KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA

COLLEGE OF SCIENCE

DETERMINATION OF ANTIBIOTICS RESIDUES IN BEEF AND MUTTON FROM SOME SELECTED MARKETS IN KUMASI –GHANA

BY

DANIEL AWENELA ABAVELIM (BSC. CHEMISTRY)

A THESIS SUBMITTED TO THE DEPARTMENT OF CHEMISTRY,

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE

OF

MASTER OF PHILOSOPHY (MPHIL) IN ANALYTICAL CHEMISTRY

NOVEMBER, 2014

DECLARATION

I hereby declare that this submission is my own work towards the MPhil and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

…… ……

ABAVELIM DANIEL AWENELA Date

(PG8040912)

STUDENT

Certified by

…… ……..

Mr. Edward Ebow Kwaansa – Ansah Date

(Supervisor)

Certified by:

…… ……

Dr. Godfred Darko Date

(Head of Department)

DEDICATION

This project is dedicated to my Beloved father MR. GEORGE AJUPUING ABAVELIM. You are a blessing to me.

ACKNOWLEDGEMENTS

To God be the glory, great things has He done. God has been merciful and gracious unto me throughout my life and I am forever grateful unto Him. My profound gratitude goes to my supervisor Mr. E. E. Kwaansa-Ansah as he was cooperative and supportive to bring this work to completion. I would want to thank Mr. Francis Opoku for his contribution. I am indebted to my family especially my father Mr. George Ajupuing Abavelim, my mother Stephany Damanira Abavelim and brothers.I will love to express my utmost gratitude to my friends and my love ones. I am particularly grateful to all catholics, the Pope, the Bishops, the Priests etcetera on campus and elsewhere for their prayer, support, love, and spiritual food given to me during my study.

ABSTRACT

A study was conducted between October 2013 and January 2014 to determine antibiotics residues levels in beef and mutton from some selected markets in Kumasi-Ghana. A total of 60 samples comprising beef and mutton were bought using the random and systematic sampling methods.

Antibiotics are substances either produced naturally by living organisms or synthetically in the laboratory, and they are able to kill or inhibit the growth of micro-organisms. Antibiotics are also used as feed additives for the purpose of livestock health maintenance. Antibiotics residues in feedstuffs (beef and mutton) are currently a problem of some magnitude in different parts of the world (Ghana), particularly due to associated public health concerns that include hypersensitivity reactions, antibiotic resistance, toxicity, teratogenicity, mutagenicity, reproductive disorder and carcinogenicity.

In Ghana as in other parts of the world, antibiotic residues in animal-derived foods have been extensively recorded, these residues have exceeded the World Health Organization (WHO) maximum residues limits in most African countries. Tetracyclines are the most predominantly prescribed antibiotics in Africa and for that matter Ghana followed by β-Lactams.

The mean concentrations for chloramphenicol in the beef and mutton for the three markets Central, Asafo, and Central Abattoir were 217.92 mg/kg, 213.19 mg/kg and 164.36 mg/kg respectively for beef and that for mutton were 259.63 mg/kg, 154.16 mg/kg, and 270.22 mg/kg respectively for the markets above. In all 30 beef and mutton samples were bought and for beef 24 samples had detectable chloramphenicol levels (80%) and for mutton 25 samples (83%) had detectable chloramphenicol levels.

The mean concentrations for Oxytetracycline in the beef and mutton for the three markets mentioned above were 86.18 mg/kg, 87.17 mg/kg, and 480.25 mg/kg respectively for the three markets mentioned earlier and that for mutton were 181.13 mg/kg, 239.70 mg/kg, and 105.08 mg/kg respectively for the three markets. Out of 30 samples each for beef and mutton, 15 samples (50%) had detectable Oxytetracycline residues for both beef and mutton. Penicillin G was not detected in any of the samples.

The risk assessment analysis performed on the samples had risk quotients less than 1 (RQ<1) indicating that the consumption of such meat may pose no danger or harm to its consumers for all the samples except Oxytetracycline in beef from the Central Abattoir Market with a RQ of 1.04 indicating that there may be harm in consuming such meat.

