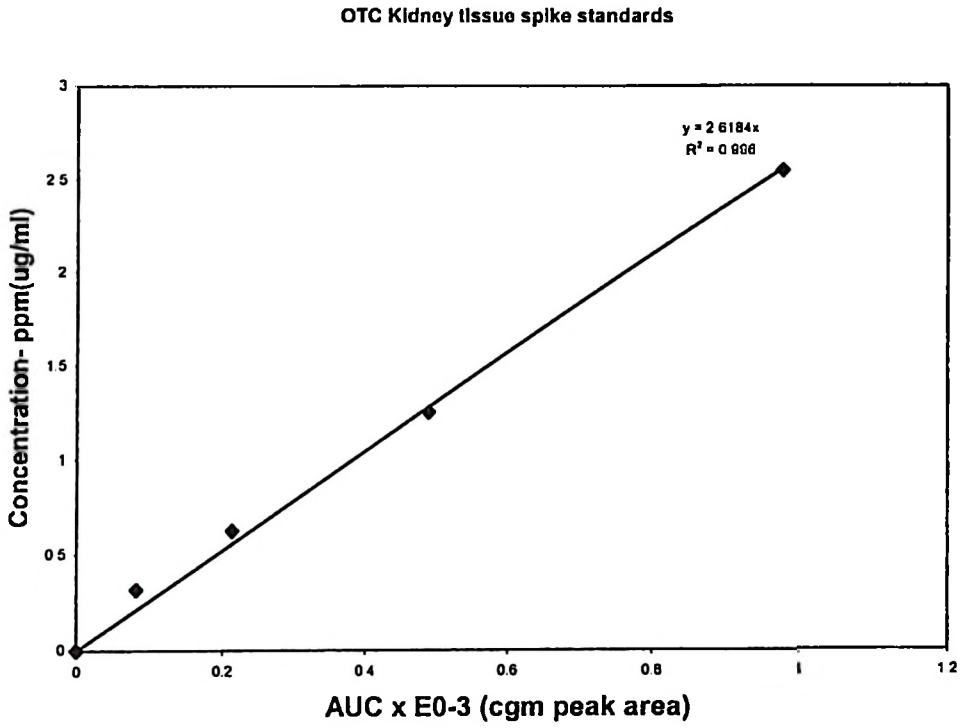
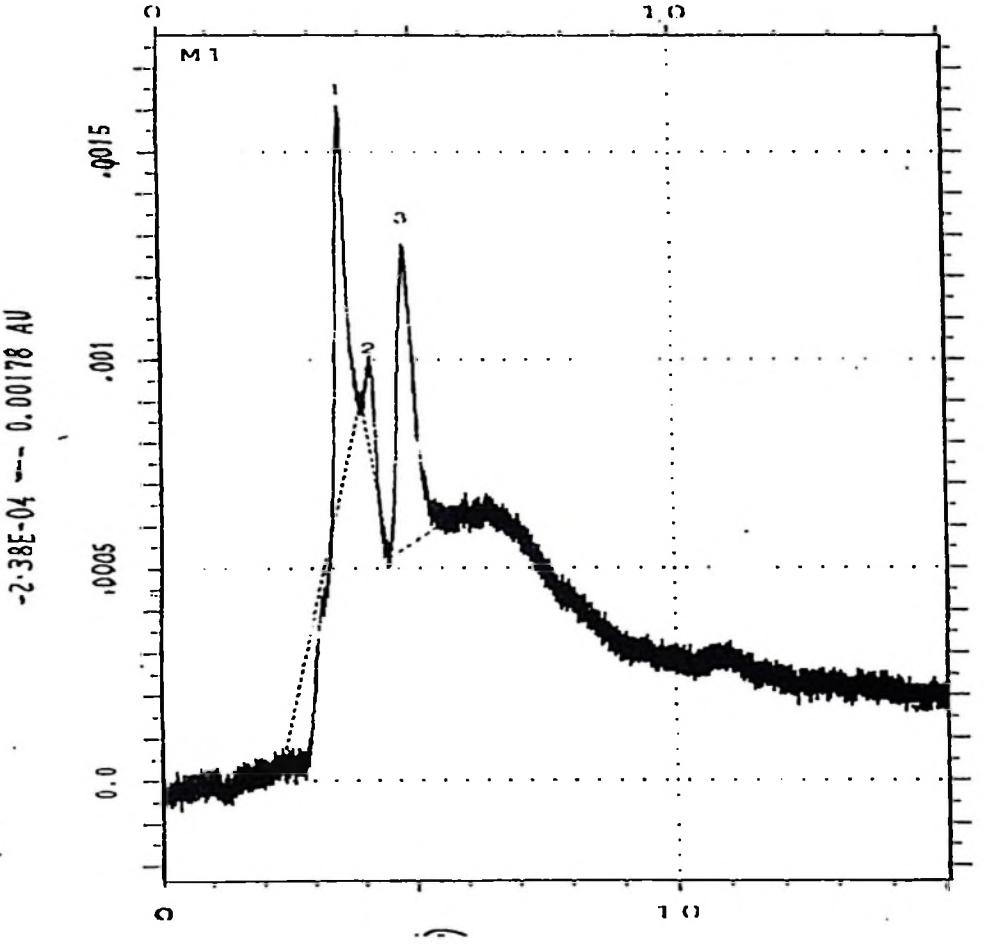


Figure 11: The correlation between OTC concentration and AUC after OTC extraction and analysis by HPLC

The correlation coefficient $R^2 = 0.996$ indicated a very strong correlation between the two parameters.

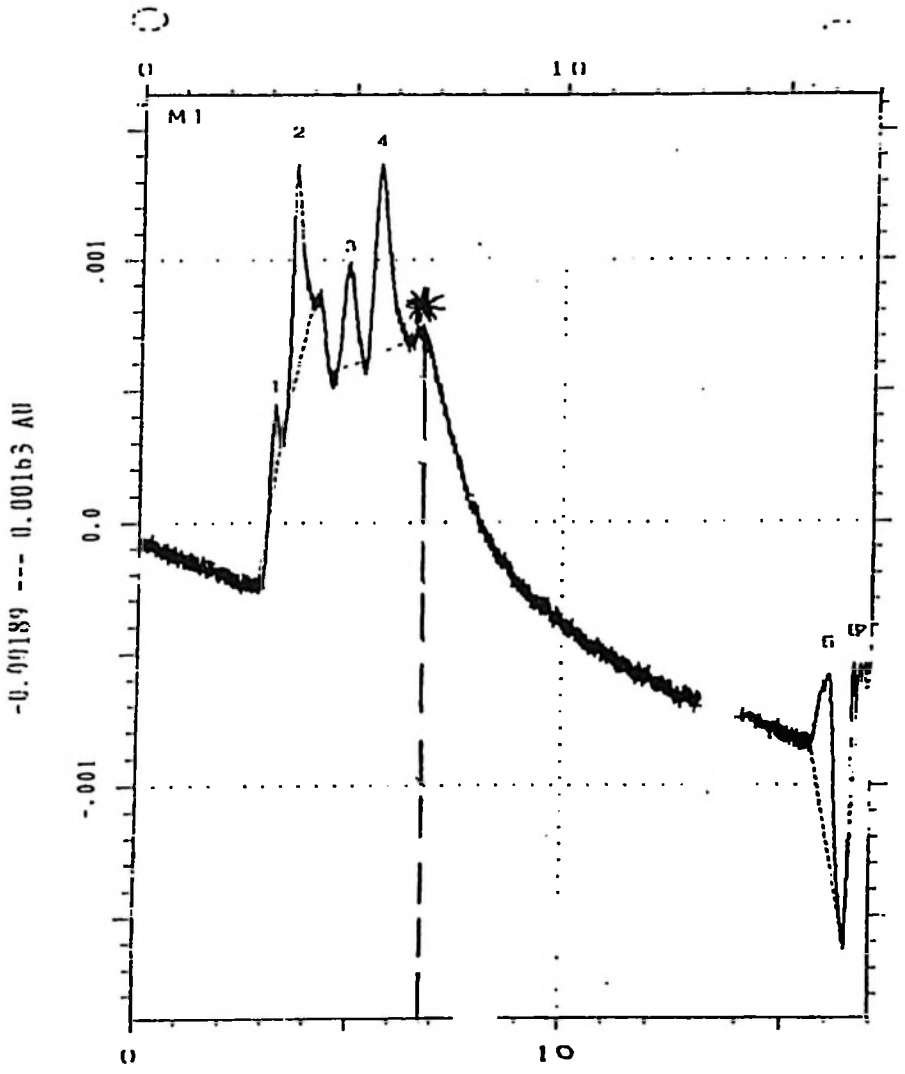
Chromatograms

HPLC quantification of Oxytetracycline using spike standard solutions of OTC at concentrations 2.5ppm, 1.25ppm, 0.625ppm, 0.32 (0.3175) ppm and 10ppm was carried out by injection of 20µl of each concentration in turn. The peak heights, AUC and retention time tr for each injection concentration was recorded. The tr for 10ppm was 6.67 minutes, 6.68 for 2.5 ppm, 6.69 for 1.25ppm, 6.68 for the 0.625ppm and 6.68 for the 0.32 ppm concentration. The cgms, and cgm reports are shown on Figures 12 – 18.



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	3.59	0.00090	2.42	4.00	1.149E-04	27.6	I
2	4.16	0.00017	4.00	4.52	3.109E-05	7.5	I
3	4.85	0.00072	4.52	5.47	2.705E-04	64.9	I

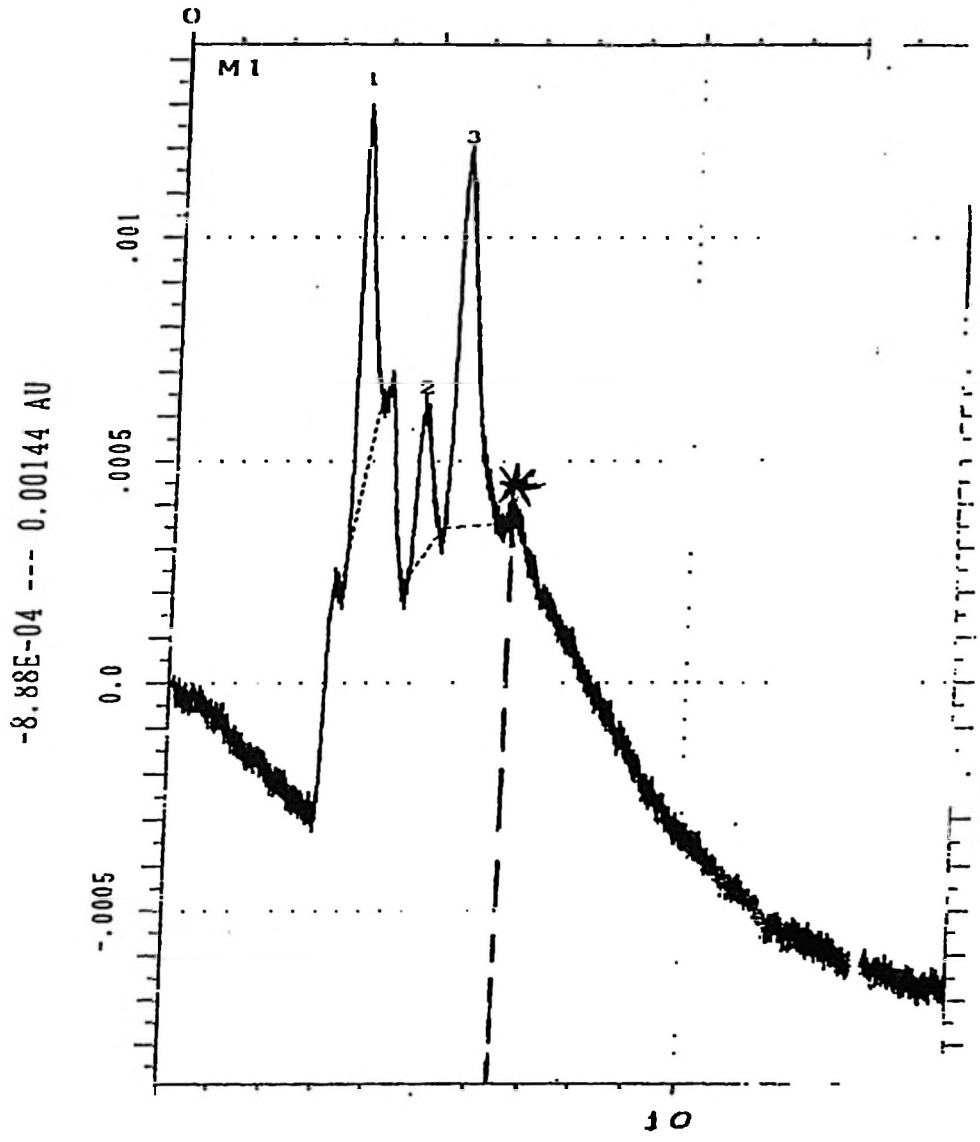
Figure 12: Chromatogram STD OTC SP-K Blank



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	3.18	0.00023	2.80	3.34	4.160E-05	4.2	I
2	3.61	0.00084	3.34	4.06	2.225E-04	22.6	I
3	4.89	0.00034	4.51	5.29	1.173E-04	11.9	I
4	5.63	0.00070	5.29	6.33	2.970E-04	30.2	I
5	16.02	0.00064	15.60	16.41	2.299E-04	23.4	I
6	16.69	0.00047	16.41	16.67	5.661E-05	5.8	I
7	16.74	0.00020	16.67	16.81	1.781E-05	1.8	I