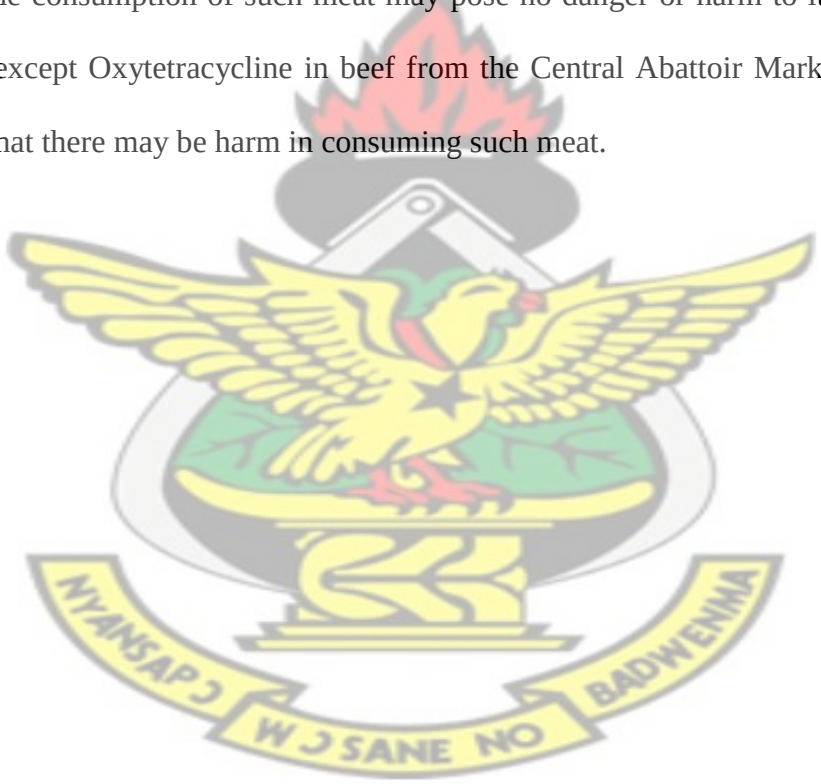


TABLE OF CONTENTS

TITLE PAGE

LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURES

FIGURE **PAGE**

CHAPTER ONE

1.0 INTRODUCTION

Antibiotics are substances either produced naturally by living organisms or produced synthetically in the laboratory, and they are able to kill or inhibit the growth of microorganisms. Antibiotics are drugs used in human health care and in veterinary practice to treat bacterial infections. Antibiotic residues are small amounts of drugs or their active metabolites which remain in meat or milk after treating the animal (CAC, 1998).

Concerns about food safety especially, with animal source food are increasing in developing countries where urbanization, increasing incomes and changing life-styles are associated with greater dependence on marketed foods by an increasing number of people (Delgado et al., 1999).

The safety of human food is threatened by various agents including pathogenic microorganisms, aflatoxins, pesticides, and antimicrobial agents. Pathogenic microorganisms constitute the most important food related threat to public health (Moat, 1988). Relatively, little is known about food safety in relation to antimicrobial agents, in the developing world. While pasteurization and other forms of heat treatment eliminate pathogenic microorganisms from animal source food, these procedures have limited or variable effects on drug residues in animal source food.

Various antibiotics used in the treatment of animal diseases have been shown to occur in animal products used as human food (Wassenaar, 2005), and are usually attributed to non-observance of withdrawal periods before sale of animal source food (Roundant and Moreitain, 1990; Shitandi, 2004). Additionally, the drugs may be introduced through the use of antibiotics in animals for therapeutic and growth purposes (Wassenaar, 2005).

Behavioral practices such as overuse of drugs and lack of understanding about drug usage also contribute to food contamination. The presence of antibiotics in human food is associated with several adverse public health effects including hypersensitivity, tissue damage, gastrointestinal disturbance, and neurological disorders (Lee et al., 2000; Wassenaar, 2005). Additionally, the use of antibiotics in animal husbandry and its occurrence in related food, may lead to selection of resistance in bacterial populations that do not respond to treatment commonly used for human illnesses (Lee et al., 2000).

Reported occurrences of antibiotics in human food vary widely among various countries and are known to be low or non-existent in places where quality assurance programmes are effective (Aning et al., 2007; EC, 2005; Henzelin et al., 2007; Kang'ethe et al., 2005; Kurwijila et al., 2006). Such programmes include mainly educational programmes, widespread testing of foods for antibiotic residues, and financial penalties. Implementation of quality assurance programmes to protect public health against adverse effects of antibiotics is a major challenge for developing countries where there is veterinary misuse of such drugs, and sales of animal source food are primarily informal.

Antibiotics can be also classified according to their effects as either bactericidal or bacteriostatic or depending on the range of bacterial species against which they are active and also according to their range of efficacy as broad or narrow in spectrum. The former fall into four categories: damage to cell membrane function, inhibition of protein synthesis, inhibition of cell wall synthesis, and inhibition of nucleic acid synthesis or function. The use of antibiotics in animals shortly followed their use in humans for the purpose of disease prevention and treatment (Gustafson, 1993).

Presently, antimicrobial drugs are used to control, prevent, and treat infection and to enhance animal growth and feed efficiency (Tollefson, and Miller, 2000). Currently, approximately 80% of all food-producing animals receive medication for part or most of their lives. The most commonly used antimicrobials in food producing animals are the β-lactams, tetracyclines, aminoglycosides, lincosamides, macrolides, pleuromutilins, quinolones, chloramphenicol and sulphonamides (Lee et al., 2001).

The use of antibiotics in food-producing animals may leave residues in foodstuffs of animal origin like meat, milk, and eggs. The occurrence of these residues may be due to any one of the following: a failure to observe the withdrawal periods of each drug, extra-label dosages for animals, contamination of animal feed with the excreta of treated animals, or the use of unlicensed antibiotics (Paige, 1994).

Antibiotic residues in foods of animal origin may be the cause of numerous health concerns in humans. These problems include toxic effects, transfer of antibiotic resistant bacteria to humans, immunopathological effects, carcinogenicity (e.g., sulphamethazine, oxytetracycline, and furazolidone), mutagenicity, nephropathy (e.g., gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity (e.g., chloramphenicol), estrogenic, neurotoxicological effects, teratogenicity, and allergy (e.g., penicillin) (Nisha, 2008).

Tetracyclines are antibiotics with broad antibacterial spectrums and bacteriostatic activity against both Gram-positive and Gram-negative bacteria as well as intracellular Mycoplasma, Rickettsia and Chlamydia (Botsoglou and Fletouris, 2001; Cinquina et al., 2003; Oh, and Han, 2006). These antibiotics are widely used in animal husbandry. In food-producing animals, tetracyclines may be administrated orally in food or drinking water, parenterally, or through the intramammary

infusion. Due to the enterohepatic circulation, tetracycline antibiotic (TC) residues may persist in the body long after the administration. The levels of tetracycline residues in animal products depend on the initial dosage and the duration between the drug administration and animal product collection. This time frame is called the withdrawal or washout period (Botsoglou and Fletouris, 2001).

Antibiotic residues, such as residues of other drugs, can remain in an animal's body even after the slaughtering if the antibiotic withdrawal period is insufficient (Cinquina et al., 2003). Antibiotic residues in foods can influence the bacterial composition and the metabolic activity of the intestinal micro flora of the consumer as well as the consumer's metabolism of endogenous compounds (Navratilova et al., 2009). Tetracycline residues in meat may stain the teeth of young children (Navratilova et al., 2009).

The presence of antibiotic residues in meat, milk and other food products may drive the development of resistant strains of bacteria due to the ingestion of sub therapeutic doses of antibiotics (Dayan, 1993; Mateu and Martin, 2001; Teale, 2002; Wilson et al., 2003; Hardman and Limbird, 2007). To ensure the human food safety, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have set standards for acceptable daily intake (ADI) and maximum residue limits (MRLs) in foods. Additionally, the European Union (EU), the United States of America (USA), Canada and some other countries have set their own MRLs (Cinquina et al., 2003). The acceptable MRLs for tetracycline residues (individually or in combination), as recommended by the Joint FAO/WHO Expert Committee on Food Additives, are 200, 600 and 1200 ng/g for muscles, liver and kidney, respectively (WHO, 1999).

Oxytetracycline residue was found to be present in levels higher than those tolerated by the EU and FDA in seven of ten cured meat samples from Turkey analyzed through an HPLC method (Senyuva et al., 2000). In 2001, 45.6% of meat samples from Nairobi slaughterhouses had detectable tetracycline residues and 20% of them had residue levels above the WHO standard (Muriuki et al., 2001). Dipleolo (2002) reported 15.6% positive tetracycline residues in goat meat samples from two states in Nigeria. In a study carried out in 2006 in Hanoi, 5.5% of all meat samples were positive for tetracycline residues and the MRLs were exceeded in 0.69% of the samples (Nhiem et al., 2006). Studies in Kuwait showed that none of the tested meat samples had tetracycline residues above the acceptable limits (Al-Mazeedi et al., 2010). Additionally, 21.7% of all samples and 5% of kidney and liver samples from slaughterhouses in Tabriz, (Iran) contained tetracycline residues above the MRLs set by the WHO (Mesgari et al., 2009). In addition, Ehsani et al (2010) reported the results of a study on broiler meats in Ahvaz, Iran. They showed that 60% of the samples were contaminated and tetracycline residue was significantly higher than European legal concentration (100 ug/Kg) in 10% of samples.

The aim of anti microbiological therapy is to rapidly produce and then maintain an effective concentration of drug at the site of infection for sufficient time to allow host specific and nonspecific defenses to eradicate the pathogen (Benet and Belleemain, 2005). Antibiotics are administered to animals by injections (intravenously, intramuscularly, or subcutaneously), orally in food or water, topically (on the skin) and by intramammary and intrauterine infusions (Bigby et al., 1986). Theoretically, all these routes may lead to residues appearing in foods of animal origin such as milk, meat and eggs (CEC, 2005).