Figure 13: Chromatogram STD OTC SP-K 0.08ppm

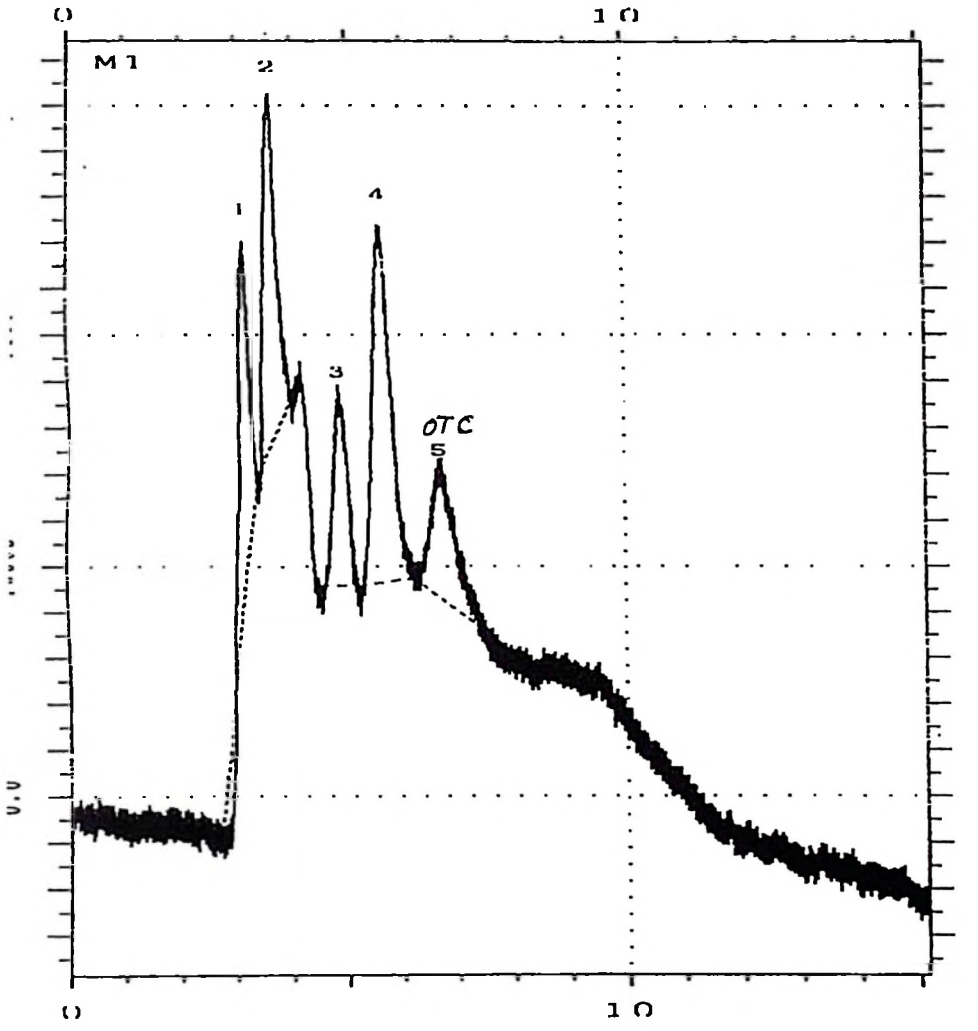
* OTC concentration too low for integration



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	3.60	0.00087	3.30	4.01	2.349E-04	33.6	I
2	4.86	0.00030	4.55	5.24	9.916E-05	14.2	I
3	5.61	0.00080	5.24	6.44	3.656E-04	52.3	I

Figure 14: Chromatogram STD OTC SP-K 0.16ppm

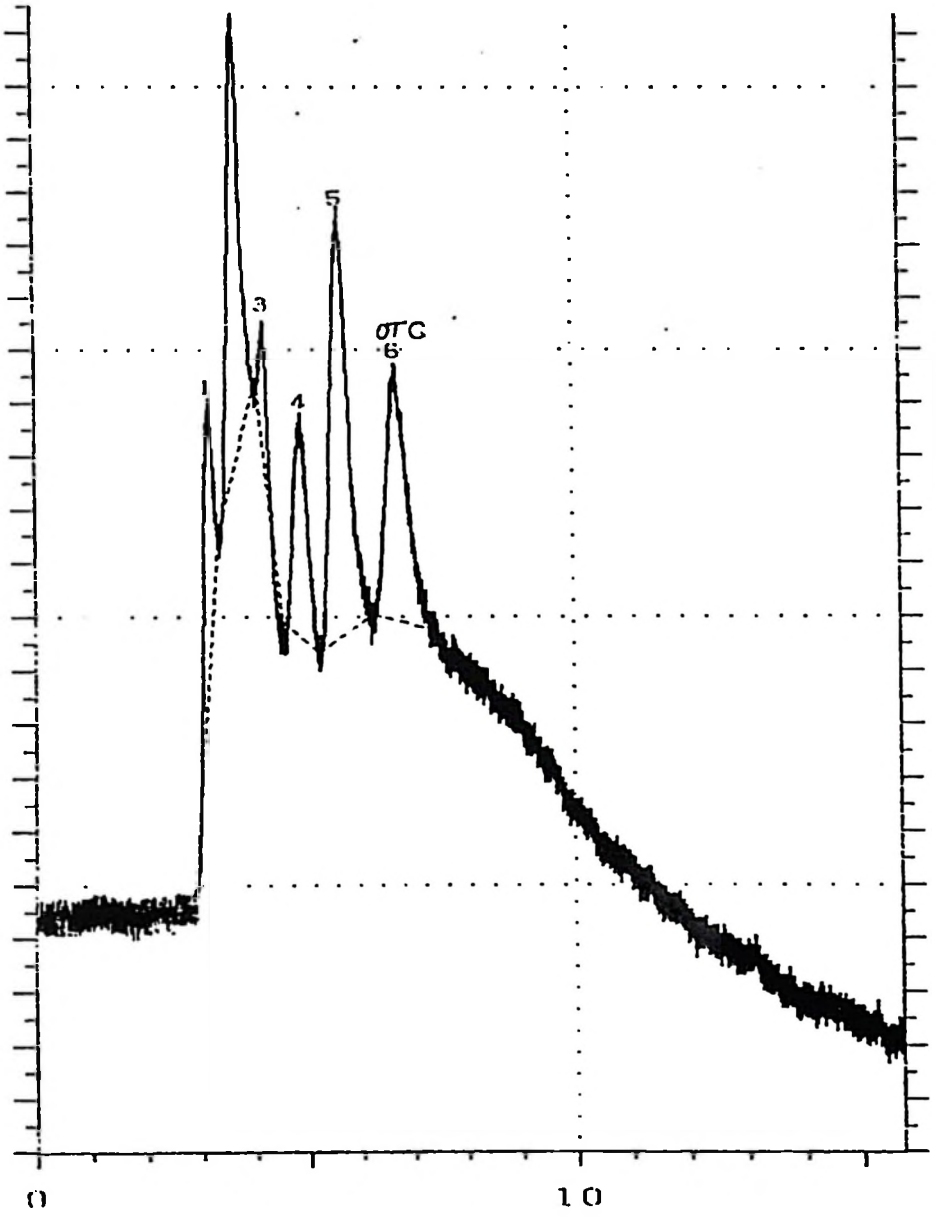
* OTC concentration too low for integration



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	3.14	0.00080	2.75	3.58	1.464E-04	16.5	I
2	3.61	0.00076	3.38	4.04	1.974E-04	22.3	I
3	4.84	0.00039	4.55	5.24	1.160E-04	13.1	I
4	5.59	0.00077	5.24	6.24	2.910E-04	32.9	I
5	6.68	0.00025	6.24	7.89	1.348E-04	15.2	I

Figure 15: Chromatogram STD OTC SP-K 0.32 (0.3175) ppm

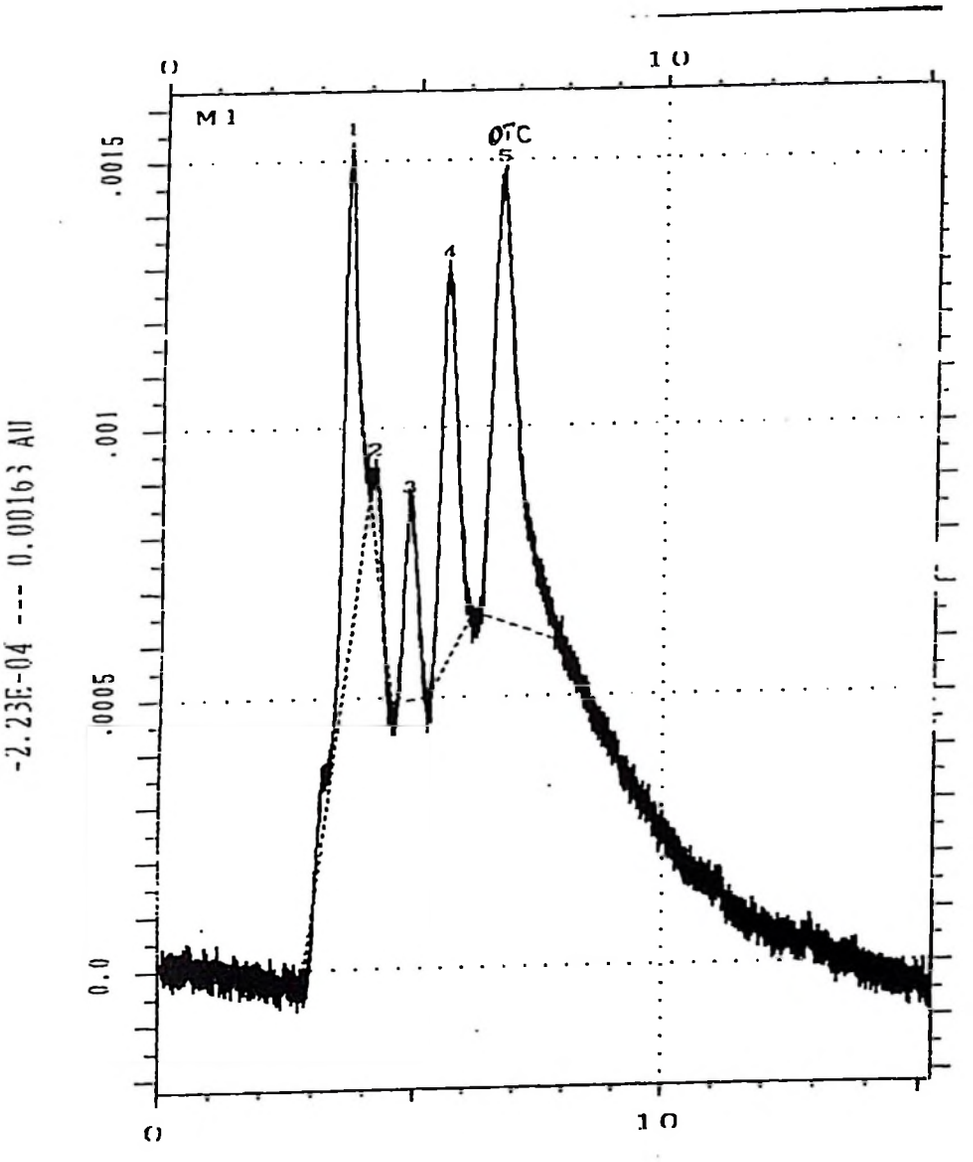
OTC-t_r = 6.68



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	3.13	0.00049	2.84	3.35	1.013E-04	10.4	I
2	3.60	0.00084	3.35	4.04	2.223E-04	22.8	I
3	4.17	0.00018	4.04	4.55	2.142E-05	2.2	I
4	4.86	0.00036	4.55	5.23	1.154E-04	11.8	I
5	5.58	0.00075	5.23	6.24	3.015E-04	30.9	I
6	6.68	0.00043	6.24	7.35	2.133E-04	21.9	I

Figure 16: Chromatogram STD OTC SP-K 0.625 ppm

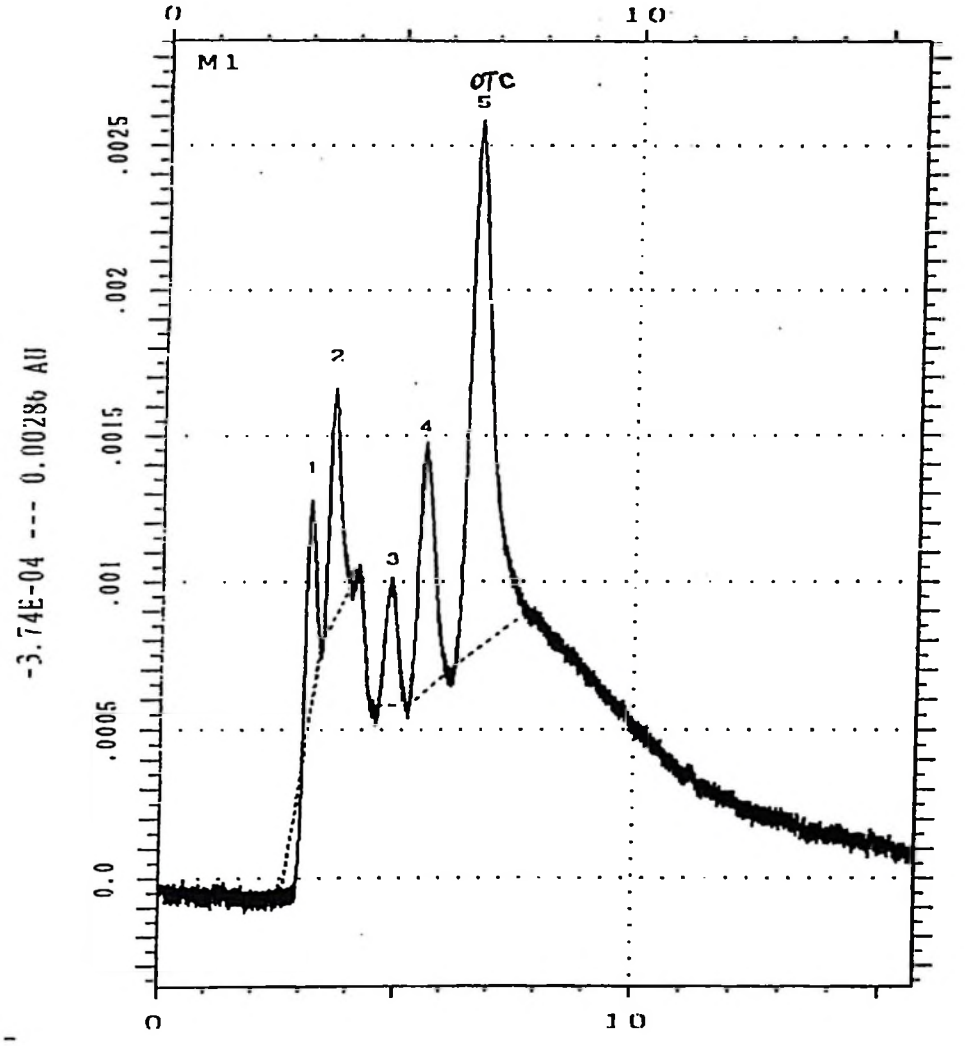
OTC-t_r = 6.68



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	3.61	0.00094	2.85	4.04	3.209E-04	26.7	I
2	4.15	0.00011	4.04	4.52	1.626E-05	1.4	I
3	4.84	0.00033	4.52	5.24	1.042E-04	8.7	I
4	5.59	0.00070	5.24	6.14	2.698E-04	22.5	I
5	6.69	0.00080	6.14	7.97	4.885E-04	40.7	I

Figure 17: Chromatogram STD OTC SP-K 1.25 ppm

OTC-t_r = 6.68



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	3.14	0.00075	2.60	3.38	8.476E-05	5.0	I
2	3.62	0.00080	3.38	4.04	2.004E-04	11.9	I
3	4.89	0.00039	4.55	5.24	1.207E-04	7.1	I
4	5.58	0.00080	5.24	6.16	3.073E-04	18.2	I
5	6.68	0.00177	6.16	7.86	9.772E-04	57.8	I

Figure 18: Chromatogram STD OTC SP-K 2.5 ppm

OTC-t_r = 6.68

4.2.2 HPLC results for oxytetracycline residues analysis in meat samples

A total of 131 beef samples were analysed for OTC residues in this study (Table 43). A total of 71 samples i.e about 54.2%, were obtained from the Morogoro Municipal abattoir while the remaining 60 samples i.e. 45.8%, of the total were from Dodoma municipal abattoir.