Acquisition of resistance to antimicrobial agents by consuming food of animal origin has been receiving increasing attention in the literature, also raising awareness of the importance of minimizing exposure to antibiotic residues in food (Delgado et al., 1999; CEC, 2005). The most common causes for the presence of antibiotic residues in food of animal origin are violation of withdrawal periods, overdosing of antibiotics and use of antibiotics banned for treatment of economic animals (Lee et al., 2000; Kang'ethe et al., 2005; Koenen-Dierick et al., 1995; Kurwijila et al., 2006). Microbiological, immuno-enzymatic and chemical methods are used for detection of antibiotic residues in food of animal origin and the protocol of control is usually based on two steps: screening for presence of different antibiotic groups and confirmation with identification of specific antibiotic in the sample and more accurate quantitative analysis. An ideal screening method would detect all antibiotics at or below their MRLs and should be robust, rapid, simple and cost effective (MacNeil, 2005).

Antibiotics used as growth promoters are administered at low doses for extended periods. As prophylactics, antibiotics are used at low doses to prevent disease. Although the duration of antibiotic use differs for growth promotion and prophylaxis, the dosage for both is typically less than 200 g/ton, and is considered subtherapeutic.

The therapeutic regimen is dictated by label instructions by the manufacturer or in accordance with extra-label instructions by a veterinarian. The use of antibiotics therapy to treat and prevent udder infections in cattle is a key component of mastitis control in many countries. The concerns arise mainly from the possibility that antibiotic -resistant bacteria may be transferred from animals to humans, through contact, through the environment (e.g., water, manure, air) or through contaminated meat products (CAC, 1998).

Residues are illegal and meat supplies containing detectable concentrations are not acceptable. Many antibiotics used in animal husbandry are poorly absorbed in the animal gut. It is estimated that 25% to 75% of the antibiotics administered to feedlot animals could be excreted unaltered in faeces (Feinman and Matheson, 1978) and can persist in soil after application on land (Donoho, 1978). There is little information available concerning the fate of antibiotics in the environment and their link to the emergence of resistant genotypes found there.

Antibacterial drugs such as oxytetracycline and penicillin G are routinely used in veterinary medicine for prevention and control of disease. Oxytetracycline is applied for the purpose of prevention or treatment of diseases such as bronchopneumonia, mastitis and metritis in cattle. As a result, there is concern that residues of these compounds may be presented in meat and meat products. The penicillins are widely used to treat or prevent local and systemic infections of farm animals. The use of penicillins as intramammary infusions or formulations to treat or prevent bovine mastitis is widespread (Haapapuro et al., 1997).

To detect antibiotic residues, different kinds of methods were developed. These consist of screening methods and chromatographic techniques to detect as many antibiotics as possible. The screening method is generally performed by microbiological, enzymatic and immunological methods. The screening methods are based on the various susceptibility of bacteria to different antibiotics. The antibiotic residue detection assays that are currently available use different methods and test microorganisms (Mitchell *et al*., 2002). Microbiological assays for the detection of antibiotic residues utilize bacteria such as *Bacillus stearothermophilus* because of its high sensitivity to the majority of antibiotics.

Both microbiological and chromatographic methods have been described for monitoring tetracyclines, chloramphenicol, and penicillins in milk and animal tissues. Although the microbiological assay techniques have been recommended as official and conventional methods because of their simplicity, the bioassay methods lack specificity and provide only semi-quantitative measurements of residues detected and sometimes produce false positives, they are also time consuming. Therefore, chromatographic techniques, such as thin layer chromatography (TLC), and high performance liquid chromatography (HPLC), and capillary electrophoresis (CE), have been developed to replace microbiological assays also because they are quantitative, accurate and give reliable measurements of antibiotic residues in animal tissues or muscles (Chen and Gu, 1995; Cinquina *et al*., 2003; Posyniak *et al*., 2005; Zhao *et al*., 2004). Some of the disease that affect farm animals (cattle and sheep) are mastitis, metritis, enteritis, dystocea, retained fetal membrane, metabolic problem and foot problem. Dry cow therapy is used to control mastitis.

In Ghana, very little is known about the usage of antibiotics in animal husbandry, and the public health effects on food safety. A study by Aning et al. (2007) on raw milk indicated that antibiotics may be translocated at high rates into raw milk, though the study did not elaborate the associated causal factors. Apart from raw milk, there is no data on the risk associated with drug residues through animal source food, though majority of Ghanaians consume animal source foods on a regular basis, and there are concerns that some of the drugs being used in animal husbandry may not be safe for humans (Wassenaar, 2005). Currently, there are no major quality assurance programmes in place in the country to protect public health against the adverse effects of antibiotics used in animal husbandry, which is partly due to the lack of research data to inform policy.

1.1 STATEMENT OF PROBLEM

Antibiotic residues in beef and mutton above the maximum residue limits (MRL) raise health concerns (Donoghue, 2003).

In order to safeguard human health, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have set standards for acceptable daily intake and maximum residue limits in foods (FAO, and WHO, 1995). In Africa, in parallel to the incautious use of antibiotics in human medicine, agricultural sectors consume a large portion (50%) of antibiotics in animal farming to treat or to minimize potential outbreaks of diseases or to promote animal health (Miller et al., 2003). However, there is no clear regulation controlling antibiotic contamination of foodstuffs in many African countries. Additionally, there is a clear lack of available information about antibiotic residues in animal-derived foods in Africa.

1.2.1 RESEARCH OBJECTIVES

MAIN OBJECTIVE

1. To determine the levels of antibiotics residues in beef and mutton from some selected markets in the Kumasi metropolis.

SPECIFIC OBJECTIVES

1. To determine quantitatively the presence of chloramphenicol, penicillin G and oxytetracycline in beef and mutton using HPLC.
2. To estimate the health risk associated with the consumption of beef and mutton.
3. To compare the results with the internationally accepted standards worldwide.

1.3 JUSTIFICATION

Antibiotic residues are considered public health hazards (Kabir et al., 2004).

Levels of the drug and their metabolites may persist at unacceptable levels and consumers can be exposed to them. The presence of residues may result from failure to observe the mandatory withdrawal periods, illegal or extra-label use of drugs and incorrect dosage levels. Unauthorized antibiotic use may result in residues of these substances in beef and mutton (Ivona, and Mate, 2002).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.1 OCCURRENCE OF ANTIBIOTIC RESIDUES IN FOODS

The introduction of antibiotics to the veterinary field started soon after the use of antibiotics for the treatment of bacterial diseases in humans. The main use of antibiotics in animal rearing was for the treatment and prevention of diseases. Indeed, antibiotics have been used for the treatment of mastitis, arthritis, respiratory diseases, gastrointestinal infections, and other infectious bacterial diseases (Draisci et al., 2001).

Indeed, antibiotics may improve growth rate by the following means: the thinning of mucous membranes in the gut, which facilitates absorption; alteration of gut motility, which enhances assimilation; production of favorable conditions for beneficial gut microbes by destroying harmful bacteria; and partitioning of proteins for muscle growth via cytokine suppression. Antibiotics also favor growth by decreasing the activity of the immune system, reducing the waste of nutrients, and reducing toxin formation. In most cases, however, only young growing animals and poultry are responsive to antibiotic-mediated health maintenance (Nisha, 2008).This approach actually is problematic as these feed additives are usually used without prescription and for very long periods, in both large and small doses, which leads to drug residues entering animal-derived food.

It is a common practice among livestock producers to treat entire groups of livestock, such as birds, fish, or other animals despite there being only a few affected individuals. Such practices unintentionally and unnecessarily expose healthy individuals to antibiotics. Additionally, many

livestock producers use subtherapeutic doses of antibiotics to prevent diseases and this of course will lead to antibiotic residues entering the human food chain. Moreover, antibiotics are prescribed inappropriately in cases of viral infection, which do not respond to such drugs.

All licensed antibiotics intended for animal use have clear cessation of use periods, pharmacokinetics and pharmacodynamics. Failure to observe the instructions for antibiotic use can lead to antibiotic residues entering animal-derived foods. Improper maintenance of treatment records or a failure to identify treated animals adequately may lead to the omission of these animals (Sundlof, 1989). Residues may also transmit vertically to calves consuming milk from cattle receiving antimicrobials (Guest, and Paige, 1991). Fecal recycling, where the drug excreted in the faeces of treated animals contaminates the feed of untreated animals, can be the cause of traces of certain antimicrobial substances being passed on (McCaughey et al., 1990). Contamination of animal feed with a variety of compounds may also occur. The significance of this contamination depends on the pharmacodynamics of the compound and the species affected (McEvoy, 2002; Myllyniemi et al., 2000).

2.1.2 INCIDENCE OF ANTIBIOTIC RESIDUES IN FOOD IN AFRICAN COUNTRIES

In many African countries, antibiotics may be used indiscriminately for the treatment of bacterial diseases or they may be used as feed additives for domestic animals and birds. The ongoing threat of antibiotic contamination is one of the biggest challenges to public health that is faced not only by the African people, but also by the human population worldwide (Cars et al., 2008). Such residues are spreading rapidly, irrespective of geographical, economical, or legal differences between countries (Harbath and Samore, 2005).