Table 43: Concentration of oxytetracycline in meat from Morogoro and Dodoma municipal abattoirs

Abattoir	Tissue	Number analysed		Positive		Min Conc µg/g	Max Conc µg/g	Mean Conc µg/g	Std. deviation	Std. Error of mean
		n	%	n	%	Concentration range				
	Muscle	20	28.2	9	45.0	0.00	6.86	0.8774	1.8589	0.4157
Morogoro	Liver	25	35.2	9	36.0	0.00	8.33	1.5025	2.7693	0.5539
	Kidney	26	36.6	12	46.2	0.00	8.98	1.5660	2.6291	0.5156
	Total	71	100.0	30	42.3					
Dodoma										
	Muscle	15	25.0	4	26.7	0.00	2.24	0.4302	0.8208	0.2119
	Liver	20	33.3	9	45.0	0.00	3.54	0.8360	1.1481	0.2567
	Kidney	25	41.7	11	44.0	0.00	6.45	1.1765	1.9421	0.3884
	Total	60	100.0	24	40.0					

Min Conc = Minimum concentration; Max Conc = Maxmum concentration;
MeanConc = Mean concentration Std. = Standard; n = Number of samples.

Further more, Table 43 shows the proportion, occurrence and mean concentration of OTC residues in muscle, liver and kidney samples from Morogoro Municipal abattoir. A total of 20 (28.2%) of Morogoro samples were muscle tissue samples, 25 (35.2%) were liver and 26 (36.6%) samples were kidney samples. About 9 (45.0%) of Morogoro muscle samples had detectable levels of oxytetracycline residues. The mean $\pm$ (SEM)

concentration of OTC in positive samples was 0.8774± 0.4157 ppm. OTC concentrations in the positive samples ranged from 0.06 ppm to 6.86 ppm.

A total of 9 (36.0%) of Morogoro liver samples were positive for OTC residues. The mean concentration of OTC in these samples was 1.5025± 0.5539 ppm. The OTC contamination of liver sample was high, and ranged from from 0.329 ppm to 8.33 ppm. A total of 12 (46.2%) of Morogoro kidney samples were found to be positive for OTC residues. Mean OTC concentration in the positive samples was 1.5660± 0.5156 ppm. OTC concentrations in the positive kidney samples was high and ranged from 0.004 ppm to 8.98 ppm. The kidneys contained the highest concentrations of the 131 meat samples analysed.

The proportion, occurrence and mean concentrations of OTC in muscle, liver and kidney samples collected from Dodoma municipal abattoir is also shown in Table 43. About 15 (25.0%) of the samples were muscle tissue, 20 (33.3%) were liver tissue and 25 (41.7%) were kidney tissue. A total of 4 (26.7%) of muscle samples were positive for OTC residues. The mean concentration of OTC was 0.4302 ± 0.2119 ppm. The concentrations in muscle samples were moderately high, and ranged from 0.522 ppm to 2.340 ppm. A total of 9 (45.5%) of Dodoma liver samples were found to be positive for OTC residues. Their mean OTC concentration was 0.8360± 0.2567 ppm. Concentrations were moderately high and ranged from 0.446 ppm to 3.54ppm.

A total of 11 (44.0%) of kidney samples from Dodoma were positive for OTC residues. The mean OTC concentration was 1.1765 ± 0.3884. Concentrations were high and ranged

from 0.023 ppm to 6.45 ppm. A student's t-test was carried out to test for the significance of the differences of the means of OTC concentration for each of the 3 types of samples and between the two abattoirs, as shown in Appendices VI –VIII. The results revealed that there was no significant differences (P>0.05) between the two mean OTC concentrations of muscle samples from the two abattoirs. There was also no significant difference (P>0.05) between the mean OTC concentrations of liver samples from the two abattoirs. Further, there was no significant differences (P>0.05) between the mean OTC concentrations of kidney samples from Morogoro and Dodoma abattoirs.

A further statistical analysis of the OTC residue data was carried out using X^2 tests to find out whether there was a significant relationship between occurrence of OTC residues in the meat samples and location (i.e. abattoirs) and also between occurrence of OTC resides and type of tissue sample. Results of the X^2-test (Appendix IX) show that there was no significant difference (P>0.05) between abattoirs on the occurrence of OTC residues in meat irrespective of the type of tissues. There was no significant difference (P>0.05) in the occurrence of OTC residues between the three types of tissue samples irrespective of their abattoir source (Appendix X). There was no significant difference (P>0.05) between the muscle, liver and kidney samples collected from Morogoro abattoir on the occurrence of OTC residues (Appendix XI). There was no significant difference (P>0.05) between the muscle, liver and kidney samples collected from Dodoma abattoir in the occurrence of OTC residues (Appendix XII).

In addition, while there was no significant difference (P>0.05) between the muscle samples collected from Morogoro abattoir versus those from Dodoma abattoir in the

occurrence of OTC residues (Appendix XIII), there was also no significant difference (P>0.05) between the liver samples collected from Morogoro abattoir and those from Dodoma abattoir in the occurrence of OTC residues (Appendix XIV). No significant differences (P>0.05) were observed between the kidney samples collected from Morogoro abattoir and those from Dodoma abattoir in the occurrence of OTC residues (Appendix XV).

4.2.3 Samples with violative residue levels

The number of samples with violative OTC residue levels are shown on Table 44. The total number of samples with violative OTC residue levels was 41 out of 131 samples analysed, constituting 31.3%. But of the 41 violative samples 24.4% were muscle, 36.6% were liver, and 39.0% were kidney tissue samples.

Table 44: The number of tissue samples with violative OTC residue levels

Location	Tissue			
	Muscle	Liver	Kidney	Total
Morogoro	6	7	8	21
Dodoma	4	8	8	20
Total	10	15	16	41

The maximum residue levels for (MRLs) OTC for muscle, liver and kidney tissues are 0.2mg/kg, 0.6 mg/kg and 1.2 mg/kg respectively (FAO/WHO, 1999).

CHAPTER FIVE

5.0 DISCUSSION

The analysis of beef samples collected from Dodoma and Morogoro abattoirs established that there were OTC residues in the meat samples, 31.3% of which had violative OTC levels. This implies they must have originated from the abusive use or improper use of OTC. Their occurrences were consummate with the results obtained from the studies done in the field in the two regions on the OTC regime of use and mode of application.

The regimen of use and mode of application of OTC in the field was studied under the following parameters; use and level of use of OTC in the field, the dose or amount of OTC administered to cattle, the basis of OTC dose determination, duration of treatment with OTC, observance of the OTC withdrawal period, adherence to OTC expiry dates, and the stoking of drugs which reflects the intensity or likelihood of the use of OTC for treatment. The mode of application of OTC was studied under the parameters of; route of administration and use of drug combination because of the interactive tendencies of OTC with other substances (Kamel and Brown, 2002).

The main users of OTC for the treatment of livestock diseases in the area of study were the livestock farmers themselves and the LFOs/AFOs. That these two groups were the main users of OTC in this study was clear from the fact that these two groups were those who did most of the therapeutic work where overall 49% of the farmers said they treated their sick animals on their own and 42% called LFOs treatment assistance in the two region. However at the district level the proportions differed significantly ($p>0.05$) while

in Morogoro rural district 70% of livestock owners treated their sick animals themselves. In Morogoro urban district only 12.2% did the same (Table 13).

From the results of the study, OTC was not only widely used but it was the principal drug used in the field, hence its connotation by the Maasai livestock keepers as the wonder drug which treats "100" diseases. Overall about 81.6% of the farmers admitted to using the various formulations of OTC as a single drug (Table 18) and 113.6% used it in combination with other drugs Table 19. The corresponding cumulative proportions for the LFOs/AFOs were 146% and 209% respectively (Tables 20 and 21). And its principal users are mainly the livestock keepers and to some extent the LFOs/AFOs. The widespread use of OTC as the principal therapeutic drug meant that any abuse or violation of its use would certainly lead to occurrence of its residues or those of its metabolites in the affected animal food products.

The background characteristics profile of the livestock farmers (Table 7) established that overall about 55% of them had never been into a classroom. However with Morogoro rural district whose livestock keepers consisted mainly of the "Maasai" pastoralists the illiteracy rate was as high as 82% of those interviewed. Unfortunately these were the people who carried out most of the treatment of their sick animals. A high farmers illiteracy rate is known to be a factor in drug mishandling and abuse (Gracey, 1986), since they could not read and follow the manufacturers data sheet and hence contributing to non-observance of withdrawal periods.

The risk of drug abuse is definitely very high. For instance, about 90% of the Maasai ethnic group in Morogoro rural district administered OTC to their animals themselves. This same Maasai livestock keeper group has a 100% chance of possessing inadequate knowledge on drug handling and use. Hence the high probability or risk involved in drug abuse leading to drug residues in animal food products such as meat, milk and cheese. This contrasts with the situation in internsive zero grazing areas where treatment is done by qualified veterinarians (Lindsay *et al.*, 1975; Hamilton *et al.* 1980,; Muriuki *et al.*, 2001).

For the LFOs/AFOs user group, there was also some degree of abuse but because of their veterinary training and knowledge on drug use, the risk factor on abuse was expected to be lower leading to a lower risk of drug residues than with the farmers. However the risk in the LFOs/AFOs group should be higher if one considers the AFOs separately. Additionally they administered OTC and other drugs to far fewer animals than the livestock owners, further lowering their risk of abuse factor. AFOs are certificate holders in agriculture, essentially in crop production. They were retrained for about six months in livestock production/animal health in order to confer them with skills in disease control (MALD, 1994).

The question on basis of OTC dose estimation was an attempt at finding the accuracy of the doses used by farmers and LFOs/AFOs in the field. Overall 76% of the livestock keepers (Table 26) said their dose estimates were based on the body size or weight of the animal while 15% said the doses were determined by LFOs/AFOs.

Another 6% said they didn't know i.e. they administered whatever came into their mind. About 5% estimated on the basis of the age of the animal. However even with those who estimated dosage rates on the basis of body weight there was the problem of accuracy of estimation of an animal's body weight. And even then they could not carry out the simple arithmetic of the total amount of OTC in mg to be administered to the respective animal. For example overall 83% and 66% of farmers did not know the dose rate for an adult cow for OTC 10% and 20% formulations respectively (Table 30) As such they ended up estimating the volume in millilitre of OTC to be administered often with a wide range and many times without differentiating OTC 10% from OTC 20% i.e. the 80% DNK group (Table 30). There was the risk of overestimation or underestimation of the volume of OTC administered. The LFOs/AFOs have undergone some training on how to estimate the body weight of an animal and further they were able to read the directions of use of an OTC formulation on the data sheet of the drug because of their understanding of the English language. The results of the doses used by them were accurate, though with some errors, compared to those used by livestock farmers. The level of underdosing or overdosing was far less compared to that of farmers whereby overall only 45% of those interviewed did not know the dose rates for the 10% and 20% solutions for an adult cow. The situation was worse for Morogoro urban farmers where about 100% of the AFOs did not know the dosage rates as shown in Table 33.

The results in Table 26 show that for all practical purposes the main group of OTC users that is the livestock owners, did not observe the 28 days of OTC withdrawal periods. Their retention of the recovered animals past OTC administration was probably for reasons, which bore no relationship to its withdrawal period. Only 1% overall, and this

was from the Morogoro urban respondents, mentioned the observation of a 28 or more days withdrawal period past the last OTC administration for the OTC to be cleared from the animals body before selling it for slaughter.

Hence, generally most of the farmers interviwed did not observe an OTC withdrawal period before selling or slaughtering their animals. This was also echoed by the LFOs/AFOs whereby unfortunately a high proportion of them (63%) did not have any duration of time for the withdrawal time for OTC after administration to an animal. Only 2.5% of this livestock technical cadre which is closest to the livestock farmers said they will look for the OTC withdrawal period on the manufacturer's data sheet. While this was correct it was still a disappointment, and dangerous that at the time of interview they did not know it. This was so because while they admitted to using this drug more routinely for treatment than any other drug, they did not have the proper advise on the OTC withdrawal period to extend to livestock farmers, leaving consumers at the risk of consuming OTC residues. The WP of a drug is the most important parameter in the control of drug residues in animal food products. Failure to observe withdrawal periods has been the main source of drug residues in many countries (Tayemama, 1988; Rogsta, 1989). A closer look at the 63% overall DNK bracket, i.e. those who did not know, shows that while 40% of LFOs/AFOs of Dodoma region belonged to this group, of those who know nothing about the OTC withdrawal period, in Morogoro region this group constituted 85%. In Morogoro rural district those LFOs/AFOs who did not know were 88% (Table 28). It seems that the important message on OTC withdrawal period was not being passed down to the livestock farmers and the beef-consumers by the extension staff. Without the observation of the OTC withdrawal period of 28 days for beef and 7 days for milk,

consumers of animal food products are left to consume OTC residues unknowingly. The Maasai on the other hand, due to their love for their animals' well being, adhere to drug expiry dates more than the LFOs/AFOs.