In one study, ceftazidine residues were recorded in the tissues of rabbits reared in Egypt (Table 2.1), with high concentrations seen in kidney, liver, heart, and muscle tissues (Abd El-Aty et al., 2001). Erythromycin was rapidly passed from blood to milk in experimentally treated lactating ewes (Goudah et al., 2007). Amoxicillin is commonly used in hen farms in Egypt for the prevention and control of many bacterial diseases.

TABLE 2.1 ANTIBIOTIC RESIDUES IN VARIOUS FOODSTUFFS CONSUMED IN AFRICAN COUNTRIES

COUNTRY	ANTIBIOTIC	FOODSTUFF	REFERENCE
Egypt	Tetracyclines	Chicken meat	Salama et al., 2011
		Bovine carcasses	Morshdy et al., 2013
	β-Lactams	Eggs	Khattab et al., 2010
	Cephalosporines	Rabbit meat	AbdEl-Aty et al., 2001
		Rabbit liver	
		Rabbit kidney	
	Macrolides	Milk	Goudah et al., 2007
Sudan	Quinolones	Animal-derived foods	El-tayeb et al., 2012
	Tetracyclines	Animal-derived foods	
Kenya	Tetracyclines	Beef, liver and kidney	Murinki et al., 2001
	β-Lactams	Milk	Shitandi and Sternyo, 2001
Ethiopia	Tetracyclines	Edible tissues	Myllyniemi et al., 2000
Tanzania	Tetracyclines	Milk	Kurwijila et al., 2006
		Eggs	Nonga et al., 2010
	Chloramphenicol	Eggs	
Nigeria	Tetracyclines	Meat	Olufemi and Agboola, 2009
		Eggs	Ezenduka et al., 2011
	Nitrofurans	Animal-derived foods	
	Chloramphenicol	Eggs	Omeiza et al., 2012
	β-Lactams	Beef	Ibrahim et al., 2010
Ghana	Tetracyclines	Milk	Addo et al., 2011
South Africa	Tetracyclines	Milk	Bester and Lombard, 1979

The cessation of use time of amoxicillin is seven days. A study was undertaken to detect amoxicillin residues in laying chicken and commercial eggs. The effects of cooking and storage on amoxicillin contamination in the eggs were also assessed. Levels were detected in both egg yolks and egg whites for six successive days after last exposure to the drugs (Table 2.1). It was found that amoxicillin residues remained until the seventh day after drug administration in eggs stored at room temperature and at 4°C. Amoxicillin residues were not affected after these eggs were boiled for ten minutes (Khattab et al., 2010).The incidence of tetracycline residues (e.g., oxytetracycline, tetracycline, chlorotetracycline, and doxycycline) in fresh chicken samples (meat and liver) collected over the course of one year from retail shops in Cairo was also recorded (Table 2.1). The results revealed that 66 samples (44%) contained tetracycline residues including 21 breast (42%), 19 thigh (38%), and 26 liver (52%) samples. The corresponding ranges of contamination were 124-5812, 107-6010, and 103-8148 μgkg^{-1}. A respective 8%, 7%, and 13% of samples of breast, thigh, and liver had tetracycline residues above the maximum residue limits. Liver samples had a higher incidence and content than those from breast or thigh (Salama et al., 2011). Oxytetracycline residues were examined in 600 samples (made up of 200 samples from muscle, liver, and kidney) collected randomly from bovine carcasses slaughtered at the Mansoura Abattoir (Dakahlia Province, Egypt). Two percent of samples tested positive for residues. Oxytetracycline residues exceeded the maximum limits in 1.3% of the samples examined (Morshdy et al., 2013).

In Sudan, the most commonly used antibiotics by farmers in Khartoum are quinolones and tetracyclines. The majority of farmers use antibiotics for prevention and control of disease; only 5% of farmers use antibiotics for livestock health maintenance (Eltayb et al., 2012).

In Kenya, tetracyclines, sulphonamides and trimethoprim, nitrofurans, aminoglycosides, β-lactams, and quinolones are the most commonly used drugs in food-producing animals. A total of 250 beef samples were collected from five slaughterhouses in and around the city of Nairobi. Out of the 250 samples that were analyzed for tetracycline residues, 114 (45.6%) had detectable tetracycline residues. Of those 114 samples, 60 (24%) were from liver, 35 (14%) from kidney, and 19 (7.6%) from muscle. The mean tetracycline levels of samples from the five slaughterhouses in the study were as follows: Athi River, 1,046 µg/kg; Dandora, 594 µg/kg; Ngong, 701 µg/kg; Kiserian, 524 µg/kg; and Dagoretti, 640 µg/kg. Of the 250 samples analyzed 110 (44%) had oxytetracyclines while 4 (1.6%) had chlortetracyclines. The mean levels of the detected tetracyclines were higher than the recommended maximum levels in edible tissues (Table 2.2) (Muriuki et al., 2001; WHO, 1999). In milk, a higher prevalence of antimicrobial residues was also reported. For example, 11% of raw bulk milk samples sold in Nakuru was found to have penicillin-G residues (Shitandi and Sternesjo, 2001) and 9-16% of the marketed milk in rural and urban households in the Dagoritti division, Nairobi, contained higher levels of antibiotics (Ekuttan et al., 2007; Kang'ethe et al., 2005). Additionally, there was a steady increase in the consumption of quinolones, which was first seen in 1998 (Mitema et al., 2001).

TABLE 2.2 MAXIMUM RESIDUAL LIMITS (MRLs) OF TETRACYCLINES IN ANIMAL-DERIVED FOODS (WHO, 1999).

ANTIBIOTIC	TISSUE	MRLs
Tetracyclines(oxytetracycline, tetracycline, chlortetracycline)	Muscle	200µg/kg
	Liver	600µg/kg
	Kidney	1200µg/kg
	Milk	100µg/kg
	Eggs	400µg/kg

In Tanzania, antimicrobial residues were detected in 36% of marketed milk samples from milk supply chains in and around Mwanza and Dar es Salaam during 1999 and 2000 (Kurwijila et al., 2006). The occurrence of antibiotic residues in commercial chicken eggs was determined in the Morogoro municipality between January and February 2007. All eggs examined tested positive for antibiotic residues (Table 2.2). The common drugs detected were oxytetracycline, chlortetracyclines, chloramphenicol, doxycycline, and flumequine (Nonja et al., 2010).

In Ethiopia, a cross-sectional study was conducted from October 2006 to May 2007 to estimate the proportion of tetracycline levels in beef; the study focused on the Addis Ababa, Debre Zeit, and Nazareth slaughterhouses. Out of the total 384 samples analyzed for tetracycline residues, 71.3% had detectable oxytetracycline levels. Among the meat samples collected from slaughter houses in three cities Addis Ababa (93.8%), Debre Zeit (37.5%), and Nazareth (82.1%) tested positive for oxytetracycline. The mean levels of oxytetracycline in muscle from the three slaughterhouses were as follows: Addis Ababa, 108.34 μg/kg; Nazareth, 64.85 μg/kg; and Debre Zeit, 15.916 μg/kg. Regarding kidney samples, oxytetracycline levels were found to be 99.02 μg/kg in Addis Ababa, 109.35 μg/kg in Nazareth, and 112.53 μg/kg in Debre Zeit. About 48% of the edible tissues had oxytetracycline levels above the recommended maximum limits (Table 2.2) (Myllyniemi et al., 2000; WHO, 1999).

In Nigeria, a study was designed to determine the prevalence of antibiotics in eggs from poultry farms and retail outlets in Enugu State. Eggs from 25 selected commercial farms and ten retail outlets were screened for the occurrence of antibiotic residues. All 25 farms surveyed used oxytetracycline (Table 2.1). Eggs from nine of the surveyed farms tested positive for antimicrobial residues and three of the ten outlets also tested positive for antimicrobial residues. Drugs like nitrofurans, which have been banned in food animals, are still very much in use in

Enugu State, Nigeria (Ezenduka et al., 2011). Chloramphenicol, despite being banned in food-producing animals, is still used in poultry farms in Nigeria. In a survey of chloramphenicol use in poultry farms in Kaduna state, 21 farm authorities (20.0%) admitted the use of chloramphenicol in both human and veterinary preparations, while 15 (62.5%) admitted to the use of a chloramphenicol preparation intended for humans. The presence of antimicrobials was confirmed in eight out of 144 pooled egg samples (10 eggs per sample). The only positive chloramphenicol sample was recorded on a farm that used a human chloramphenicol preparation (Omeiza et al., 2012). Similar reports from different parts of the world, although experimental, have demonstrated the presence and persistence of chloramphenicol residues in tissue from poultry and cattle. (Anadon et al., 1994; Korsud et al., 1987; Ramos et al., 2003). Meat and other edible tissue from cattle slaughtered in the Akure metropolitan abattoir were analyzed from January to June 2008 for oxytetracycline residues. Out of a total of 180 beef samples analyzed in this study, 98 (54.44%) had detectable levels of oxytetracycline of which 62 (34.44%) were contaminated to levels considered violative by the WHO/FAO (Table 2.2) (Olufemi and Ehinmowo, 2009; WHO, 1999). In another report, a microbiological screening was performed for 50 cattle slaughtered in the Sokoto metropolitan abattoir, Nigeria, in order to detect antibiotic residues in meat. A total of 44% of the slaughtered cattle tested positive. Penicillin was the drug with the highest rate of occurrence (14%) followed by tetracycline (8%) and streptomycin (4%) in the samples in question (Ibrahim et al., 2010).