The results of this study indicated that overall all livestock farmer respondents did stock drugs. The Maasai pastoralists stocked OTC, especially OTC 20%. Stocking of a drug posed a higher probability or more likelihood of it being used in case of the occurrence of a clinical case. In the absence of a clinician the stocked OTC could be administered for a wrong disease, in wrong amounts, using a wrong route of administration, consequently giving rise to probable OTC residues. This is especially so, should the sick animal become terminally ill or die, get slaughtered and have its meat consumed, as observed in the results. For the treated terminally sik animals, about 68% of the farmers sold them for slaughter, 11% continued with treatment and consumed the animal upon death and 7.2% did not know what to do with their animals (Table 13). Overall 48.5% (cumulative) of LFOs/AFOs advised slaughter and consumption, while 2.5% did not know what to advise. About 23% advised discontinuation of treatment and condemnation upon death, and 18% advised continued treatment and condemnation should death occur (Table 14).

This means the LFOs/AFOs may administer OTC if called upon to attend a case, such as like anaplasmosis. Some LFOs/AFOs (68%) stocked OTC and other drugs. Stocking drugs in their (LFOs/AFOs) homes is an acceptable procedure (Directorate of Livestock Development, personal communication 2003). The drug stocking rate of the LFOs/AFOs was less compared to the Maasai pastoralists whose OTC stoking rates were 100%; and the drug was used in almost every clinical case attended.

The duration of OTC treatment by all the pastoralists in Dodoma was not more than 2 days. They administered a sub standard starting dose on the first day which was followed by a second dose the following day, and that was all. With the Maasai pastoralists their duration of treatment especially by OTC 20% by IV, IM, intra thoracic, Intra peritoneal and IM on hump, ranged from 3 days to 7 days, or dosing daily until the animal appeared to recover.

Intra thoracic, and IM into the hump, are routes of administration that are not recommended in routine therapeutics (Riviere and Spoo, 2001). Their disposition kinetics are also unknown, as well as their role in the occurence of residues in slaughtered animals.

According to Riviere and Spoo (2001) OTC 20% is administered at 48 to 72 hours intervals. Daily administration amounts to overdosing and hence there is a higher probability of occurrence of residues in meat should the animal be slaughtered in the course of treatment or soon after. This scenario was observed especially in Morogoro region where about 10% of the farmers used the dose rate of 5-20ml of OTC 20% per adult cow per day, 15% used 21-49ml and 20% of them used the 50-120ml range per cow per day (Table 30).

The dosage rate of a drug for example OTC, to food animals is an important parameter not only from the therapeutic and toxicological point of view but also from the point of view of occurrence of residues. Overdosage may lead to accumulation of OTC's in organs such as the kidney and the liver. Alternatively an under dosage may lead to non-recovery and development of microbial resistance (Chopra, 1988).

Some doses administered to animals were equivalent to 80mg/kg bwt daily. Since the Maasai wrongly use the IV route mainly for the 20% OTC formulation, it means the acummulation effect may be inevitable as the kidneys may become overwhelmed and instead of the OTC disposition kinetics obeying a first-order kinetic disposition, at some critical blood OTC concentration, the OTC disposition may begin to obey zero-order kinetics resulting in the accumulation of OTC's in various tissues especially the kidney and liver (Baggot, 1977). At such levels the prescribed WP of 28 days for the elimination of OTC's becomes insufficient and irrelevant, as this WP has been recommended on the basis of therapeutic doses. At such a high over dosage, the administered OTC will inevitably need more time for its complete elimination from the tissues and organs of the food animals. With the failure by 45% of all the LFOs/AFOs to know the proper dosage of OTC and also their failure by 97.5% overall to know the recommended withdrawal period of OTC, the occurrence of OTC residues in the beef samples analysed was probably inevitable.

The route of administration of a drug is important from the point of view of treatment, toxicity and also OTC residue occurence in food animals' tissues. The OTC 10% formulation is meant for I/V, and IM administration on a daily basis. On the other hand the OTC 20% (LA) formulation is meant for IM administration only every 48-72 hours to allow its slow release from its site of injection of amounts equivalent to daily therapeutic doses into the blood stream (Huber 1988; Riviere and Spoo 2001). However the Maasai, due to their desire to sec quick results, prefer the IV route (62%) versus the IM route (24.3%) and IT route 10% for OTC 20% solution (Table 39). This, coupled with the non-observance of WP, was probably one of the reasons for the high proportion of samples

with violative OTC concentrations. The use of the I/Thoracic route in cases of CBPP is gross abuse of the use of the drug considering the irritant effect of OTC. They do it in the hope of delivering large amounts of OTC into the affected lungs and pleural membranes. CBPP is a highly contageous disease, and affects the pleura and lungs leading to pneumonia (Radostits *et al.*, 1999). The Maasai are aware of the lesions associated with the disease. The use of the oral route is another gross abuse of the mode of application of OTC 10% because most of it will be destroyed by the volatile fatty acids (VFAs) in the rumen and the acid pH in the abomasum. No effective absorption into the blood stream from the fore stomachs occurs and hence the antibiotic is merely wasted (Brander *et al.*, 1991).

OTC residues may result from treatments by LFOs/AFOs mainly due to non- observance of the OTC WP of 28 days. This compares well with observations elsewhere, where non-observance of WP was the main cause of OTC residues in beef (Farrar, 1985; Tayemama, 1988; Rogsta, 1989). From this study, it appears that OTC over dosage, the use of wrong routes of administration and non-observance of OTC withdrawal period may be the principal causes of its residue occurence in products of animals treated by farmers. On the other hand animal products from animals treated by LFOs/AFOs contained residues mainly due to non-observance of WP. Similar studies carried out elsewhere found the misuse of drugs in animal treatment to be responsible for the occurrence of OTC residues in beef (Kerr *et al.*, 1972; Obasajo *et al.*, 1988; Mdachi *et al.*, 1991; Muriuki *et al.*, 2000). Accoding to Kerr *et al.*, (1972) out of 10,000 red meat samples analysed for tetracyclines' residues in 1971, 1500 i.e. 15% were positive for tetracycline residues due to improper drug use. Mdachi *et al.*, (1991), reported that 100% of meat samples collected from

Nairobi slaughter houses were contaminated with trypanocide residues and about 20% of them had antibiotic residues. This conformed with the results of another study by Muriuki *et al.*, (2001) in the same area where out of 250 samples analysed about 20% of them had violative OTC and CTC residues.

Due to the occurrence of mixed infections, often OTC has to be used in combination with other drugs in order to achieve good therapeutic results. For example in the management of ECF, OTC has been administered with an antitheilerial. Similarly, ticks transmit more than one type of infection, for instance babesiosis, anaplasmosis, etc. During the treatment and control of such multiple infections simultaneously, in the field, OTC has been administered in combination with diminazene diaceturate (Berenil®, Diminasan®) (Tables 19 and 21) leading to drug interactions (Kamel and Brown, 2000) and subsequent residues in animal food products. However this may have a detrimental effect of reducing the efficacy of OTC depending on the drug combinations used. Hence drug combinations must be used carefully for their use may also slow the disposition of OTC resulting in drug residues. The more serious combinations involve the direct unorthodox mixing of various drugs for instance Butalex®, Parvexon® or Berennil® with OTC prior to administration by mixing several millilitres of those drugs with a vial of OTC.

A total of 131 beef samples consisting of 35 muscle, 45 liver and 51 kidney samples from Morogoro and Dodoma abattoirs were analysed for OTC and 54 (41.2%) were found to be positive for OTC residues. The Maximum residue levels (MRLs) for oxytetracycline recommended by the Joint FAO/WHO Expert Committee on Food Additive (JECFA) in 1999 were muscle 0.2 mg/kg, liver 0.6mg/kg, and 1.2 mg/kg for kidney tissues. Of the 131

samples, 41 (31.3%) had oxytetracycline levels, which were above (violative) the JECFA MRLs. About 10 of the violative samples were muscle, 15 were liver and 16 were kidney samples. The 31.3% prevalence of violative OTC samples is much higher than for most other countries where similar studies have been conducted in countries such as Germany (Vaughan *et al.*, 1981) 0.05%, Netherlands (Whider *et al.*, 1977) 0.1%, Canada (Tittiger *et al.*, 1980) 0.2%, USA (Ryan *et al.*, 1974) 0.025%, and Kenya (Muriuki *et al.*, 2000) 20%.

The study indicated that the occurrence of OTC residues in beef samples in the two abattoirs were the same. The main reason for this trend is probably the source of the animals slaughtered in the two abattoirs. While cattle slaughtered in the Dodoma abattoir originated mainly from Manyoni, Dodoma rural, Dodoma urban, Mpwapwa, Kongwa and Kiteto districts' livestock markets, animals slaughtered at Morogoro abattoir originated from Morogoro rural, Kilosa, Mpwapwa, Kongwa, Kiteto and Morogoro urban livestock markets. This means there was a mixture of cattle from the Gogo and Nyaturu pastoralists who were observed to be poor to moderate users of OTC with cattle from the Maasai pastoralists who were observed to be intense users of OTC. The high standard deviation of the mean concentrations of the samples may also be explained by the fact that animals for slaughter in the two abattoirs originated from both low and high intensity OTC user areas.

Further X^2 tests revealed that there were no significant differences ($P>0.05$) on the occurrences of OTC residues between the muscle, liver and kidney tissues irrespective of the two locations. Even the mean occurences of OTC residue between the three types of tissues from the same abattoir revealed a non-significant ($P>0.05$) differences of occurence between them. A comparison of the occurences of each of the 3 types of tissues

in paired X^2 tests between the two abattoirs revealed a non significant difference (P>0.05) in the occurence of OTC residues for all the three tissues between the two abattoirs. Though we could have expected a higher occurence rate for the liver and kidney, this was not the case due to the following reasons. The first reason was during the period of taking the samples there was a severe out break of CBPP and CCPP in both Dodoma and Morogoro regions and a lot of animals were dying. Again in Morogoro rural and Kilosa districts there was a severe out break of lumpy skin disease. The livestock owners in Dodoma and Morogoro regions were using very high OTC doses including blind treatments to cover non-clinical cases. And before animals were sent to livestock markets they were covered with a blind treatment of OTC in order to protect them from diseases during trekking. The second reason was the fact that a few of the muscle samples were taken from sites of injection i.e. the gluteal and neck muscles. When an animal is administered an OTC 20% formulation dose which is more than 10 times the therapeutic dose by IV, IM and I/Thorax simultaneously OTC residues are bound to occur even if the recommended OTC WP of 28 days is observed because the first-order, 3 compartment – open model disposition kinetics of OTC will no longer hold (Baggot *et al.*, 1977).

The Maasais admitted, upon questioning, that some of the animals collapsed and some died when administered such high doses by the IV route. This is consistent with the findings of Gyrd-Hansen *et al.*, (1981) who reported that animals died from cardiac failure due to extensive intravascular chelation of Ca^{++}.

It is apparent that the high concentrations of OTC residues in the samples from Dodoma and Morogoro abattoirs were due to rampant misuse of OTC 10% and 20% formulations,

but mainly the 20% formulation. This is consistent with studies conducted in other pastoralist areas where farmers/livestock keepers were found to overdose their animals with antibiotics and other drugs and failed to observe the drug withdrawal period (Lindsay *et al.*, 1975; Hamilton *et al.*, 1980).

From the pharmacological and environmental point of view drug residues in animal food products are not the only problem from the massive doses of OTC used for prolonged periods of time (Aalback *et al.*, 1991; Nijsten *et al.*, 1996). Prolonged use of antibiotics may lead to development of resistance through various mechanisms. If this trend of use of OTC is unchecked OTC resistant bacteria and protozoa will be rampant and the antibiotic will no longer work. The possibility also exists of the development of multiple resistance of some bacteria to other antibiotics (Russel and Chopra, 1990; Aalback *et al.*, 1991; Nijsten *et al.*, 1996).

The problems of OTC residues in beef and in other animal products such as in milk (Kurwijila *et al.*, 2000) and the danger of development of microbial resistance in Tanzania may be overcome by employing various measures including the control on the sale and use of antibiotics by strong legislation and follow up, by frequent monitoring and screening of livestock products, the use of specific therapy, education to the farmers, and livestock consumers on the adverse effects of OTC in food products. The screening and monitoring for antibiotics residues could be done using the Charm Aim - 96 inhibition test for milk (Kurwijila *et al.*, 2000) and the Charm ROSA test for meat. The tests would be useful as quick qualitative tests.

For the quantitative analysis of OTCs and other antibiotics the HPLC method, though expensive and highly demanding, is the most accurate method in the quantitative analysis of OTCs on the basis of its sensitivity, specificity and accuracy. With the use of an LC-18 SPE extraction method using McIlavine buffer pH 4.0, the recovery of OTCs cannot escape detection (Mac Neil, 2000; Muriuki *et al.*, 2001) at the recommended detection levels.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1) This study has shown that oxytetracycline was the most widely used veterinary drug in Dodoma and Morogoro regions.
2) Oxytetracycline was not only the most widely used veterinary field drug but it was also the most widely abused due to lack of institutional state control.
3) Tetracycline is still a viable field drug for those syndromes, which are responsive to it according to all field extension staff interviewed.
4) The sale and handling of veterinary drugs in this country practically has no institutional control at all and this is probably the reason why drugs are being sold every where e.g. in the livestock markets, by the "machingas", and also in the so called "pembejeo" shops mostly by unqualified personnel.
5) Out of the 131 beef samples analysed, 54 (41.2%) had detectable OTC residues, while 41 (31.3%) of the total had violative OTC residues comparative to the JECFA values of 1999 of 200µg/kg for muscle, 600 µg/kg for the liver, and 1200 µg/kg for the kidney. Hence the meat that we eat in Morogoro and Dodoma is not safe for health; it has high OTC levels.
6) From available literature, and from the results of this study, the McIlavaine buffer (pH 4.0) –EDTA was a very efficient extractant for oxytetracyclines at trace levels.
7) The Solid phase extraction (S.P.E) method was one of the most suitable methods for OTC extraction and could probably be modified for other drugs, toxins, hormones and various other compounds.

8) Due to the high level of veterinary drug abuse, the long-term effect of OTC abuse and its attendant residues are difficult to gauge in terms of public heath. The possibility exists that adverse public health effects are widespread in Tanzania.

9) The high levels of occurrence of violative OTC residues and high concentration levels of these OTC residues were a direct consequence of the level of OTC abuse at the level of sale, handling and administration.

10) The most widely abused parameters of OTC administration and which were the most responsible for the occurrence of violative drug residues were over dosing, wrong route of administration, and non-observation of the OTC withdrawal period WP.

11) Most of the livestock keepers in Dodoma and Morogoro regions especially the pastoralists were mainly illiterate. Only 55% of those interviewed had ever entered a classroom. The high level of illiteracy may have greatly contributed to drug abuse and thus high level of OTC in meat.

12) Pastoralists and especially the Maasai were the leading group in the abuse of OTC. This abuse was mainly on overdosage of up to 10 to 20 times the therapeutic dose, the use of wrong route of administration and non-observance of OTC withdrawal period. These were possibly responsible for the high prevalence and high concentration of OTC residues.

13) LFOs/AFOs were the second group in line in the abuse of OTC. Though a high proportion of them, about 45%, did not know the exact dose of OTC, the biggest threat to residue occurence was their non-observance of OTC withdrawal period. It appeared the extension officers were not adequately trained.

14) LFOs/AFOs were more likely to use expired OTC than the Maasai pastoralists who never used expired OTC.

15) The high preferance of some pastoralists especially the Masaai for the 20% LA OTC formulation was one of the main causes of residue occurrence in meat. The recent appearance of OTC 30% LA on the market may further complicate the situation.

6.2 Recommendations

1) The results of this study have shown that there was a high prevalence (31.3%) of violative oxytetracycline residues and relatively high levels (>8mg/kg) compared to other countries even when compared with our Kenyan neighbours. The government should therefore deliberately embark on a screening programme in several zones of this country to establish the extent of the OTC and other drugs residue problem and set national maximum residue levels (MRLS) for OTC for various tissues and species of animals.

2) From this study it was established that the best users and abusers of OTC and other livestock drugs were the livestock farmers especially the pastoralists. It was also established that their OTC abuse was mainly dosage rate, use of the wrong route of administration and non-observance of withdrawal period. The rules governing the sale and use of OTC and other drugs should be strengthened and adhered to in order to reduce its abuse.

3) Livestock farmers and consumers in general should be educated on the dangers of OTC and other drugs abuse and their attendant residues to public health.

4) From this study, it has been established that the LFOs/AFOs were the second group in line in the use and abuse of OTC especially on their failure to appreciate and advise farmers on the importance of OTC withdrawal periods. They should be

educated on its importance so that they extend the right message to the livestock farmers. Aspects of food safety and drug residue occurrence should be part of their training curriculum.

5) Livestock farmers and LFOs/AFOs should be emphasized to concetrate more on good animal husbandary techniques and use of vaccines to reduce the occurrence of diseases and reduce the need for use of drugs and their attendant residues.

6) A screening and monitoring programme of OTC residues in meat and in milk for the prevalence of OTC residues qualitatively should be instituted using the Charm ROSA and Charm AIM 96 for meat and milk respectively, in order to institute the necessary measures. Quantitavely and qualitatively as well they could be monitored using the reversed phase HPLC analysis which is very sensitive, accurate and has a high precision.

7) The sale of veterinary drugs in livestock markets, and by the "machingas" should be banned otherwise if the present trend continues unabated disaster from plasmid and transposon mediated resistance to OTC, other tetracyclines and multiple resistance to other antibiotics, might occur.

8) The Tanzania Food And Drugs Authority (TFDA) should strengthen its regime on the control of importation, sale, handling and use of veterinary drugs.

9) Veterinary drugs such as OTC and some of the more sensitive ones to public and animal health should cease to be viewed merely as "Pembejeos" (farm inputs) but rather as sensitive chemicals to be handled by pharmacies and veterinary clinics only. At present they are merely "pembejeos" and this may be the reason why they found their way to livestock markets and "machingas".

10) The regional veterinary drug licensing authorities should be more serious in the issuance of licenses on the sale of OTC and other sensitive drugs. TFDA should facilitate the regional and district inspectors financially and logistically to enable them perform their duties properly.

11) Due to the high prevalence and high levels of OTC found in the sampled meat, consumers should be advised to cook and roast meat thoroughly before consumption in order to reduce the amount of OTC, which may find its way into the gastrointestinal tract.

12) More veterinarians and paraveterinarians should be trained and employed by the state and located to serve the pastoral areas. However, concurrent programmes should be developed to produce more veterinary surgeons to replace the LFOs/AFOs.

13) The State should provide incentives to both private and state employed veterinarians and paraveterinarians to encourage them to move and work in the remote pastoral areas. This may reduce the appetite and need for pastoralists to acquire drugs, which they eventually abuse with their attendant residue problems.

14) The presently state employed veterinarians and paraveterinarians who are mostly located in the towns and trading centers essentially trading in their "pembejeo "shops should be moved and redistributed in to the pastoral areas with incentives to work there.

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APPENDICES

Appendix 1: Questionnaire for livestock farmers

I: Identity

1. Identification No.....
2. Name.....
3. Sex.....
4. Region.....
5. District.....
6. Ward.....
7. Village.....
8. Occupation.....
9. Level of education (Primary/Secondary School).....
10. Level of found livestock keeping training.....

II: Livestock keeping experience

11. When did you start livestock keeping?.....
12. Is it the sole business on which you livelihood depends or is it just one of them?
13. What are the other?.....
14. What problems have you encountered in you livestock keeping business
    .....
15. What do you do when some of your animals get sick?.....
16. Which diseases are normally encountered?
    (a) .....
    (b) .....
    (c) .....
17. During which time or season of the year does each of the diseases above NO 16 above occur? (Dry or Rainy season).....
18. What do you do next?.....
19. Which drugs do you normally use to treat each of the diseases named in question No above?
    (a) .....
    (b) .....
    (c) .....
20. Other treatments?.....
21. How do you dispense the drug i.e. which route of administration; Orally, by injection-IM, I/V or S/C?.....
22. How do you dispense the drug i.e. which route of administration; Orally, by Injection- I/M, I/V or S/C?.....
23. How do you determine the amount of drug (antibiotic) which you dispense to an animal?.....

24. How much antibiotic do you administer to an adult cow.....
How much antibiotic do you administer to calves?.....
(a) 3 months like that one over there?.....
(b) months like that one over there?.....
(c) 9 months like that one over there.....
(d) 1months like that one over there?.....
25. What about when you have two adult cows of different sizes i.e. large and small, how do you determine the amount to be administered into each animal?.....
26. How much do you administer to a large bull lime that one over there which is about 500kg?.....
27. 7a smaller adult cow like that one over there?.....
28. Do you keep any veterinary drugs in stock?.....
29. How do you differentiate these bottles of drugs?.....
30. How much of the 5% formulation do you dispense for disease (X) to an adult animal?.....
31. How much of the 10% formulation do you dispense for the disease (X) to an adult animal?.....
32. How much of the 20% (L.A) formulation do you dispense for the disease (X) to an adult animal?.....
33. How much of the 5% formulation if it was a 6 months calf for the disease (X)?.....
34. How much of the 10% formulation if it was a 6 months calf for the same disease (X).....
35. How much of the 20% (L.A) formulation if it was a 6 months calf for the some disease (X)?.....
36. What criteria do you use to determine the time duration of keeping the drug?.....
37. Do you know the disadvantages of using already expired drugs? Yes/No.....
38. What are disadvantages?.....
(a) Loss in potency.....
(b) Toxicity due to degradation.....
(c) Economic loss due to the above two.....
(d) Others?.....
39. For how long do you keep this drug for use?.....

40. What criteria do you use to select you animals that are to be sent to the livestock market?.....
(a) Health ones only ( )
(b) Unhealthy only ( )
(c) A mixture of both health and unhealthy ( )
(d) Other selection procedures ( )

41. What about the sick ones, do you send them straight to the market for sale or do you treat them first until recovery and then send them to the market?.....
42. If you treat them first and they recover, how many days do you wait past the day of termination of treatment before you send them for sale?.....
43. If upon treatment an animal does not recover and death appears inevitable what do you do with such animal?.....
44. Have you ever heard about food safety?.....

45. What does it imply to you in terms of meat milk etc?.....
46. Have you ever heard about drug residues in foods of animal origin such as milk, meat etc? yes/ no.....
47. What are drug residues?.....
48. Do you think residues can be harmful to humans?.....

THANK YOU VERY MUCH FOR YOUR COOPERATION AND ASSISTANCE

Appendix II: Questionnaire for livestock field officers

I: IDENTITY

1. Identification No. .....
2. Name of officer .....
3. Sex .....
4. Region .....
5. District .....
6. Ward .....
7. Village .....
8. Date of graduating from livestock training .....
9. Professional qualification .....

II: PROFESSIONAL EXPERIENCE

10. How many years have you worked as a livestock officer? .....
11. What has been the nature of your work? .....
12. Have farmers seeking assistance for treatment or control of animal disease consulted you? Yes/No
13. For which diseases

(a) .....

(b) .....

(c) .....

(d) .....

14. During which season of the year does each of the diseases mentioned in Q. No. 13 above become prevalent (i.e. dry/rainy season). .....
15. Do you use the same drug to treat all the above diseases? .....
16. Which drug do you use for each of the above diseases? .....
17. How do you dispense the drugs (i.e. route of administration)? .....
18. How do you determine the amount or dosage for dispensation?.....
19. Is it the same about or dose for a large sized and small sized animal?.....

20. What about the young e.g. calf and adult cow, is the dose the same in both?.....
21. Do you have any stock of drugs for your routine prophylactic and treatment work?.....
22. How much of each vial do you administer for each of the diseases mentioned in Q. No. 13 above?.....
23. How much of each vial is administered to a cow of 200kgs, 300kgs, 5000kg i.e.

(a) 5%;200kgs ....mls;300kgs. .....mls;500kgs .....mls

(b) 10%;200kgs .....mls;350kgs .....mls;500kgs.....mls

(c) 20%;(i.e L.A)200kgs .....mls;350kgs .....mls;600kgs .....mls

24. If you have been treating a farmers animal and death appears inevitable what do
25. If a butcher operator's cow is sick before slaughter and receives treatments through your assistance until recovery, what advice would you give to him/her?

.....

26. If upon treatment, the animal does not recover what advice do you give to the butcher operator? .....
27. And for how long does the animal receive treatment before a verdict of non-recovery is reached? .....
28. If the verdict of non- recovery is reached, do you advise the butcher operator (meat trader) to do the following?

(a) To euthanise it and bury .....

(b) To wait for it to die and bury .....

(c) To slaughter it .....

(d) Other advise .....

29. If the advise is to slaughter, how long does it take i.e. how many days past the last drug administration elapse before actual slaughter occurs?.....
30. For how long do you keep and use your drug beyond the expiry period? .....
31. Of the several diseases which you mentioned in Question. No 13 above which are the 3 most prevalent and how often do you come across them? .....
32. What are you views on the intensity of use or importance of oxytetracyclines and penicillin in the treatment of animal disease? .....
33. Do you use drugs other than oxytetracycline to treat animals? Yes/No .....
34. Why?.....

35. Is there any sign of development of resistance by disease microbes against these two antibiotics?.....
36. Tanzania is a big country with more than 985,000km2 of land area and 15,000,000 cattle, more than 600 divisions, more than 5,000 wards and more than 10,000 registered villages. Most of them consist of a mixture of crop and livestock farmers. With such a huge land mass/area, substantial distances between villages and so few livestock workers; what are your views on the state of provision of veterinary and extension services in terms of adequacy and efficiency?.....
37. If the services inadequate and in efficient, what do you think should be done?.....
38. Do you have livestock farmers in your area who carry out treatment work, administering drugs on their own?.....
39. What is your opinion on their level or adequacy of their knowledge on the use of drugs? .....
40. Do you envisage any danger to the community by condoning such a practice.....
41. Have you heard about food safety? .....
42. Which aspects of food safety? .....
43. Have you heard about drug residues in animal food products? .....
44. How do they occur i.e how do they come into being? .....
45. What dangers or implications do they pose to the public .....
46. Suggest some ways of reducing or even mitigating /controlling /reducing them. .....

THANK YOU VERY MUCH FOR YOUR COOPERATION AND ASSISTANCE

Appendix III: Questionnaire for meat/ beef traders

I: IDENTITY

1. Identification No .....
2. Name .....
3. Sex .....
4. Occupation .....
5. Region .....
6. District (Town/Municipality .....
7. Name of butchers (s) .....
8. Education (primary/Secondary) .....
9. Any formal training in the meat business .....

II: EXPERIENCE IN THE BUTCHER BUSINESS

10. When did you start your butcher business? .....
11. What made you start this business? .....
12. How many cattle do you purchase per week? .....
13. How many butcher shops do you own .....
14. How many cattle do you slaughter daily? .....
15. From which source do you purchase your cows for slaughter? .....
16. At the livestock market, what type of cattle do you purchase .....
17. How do you ferry you cattle from the livestock market to the slaughter house? .....
18. For how long does an animal wait before being slaughtered? .....
19. Suppose an animal falls sick what do you do with it? .....
20. Who provides the treatment? .....
21. Which diseased do affect you animals more frequently? .....
22. Which drugs do you use for each of the above diseases? .....
23. How do you dispense the drugs (i.e. the route of administration)? .....
24. How do you determine the amount of drug dispensed to each animal? .....
25. How much do you dispense to a large animal like this/that one.....
26. How much do you dispense to small animal like this that calf?.....

27. After treatment, how long does the animal wait upon recovery before being slaughtered (i.e. how many days past the last treatment)?.....
28. Have you ever heard about food safety? Yes/No.....
29. Have you ever heard about drug residues in foods of animal origin?.....
30. What are they?.....
31. What is their implication to humans?.....

THANK YOU VERY MUCH FOR YOU COOPERATION AND ASSISTANCE

Appendix IV: Questionnaire for Veterinary Pharmaceutical Dealers

1. Identification No.....
2. Name of pharmacy:.....
3. Sex of owner:.....
4. Region:.....
5. District:.....
6. Location of pharmacy (Street).....
7. Qualification of Owner:.....
8. Qualification of manager:.....

PROFESSIONAL EXPERIENCE

9. For how long have you been in the veterinary pharmaceuntical business .....years
10. Which categories of customers do you often have for veterinary drugs?
    - 0.1 Veterinarians Yes/No ( )
    - 0.2 Livestock personnel Yes/No ( )
    - 0.3 Livestock keepers Yes/no ( )

11. Which group of the 3 categories above are the most frequent customers?.....

12. Roughly indicate the % of their customer ship i.e.
    - 0.1 10% ( )
    - 0.2 25% ( )
    - 0.3 50% ( )
    - 0.4 75% ( )
    - 0.5 100% ( )

13. If livestock keepers are some of your customers do they come with prescriptions from a veterinarians or livestock personnel?
    - 0.1 Yes ( )
    - 0.2 No ( )

14. If yes, roughly give the % of those who come with prescription
    - 0.1 10% ( )
    - 0.2 25% ( )
    - 0.3 50% ( )
    - 0.4 75% ( )
    - 0.5 100% ( )

15. In your opinion how knowledgeable do you think are the livestock keepers in the use of those drugs they purchase in dosage regimen, route of administration, withdrawal/withholding periods. (Adequate or inadequate, some idea.).

16. With cattle diseases do they mention to you when they come to purchase livestock drugs?
    (a) .....
    (b) .....

17. Which drugs do they purchase for those diseases?
    (a) .....
    (b) .....
    (c) .....

18. Who in your opinion administers the drugs they purchase for them?.....

19. Which 3 particular drugs are the most important to your livestock drug customers in descending order of importance
    (a) .....
    (b) .....
    (c) .....

20. On the basis of purchases made at your pharmacy what is the intensity of oxytetracychie use in the field?
    0.1 Very high ( )
    0.2 Moderate ( )
    0.3 Low ( )

21. Do you think your customer farmers are aware of the dangers of drug misuse to public health?
    0.1 Yes ( )
    0.2 No ( )

22. Briefly mention some of the problems facing the veterinary pharmaceutical industry in Tanzania
    (a) .....
    (b) .....
    (c) .....

23. Suggest some remedies to overcome them
    (a) .....
    (b) .....
    (c) .....

THANK YOU FOR YOUR ASSISTANCE AND COOPERATION

Appendix V: Questionnaire For Veterinarians

1. Identification No:.....
2. Name of veterinarian:.....
3. Designation (Vo, Private Vet, VRO).....
4. Sex:.....
5. Region:.....
6. District:.....
7. Location of workplace (street etc).....
8. Date of graduation as a veterinarian:.....

PROFESSIONAL EXPERIENCE

9. Professional experience in the livestock sector:.....
10. What has been the nature of your work?.....
11. From you experience what type of assitance have livestock farmers been seeking from you? .....

12. If the treatment of cattle diseases is inclusive, which diseases in descending order of importance have you encountered

(a)	.....	(f)	.....
(b)	.....	(g)	.....
(c)	.....	(h)	.....
(d)	.....	(i)	.....
(e)	.....	(j)	.....

13. For each of the disease above which corresponding treatments (drugs) or combination of drugs of your choice do you use as the best treatment for the syndromes above

Disease	Drug	Dosage and Route of administration
(a)	.....	.....
(b)	.....	.....
(c)	.....	.....
(d)	.....	.....
(e)	.....	.....
(f)	.....	.....
(g)	.....	.....
(h)	.....	.....
(i)	.....	.....
(j)	.....	.....

14. Of the several drugs used in the field mention 5 drugs which are the most used or important in descending order of their importance
    (a) .....
    (b) .....
    (c) .....

15. Is their use abused or violated in anyway?
    0.1 Yes ( )
    0.2 No ( )

16. If the answer is yes, which form of abuse on the basis of manufactures directions of their use?.....

17. Of the drugs mentioned above mention the 3 which are the most abused
    (a) .....
    (b .....
    (c) .....

18. Do you think the field staff (LFO's/AFO's) have adequate knowledge on disease diagnosis and the use of drugs?
    0.1 Yes ( )
    0.2 No ( )

19. In not what are the shortcomings on their knowledge and routine use of drugs especially those mentioned in Qus. 13 above?
    (a) .....
    (b) .....
    (c) .....

20. Tanzania is a big country with more than 985,000 km^2 of land area and 15,000,000 cattle, more than 600 divisions, more than 5,000 wards and more than 10,000 registered villages, Most of them consist of a mixture of crop and livestock farmers. With such a huge land mass/area, substantial distances between villages and so few livestock workers; what are your views on the state of provision of veterinary and extension services in terms of adequacy and efficiency?.....

21. If inadequate who provides most of the treatment to animals especially cattle in the field?.....

22. What measures do you suggest to overcome the inadequacy?.....

23. Do you have livestock farmers who treat their animals on their own
    0.1 Yes ( )
    0.2 No ( )

24. If the answer is yes, do they seek a prescription from veterinarians?
    0.1 Yes ( )
    0.2 No ( )

25. With all your sincerity how many drug prescriptions per week do you issue to farmers in oral doses inject able
    doses?.....

26. How knowledgeable are the farmers in the use of those drugs?
    0.1 Adequate ( )
    0.2 Some idea ( )
    0.3 Inadequate ( )

27. What in your sincere opinion and experience are the dosage rates often used by your field staff (L.FO's/AFO's) for oxytetracycline (OTC) in the treatment of the diseases above in the OTC formulations and Body weights below?
    0.1 10% OTC; 6-9 months calf _____ml; Adult cow (300 -350kg)_____ml; Bull (500kg)_____ml
    0.2 20% (LA) OTC; 6-9-month calf _____ml; Adult cow (300-350kg)_____ml; Bull (500kg)_____ml.

28. Mention the routes of admin they use for the two formulations?
    (a).....
    (b).....

29. Are the LFO's/AFO's aware of and do they advise farmers on the withdrawal/milk with hold periods?
    0.1 Yes ( )
    0.2 No ( )

30. What are the corresponding doses used by those livestock keepers who treat their animals themselves (self service)?
    0.1 10% OTC ( )
    0.2 20% (LA) OTC ( )

31. Mention the routes of administration they use for the two formulations
    (a) .....
    (b) .....

32. Are these farmers aware of and do they observe the withdrawal/withholding periods?
    0.1 Yes ( )
    0.2 No ( )

33. Are the field livestock personnel aware of the dangers of drug abuse such as non-observance of withdrawal periods to public health?
    0.1Yes ( )
    0.3 No ( )
34. Are the field livestock personnel aware of the dangers to public health of various drug and chemicals (Xenobiotics) residues?
    0.1Yes ( )
    0.3 No ( )

35. What about the self –service farmers are they aware of the dangers?
    0.1Yes ( )
    0.3 No ( )

36. Briefly in 3 lines suggest various measures, which could be pursued by all stakeholders of the livestock industry to reduce the dangers of drug abuse.

THANK YOU VERY MUCH FOR YOUR COOPERATION AND ASSISTANCE

Appendix VI: T-test for the mean OTC concentrations of Morogoro and Dodoma abattoir muscle samples

Abattoir	Number	Mean OTC residue conc^{n} ppm	T-test value
Morogoro	20	0.8773	0.868^{ns}
Dodoma	15	0.4302	

Results of t-test for the mean concentration of OTC residues in muscle tissue samples between Morogoro and Dodoma abattoirs.

Appendix VII: T-test for the mean OTC concentrations of Morogoro and Dodoma abattoir liver samples

Abattoir	Number	Mean OTC residue conc^{n} ppm	T-test value
Morogoro	25	1.5025	1.008^{ns}
Dodoma	20	0.8360	

The t-test results above for the mean concentration of OTC residues in liver tissue sample between Morogoro and Dodoma abattoirs.

Appendix VIII: T-test for the mean OTC concentrations of Morogoro abattoir kidney samples

Abattoir	Number	Mean OTC residue conc^{n} ppm	T-test value
Morogoro	26	1.5660	0.600^{ns}
Dodoma	25	1.1765	

The t-test results above for the mean concentration of OTC residues in kidney tissue between Morogoro and Dodoma abattoirs.

Appendix IX: A comparison of OTC residue occurence in all the 131 samples tested irrespective of the type of tissue

Variable	Dodoma		Morogoro		Total	
	n=25		n=26		n=51	
	n	%	n	%	n	%
Positive OTC test	24	40.0	30	42.3	54	41.2
Negative OTC test	36	60.0	41	57.7	77	58.8

$X^2 = 0.07^{ns}$, df – 1

Appendix X: Comparison of OTC residue occurrence in muscle, liver and kidney tissues irrespective of location (abattoir)

Variable	Tissue sample							
	Muscle		Liver		Kidney		Total	
	n=15		n=20		n=25		n=60	
	n	%	n	%	n	%	n	%
Positive OTC test	13	37.1	18	40.0	23	45.1	54	41.2
Negative OTC test	22	62.9	27	60.0	28	54.9	77	58.8

$X^2 = 0.58^{ns}$, df – 2

Appendix XI: A comparison of OTC residue occurrence in muscle, liver, and kidney tissues from Morogoro abattoir

Variable	Tissue sample							
	Muscle		Liver		Kidney		Total	
	n=15		n=20		n=25		n=60	
	n	%	n	%	n	%	n	%
Positive OTC test	9	45.0	9	36.0	12	46.2	30	42.3
Negative OTC test	11	55.0	16	64.0	14	53.8	41	67.7

$X^2 = 0.63^{ns}$, df – 2

Appendix XII: A comparison of OTC residue occurence in muscle, liver and kidney tissues from Dodoma abattoir

Variable	Tissue sample							
	Muscle		Liver		Kidney		Total	
	n=15		n=20		n=25		n=60	
	n	%	n	%	n	%	n	%
Positive OTC test	4	26.7	9	45.0	11	44.0	24	40.0
Negative OTC test	11	73.3	11	55.0	14	56.0	36	60.0

$X^2 = 1.49^{ns}$, df – 2

Appendix XIII: A comparison of OTC occurrence in muscle tissue sample between Morogoro and Dodoma abattoirs

Variable	Muscle tissue samples					
	Dodoma		Morogoro		Total	
	n=25		n=26		n=51	
	n	%	n	%	n	%
OTC negative test	4	26.7	9	45.0	13	37.1
OTC negative test	11	73.3	11	55.0	22	62.9

$X^2 = 1.23^{ns}$, df – 1

Appendix XIV: A comparison of OTC occurrence in liver tissues samples between Morogoro and Dodoma abattoirs

Variable	Liver tissue samples					
	Dodoma		Morogoro		Total	
	n=25		n=26		n=51	
	n	%	n	%	n	%
OTC positive test	9	45.0	9	36.0	18	40
OTC negative test	11	55.0	16	64.0	27	60

$X^2 = 0.38^{ns}$, df – 1

Appendix XV: A comparison of OTC occurrence in kidney samples between Morogoro and Dodoma abattoirs

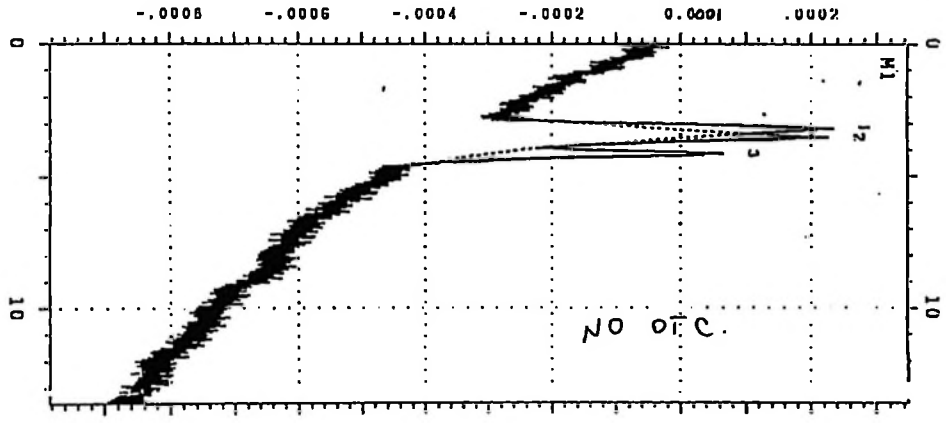
Variable	Kidney tissue samples					
Variable	Dodoma		Morogoro		Total	
	n=25		n=26		n=51	
	n	%	n	%	n	%
Positive OTC test	11	44.0	12	46.2	23	45.1
Negative OTC test	14	56.0	14	53.8	28	54.5

$X^2 = 0.02^{ns}$, df – 1

Appendices XVI: Sample chromatograms

CRYSTAL 240	< I N T E G R A T O R >			Stop at	13.56 min
Date	: Aug/05/03 09:23:50		FILE NAME	: OTC-RESEARCH	
Wavelength	: M1		Point	: 0	
Time range	: 0.00 --- 13.56 min		Smoothing	: 7	points
Interval	: NORMAL		Minimum area	: 1.0E-05	AU*min
Time double	: 30 min		Slope	: 1.0E-04	AU/min
Minus peak	: OFF		Drift	: 0.01	AU/min
Paper speed	: 5.0 mm/min		Height	: 1.0E-05	AU
Baseline correct	: OFF		Width	: 0.01	min
Sample name	: BLANK SPD		Packing material	: LICROSORB RP8 -10	
Column	: 4.6mmID* 25.0cm		Mobile phase	: MEOH/AC-N+0.01M OX AC	
Flow rate	: 2.00 ml/min		Pressure	: 150.0	PSI
Temperature	: 25.0 °C		Injection volume	: 20	µl
Flow cell	: 10.00 mm				

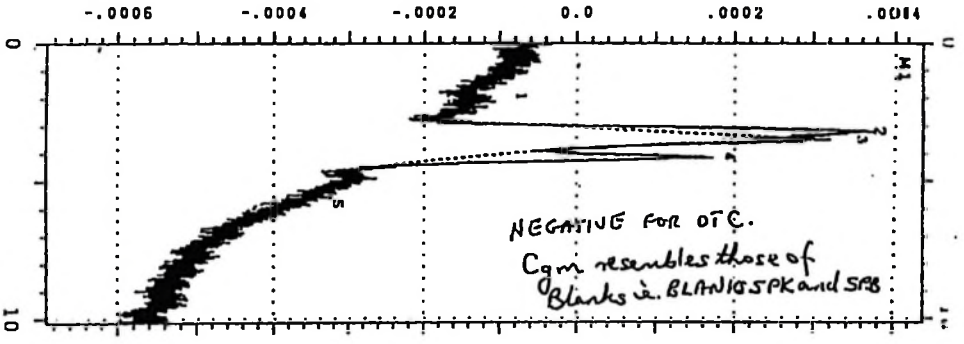
-9.83E-04 --- 3.46E-04 AU



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	3.17	0.00027	2.73	3.38	6.576E-05	32.1	I
2	3.52	0.00021	3.38	3.92	4.015E-05	19.6	I
3	4.13	0.00036	3.92	4.68	9.924E-05	48.4	I

CRYSTAL 240	< I N T E G R A T O R >			Stop at	10.15 min
Date	: Aug/05/03 09:40:49		FILE NAME	: OTC-RESEARCH	
Wavelength	: 1		Point	: 0	
Time range	: 0.00 --- 10.15	min	Smoothing	: 7	points
Interval	: NORMAL		Minimum area	: 1.0E-05	AU*min
Time double	: 30	min	Slope	: 1.0E-04	AU/min
Minus peak	: OFF		Drift	: 0.01	AU/min
Paper speed	: 5.0	mm/min	Height	: 1.0E-05	AU
Baseline correct	: OFF		Width	: 0.01	min
Sample name	: B16M		Packing material	: LICROSORB RP8 -10	
Column	: 4.6mmID* 25.0cm		Mobile phase	: MEOH/AC-Neb.01M (P'	
Flow rate	: 2.00	ml/min	Pressure	: 150.0	PSI
Temperature	: 25.0	°C	Injection volume	: 20	µl
Flow cell	: 10.00	mm			

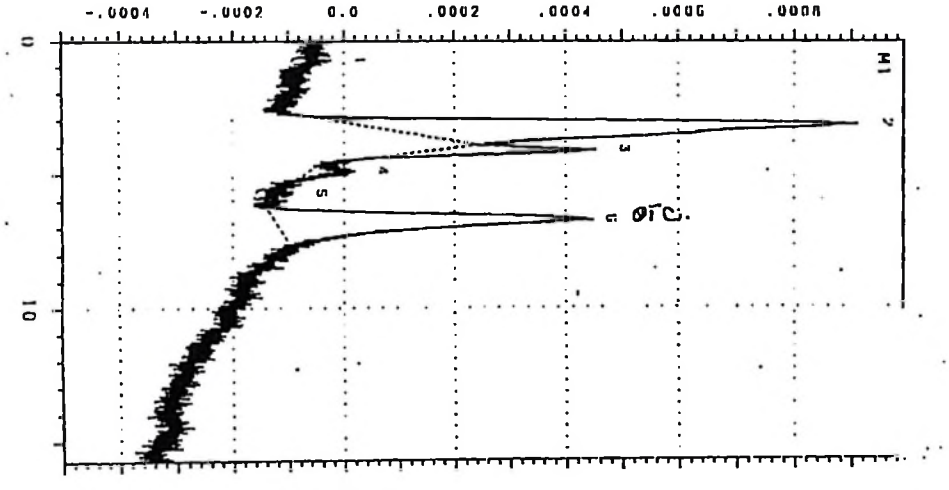
-6.9E-04 --- 4.4E-04 AU



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	1.94	0.00006	1.80	2.24	1.172E-05	5.9	I
2	3.19	0.00025	2.74	3.42	7.044E-05	35.7	I
3	3.49	0.00011	3.42	3.92	1.922E-05	9.7	I
4	4.13	0.00029	3.92	4.66	8.404E-05	42.6	I
5	5.82	0.00006	5.77	6.12	1.189E-05	6.0	I

CRYSTAL 240	< I N T E G R A T O R >		Stop at 15.71 min
Date	: Aug/05/03 09:53:19	FILE NAME	: OTC-RESEARCH
Wavelength	: MI	Point	: 0
Time range	: 0.00 --- 15.71 min	Smoothing	: 7 points
Interval	: NORMAL	Minimum area	: 1.0E-05 AU*min
Time double	: 30 min	Slope	: 1.0E-04 AU/min
Minus peak	: OFF	Drift	: 0.01 AU/min
Paper speed	: 5.0 mm/min	Height	: 1.0E-05 AU
Baseline correct	: OFF	Width	: 0.01 min
Sample name	: D17M	Packing material	: LICROSORB RP8 -10
Column	: 4.6mmID* 25.0cm	Mobile phase	: MEOH/AC-H+0.01M OX A
Flow rate	: 2.00 ml/min	Pressure	: 150.0 PSI
Temperature	: 25.0 °C	Injection volume	: 20 µl
Flow cell	: 10.00 mm		

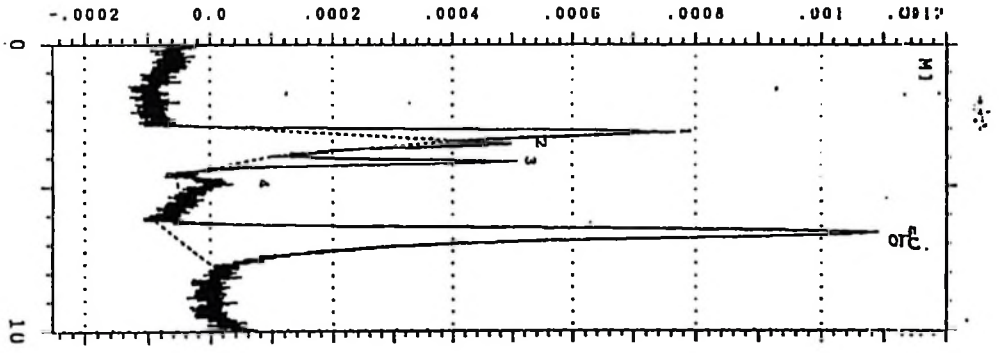
-5E-04 --- 9.9E-04 AU



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	0.73	0.00007	0.00	0.98	1.213E-05	1.3	I
2	3.18	0.00089	2.00	3.93	4.721E-04	50.9	I
3	4.12	0.00028	3.93	4.67	7.131E-05	7.7	I
4	4.88	0.00008	4.67	5.30	1.940E-05	2.1	I
5	5.71	0.00007	5.30	5.90	1.154E-05	1.2	I
6	7.23	0.00058	6.77	7.77	3.402E-04	36.7	I

CRYSTAL 240	< I N T E G R A T O R >		Stop at	10.05	min
Date	: Aug/04/03 13:54:16	FILE NAME	: OTC-RESEARCH		
Wavelength	: 1	Point	: 0		
Time range	: 0.00 --- 10.05 min	Smoothing	: 7	points	
Interval	: NORMAL	Minimum area	: 1.0E-05	AU*min	
Time double	: 30 min	Slope	: 1.0E-04	AU/min	
Minus peak	: OFF	Drift	: 0.01	AU/min	
Paper speed	: 5.0 mm/min	Height	: 1.0E-05	AU	
Baseline correct	: OFF	Width	: 0.01	min	
Sample name	: B15M	Packing material	: LICROSORB RP8 -10		
Column	: 4.6mmID* 25.0cm	Mobile phase	: MEOH/AC-N*0.01M OX A		
Flow rate	: 2.00 ml/min	Pressure	: 150.0	PSI	
Temperature	: 25.0 °C	Injection volume	: 20	µl	
Flow cell	: 10.00 mm				

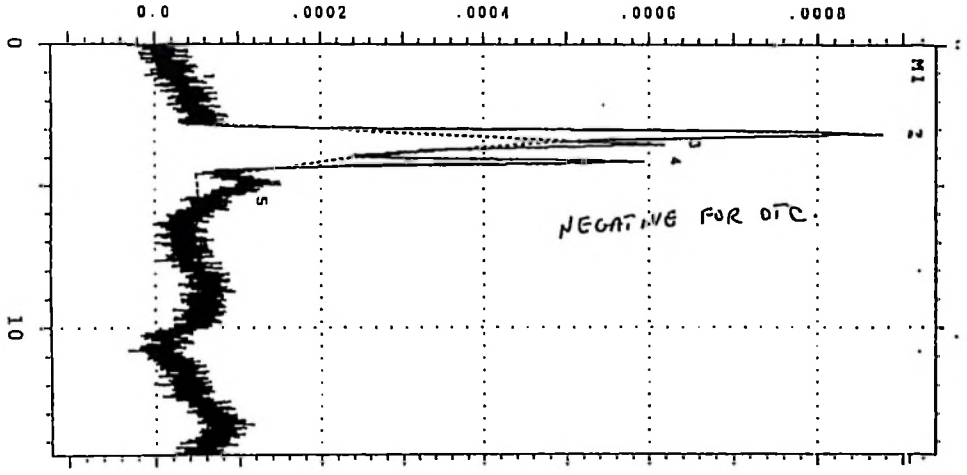
-2.51E-04 --- 0.0012 AU



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	3.13	0.00056	2.78	3.42	1.625E-04	16.9	I
2	3.53	0.00016	3.42	3.94	2.090E-05	2.2	I
3	4.14	0.00043	3.94	4.59	9.519E-05	9.9	I
4	4.90	0.00009	4.59	5.17	2.386E-05	2.5	I
5	6.61	0.00116	6.01	7.79	6.620E-04	68.6	I

CRYSTAL 240	< I N T E G R A T O R >		Stop at 14.46 min
Date	: Aug/04/03 12:51:43	FILE NAME	: OTC-RESEARCH
Wavelength	: M1	Point	: 0
Time range	: 0.00 --- 14.46 min	Smoothing	: 7 points
Interval	: NORMAL	Minimum area	: 1.0E-05 AU*min
Time double	: 30 min	Slope	: 1.0E-04 AU/min
Minus peak	: OFF	Drift	: 0.01 AU/min
Paper speed	: 5.0 mm/min	Height	: 1.0E-05 AU
Baseline correct	: OFF	Width	: 0.01 min
Sample name	: B11M	Packing material	: LICROSORB RP8 -10
Column	: 4.6mmID* 25.0cm	Mobile phase	: MEOH/AC-N*U.01M OX AC
Flow rate	: 2.00 ml/min	Pressure	: 150.0 PSI
Temperature	: 25.0 °C	Injection volume	: 20 µl
Flow cell	: 10.00 mm		

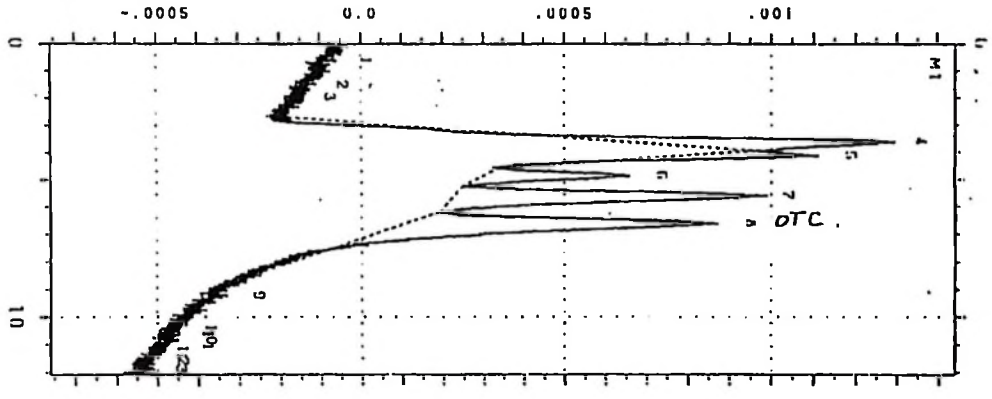
-1.2E-04 --- 9.39E-04 AU



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	1.16	0.00002	0.52	1.42	1.433E-05	4.8	I
2	3.15	0.00055	2.77	3.40	1.502E-04	50.2	I
3	3.53	0.00017	3.40	3.96	2.152E-05	7.2	I
4	4.14	0.00039	3.96	4.58	6.959E-05	23.3	I
5	5.60	0.00004	4.60	5.72	4.347E-05	14.5	I

CRYSTAL 240	< I N T E G R A T O R >		Stop at 12.05 min
Date	: Aug/04/03 10:08:01	FILE NAME	: OTC-RESEARCH
Wavelength	: Ml	Point	: 0
Time range	: 0.00 12.05 min	Smoothing	: 7 pnt.
Interval	: NORMAL	Minimum area	: 1.0E-06 AU*min
Time double	: 30 min	Slope	: 1.0E-04 AU/min
Minus peak	: OFF	Drift	: 0.01 AU/min
Paper speed	: 5.0 mm/min	Height	: 1.0E-05 AU
Baseline correct	: OFF	Width	: 0.01 min
Sample name	: STD OTC SPK 1.25PPM	Packing material	: LICROSORB RP8 -10
Column	: 4.6mmID* 25.0cm	Mobile phase	: MEOH/AC-H+0.01M OX AC
Flow rate	: 2.00 ml/min	Pressure	: 150.0 PSI
Temperature	: 25.0 °C	Injection volume	: 20 µl
Flow cell	: 10.00 mm		

-7.59E-04 --- 0.00144 AU

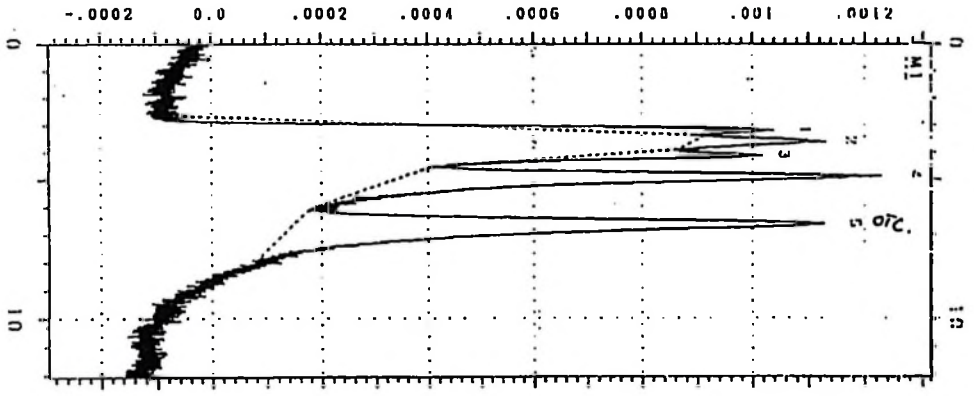


No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	0.63	0.00004	0.53	0.70	2.312E-06	0.2	I
2	1.51	0.00004	1.44	1.59	3.461E-06	0.3	I
3	1.98	0.00003	1.91	2.14	5.220E-06	0.5	I
4	3.59	0.00064	2.68	3.94	1.570E-04	14.7	I
5	4.11	0.00031	3.94	4.57	5.755E-05	5.4	I
6	4.87	0.00036	4.57	5.23	1.117E-04	10.4	I
7	5.58	0.00074	5.23	6.19	3.196E-04	29.9	I
8	6.61	0.00077	6.19	7.95	3.899E-04	36.4	I
9	9.21	0.00006	9.13	9.50	4.134E-06	0.4	I
10	10.64	0.00005	10.59	10.74	4.009E-06	0.4	I
11	10.86	0.00005	10.74	11.07	4.510E-06	0.4	I
12	11.40	0.00002	11.32	11.61	4.426E-06	0.4	I
13	11.68	0.00004	11.61	11.81	5.683E-06	0.5	I

CRYSTAL 240 < I N T E G R A T O R > Stop At 12.05 min

Date	: Aug/05/03 14:08:48	FILE NAME	: 01 -RESEARCH
Wavelength	: 81	Point	: 0
Time range	: 0.00 --- 12.05 min	Smoothing	: 7 points
Interval	: NORMAL	Minimum area	: 1.0E-05 AU*min
Time double	: 30 min	Slope	: 1.0E-04 AU/min
Minus peak	: OFF	Drift	: 0.01 AU/min
Paper speed	: 5.0 mm/min	Height	: 1.0E-05 AU
Baseline correct	: OFF	Width	: 0.01 min
Sampl[illegible]	: L18M	Peaking [illegible]	: [illegible]ROSORP RPS -10
Column	: 4.6mmID 25.0cm	Mobile phase	: MEOH/AC-N+0.01M OX AC
Flow rate	: 2.00 ml/min	Pressure	: 150.0 PSI
Temperature	: 25.0 °C	Injection volume	: 20 µl
Flow cell	: 10.00 mm		

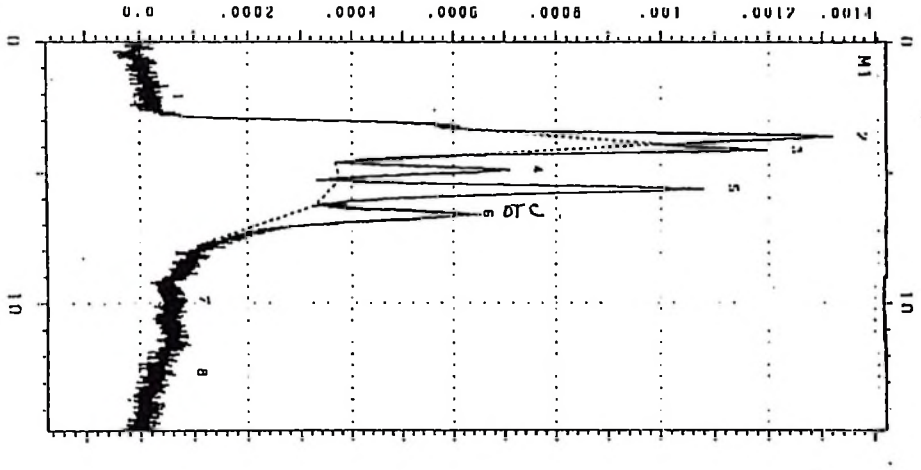
-2.93E-04 --- 0.00132 AU



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	3.18	0.00039	2.62	3.38	4.821E-05	4.0	I
2	3.59	0.00023	3.38	3.92	5.964E-05	4.9	I
3	4.12	0.00029	3.92	4.53	7.298E-05	6.0	I
4	4.87	0.00087	4.53	6.07	4.230E-04	34.8	I
5	6.61	0.00098	6.07	7.97	6.115E-04	50.3	I

CRYSTAL 340	< I N T E G R A T O R >			Stop at	14.76 min
Date	: Aug/05/03 13:33:02	FILE NAME	: OTC-RESEARCH		
Wavelength	: 1	Point	: 0		
Time range	: 0.00 --- 14.76 min	Smoothing	: 7		point
Interval	: NORMAL	Minimum area	: 1.0E-05		AU*min
Time double	: 30 min	Slope	: 1.0E-04		AU/min
Minus peak	: OFF	Drift	: 0.01		AU/min
Paper speed	: 5.0 mm/min	Height	: 1.0E-05		AU
baseline correct	: Orr	Width	: 0.01		
Sample name	: STD OTC SPK 0.625PPM	Packing material	: LICROSORB RP8 -10		
Column	: 4.6mmID* 25.0cm	Mobile phase	: MEOH/AC-N=0.01M OX AC		
Flow rate	: 2.00 ml/min	Pressure	: 150.0		PSI
Temperature	: 25.0 °C	Injection volume	: 20		ul
Flow cell	: 10.00 mm				

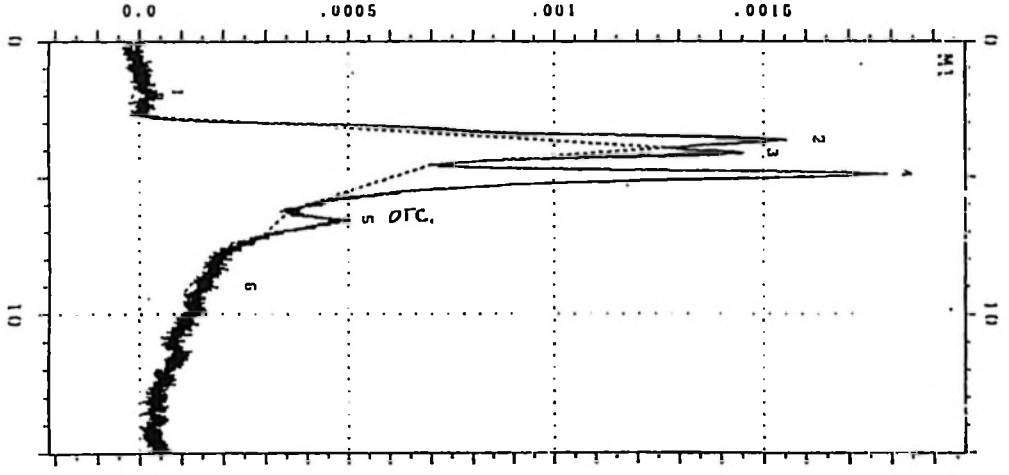
-1.7E-04 --- 0.00142 AU



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	2.09	0.00003	1.96	2.43	1.248E-05	1.5	I
2	3.58	0.00052	3.24	3.89	1.549E-04	18.3	I
3	4.11	0.00039	3.89	4.58	8.196E-05	9.7	I
4	4.87	0.00034	4.58	5.25	1.042E-04	12.3	I
5	5.62	0.00072	5.25	6.27	2.858E-04	33.8	I
OTCピーク6	6.60	0.00033	6.27	7.79	1.850E-04	21.9	I
7	9.88	0.00002	9.71	10.15	1.010E-05	1.2	I
8	12.62	0.00006	12.45	12.74	1.038E-05	1.2	I

CRYSTAL 240	< I N T E G R A T O R >		Stop at 15.01 min
Date : Aug/05/03 13:50:54	FILE NAME : OTC-RESEARCH		
Wavelength : Ml	Point : 0		
Time range : 0.00 --- 15.01 min	Smoothing : 7		points
Interval : NORMAL	Minimum area : 1.0E-05		AU*min
Time double : 30 min	Slope : 1.0E-04		AU/min
Minus peak : OFF	Drift : 0.01		AU/min
Paper speed : 5.0 mm/min	Height : 1.0E-05		AU
Baseline correct : OFF	Width : 0.01		min
Sample name : L17M	Packing material : LICROSORB RP8 -10		
Column : 4.6mmID* 25.0cm	Mobile phase : MEOH/AC-H+0.01M TX AC		
Flow rate : 2.00 ml/min	Pressure : 150.0		PSI
Temperature : 25.0 °C	Injection volume : 20		. ul
Flow cell : 10.00 mm			

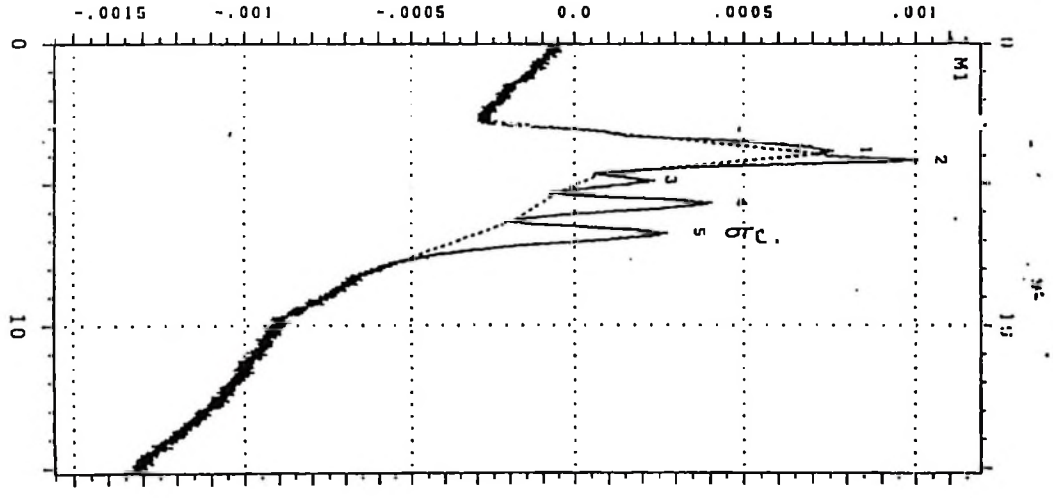
-2.15E-04 --- 0.00198 AU



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	1.90	0.00003	1.69	2.28	1.977E-05	2.0	I
2	3.63	0.00059	2.68	3.93	2.540E-04	25.5	I
3	4.13	0.00036	3.93	4.54	8.885E-05	8.9	I
4	4.90	0.00113	4.54	6.19	5.554E-04	55.8	I
OTC→ 5	6.56	0.00014	6.19	7.30	6.337E-05	6.4	I
6	9.02	0.00007	8.86	9.27	1.456E-05	1.5	I

CRYSTAL 240	< I N T E G R A T O R >			Stop at	15.16 min
Date	: Aug/05/03 08:51:54	FILE NAME	: OTC-RESEARCH		
Wavelength	: M1	Point	: 0		
Time range	: 0.00 --- 15.16 min	Smoothing	: 7		points
Interval	: NORMAL	Minimum area	: 1.0E-05		AU*min
Time double	: 30 min	Slope	: 1.0E-04		AU/min
Minus peak	: OFF	Drift	: 0.01		AU/min
Paper speed	: 5.0 mm/min	Height	: 1.0E-05		AU
Baseline correct	: OFF	Width	: 0.01		min
Sample name	: STD OTC SPK 1.25PPM	Packing material	: LICROSORB RP8 -10		
Column	: 4.6mmID* 25.0cm	Mobile phase	: MEOH/AC-N*0.01M OX AC		
Flow rate	: 2.00 ml/min	Pressure	: 150.0		PSI
Temperature	: 25.0 °C	Injection volume	: 20		µl
Flow cell	: 10.00 mm				

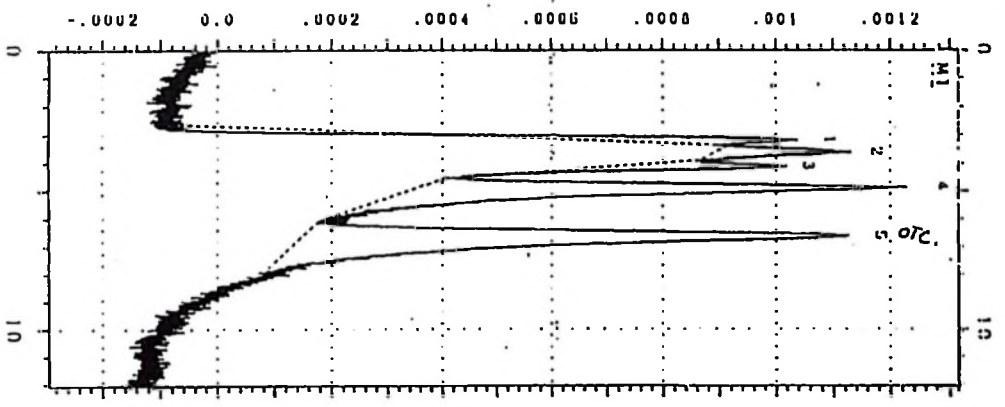
-0.00156 --- 0.0012 AU



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	3.82	0.00014	2.70	3.94	5.698E-05	6.9	I
2	4.16	0.00045	3.94	4.63	1.178E-04	14.3	I
3	4.88	0.00016	4.63	5.31	6.111E-05	7.4	I
4	5.70	0.00050	5.31	6.32	2.404E-04	29.1	I
OTC ピーク	6.75	0.00057	6.32	8.40	3.497E-04	42.3	I

CRYSTAL 240	< I N T E G R A T O R >		Stop at 12.05 min
Date	: Aug/05/03 14:08:48	FILE NAME	: OTC-RESEARCH
Wavelength	: M1	Point	: 0
Time range	: 0.00 --- 12.05 min	Smoothing	: 7 points
Interval	: NORMAL	Minimum area	: 1.0E-05 AU*min
Time double	: 30 min	Slope	: 1.3E-04 AU/min
Minus peak	: OFF	Drift	: 0.01 AU/min
Paper speed	: 5.0 mm/min	Height	: 1.0E-05 AU
Baseline correct	: OFF	Width	: 0.01 min
Sampl[illegible]	: 118M	Packing material	: [ICROSORB RP8 -10.
Column	: 4.6mmID 25.0cm	Mobile phase	: MEOH/AC-N*0.01M OX AC
Flow rate	: 2.00 ml/min	Pressure	: 150.0 PSI
Temperature	: 25.0 °C	Injection volume	: 20 µl
Flow cell	: 10.00 mm		

-2.93E-04 --- 0.00132 AU



No.	Retention [Min]	Height [AU]	Left [Min]	Right [Min]	Area [AU*Min]	Area [%]	Mark
1	3.18	0.00039	2.62	3.38	4.821E-05	4.0	I
2	3.59	0.00023	3.38	3.92	5.964E-05	4.9	I
3	4.12	0.00029	3.92	4.53	7.298E-05	6.0	I
4	4.87	0.00087	4.53	6.07	4.230E-04	34.8	I
5	6.61	0.00098	6.07	7.97	6.115E-04	50.3	I