In Ghana, a report showed that 35% of the raw milk marketed in two major cities, Accra and Kumasi, were contaminated with antibiotics (Aning et al., 2007). Additionally, 3.1% of the raw milk samples contained antibiotic levels above the European Union maximum residue limit. The

antimicrobials detected included β-lactams, sulphonamides, aminoglycosides, tetracyclines, and macrolides (Addo et al., 2011).

In South Africa, a survey was done in 1977/78 to investigate the incidence of antibiotic contamination in milk marketed in the markets of Pretoria. In milk from 1081 cattle herds, 60 tankers, and 112 pasteurized batches, antibiotics were found in 2.13% of the herd samples, 11.7% of the tanker samples, and 2.1% of the pasteurized samples (Bester, and Lombard, 1979).

In the literature discussed above, it is clear that antibiotic residues in animal-derived foods are frequently recorded in several African countries (Table 2.1). These residues exceeded the WHO limits (Table 2.2) in many cases. Tetracyclines are highly predominant antibiotics, and represent 41 % of all antibiotic contaminants, followed by β-lactams at 18% (Fig. 2.1). Great care should be taken to observe the antibiotic cessation of use periods before the production of animal-derived foods intended for human consumption.

2.1.3 THE PUBLIC HEALTH SIGNIFICANCE OF ANTIBIOTIC RESIDUES IN FOODS

In many cases the long-term effects of antibiotics on human health are not known, but they can, for example, provoke strong allergic reactions in sensitive people. An allergic reaction may be triggered by antimicrobial residues in a previously sensitized individual. In relation to primary sensitization, it is unlikely that residues could contribute to the overall immune response in view of the very low concentrations that are likely to be encountered.

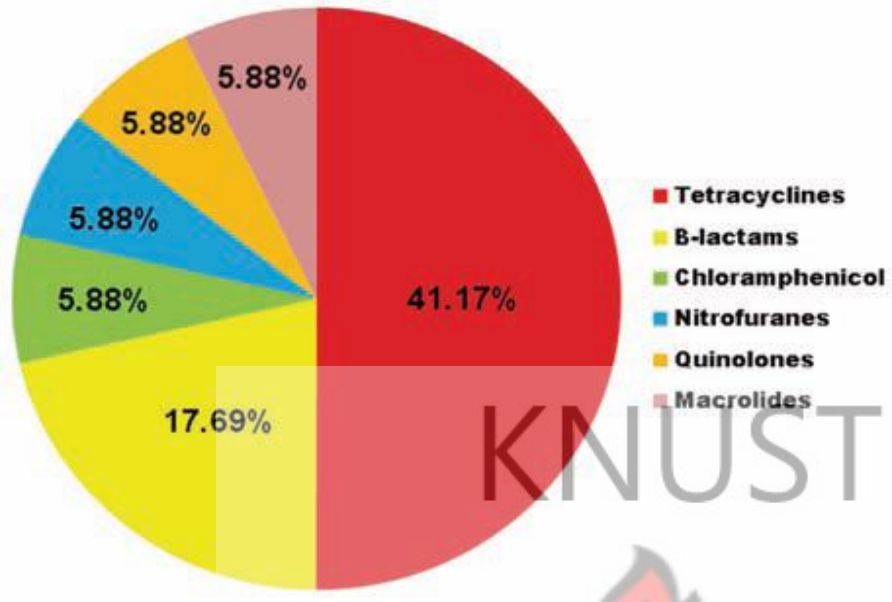


Figure 2.1 Distribution of antibiotic residues in African countries.

The duration of exposure is also short (Dewdney et al., 1991; Sundlof et al., 2000). Despite their generally non-toxic nature, β-lactams appear to be responsible for most of the reported human allergic reactions to antimicrobials (Fein et al., 1995; Sundlof, 1994; WHO, 1991). Aminoglycosides, sulphonamides, and tetracyclines may also cause allergic reactions (Paige et al., 1997). Certain macrolides may in exceptional cases be responsible for liver injury caused by a specific allergic response to macrolide metabolite-modified hepatic cells (Dewdney et al., 1991). However, only a few cases of hypersensitivity have been reported as a result of exposure to residues in meat. Anaphylactic reactions to penicillin in pork and beef have been described (Kanny et al., 1994; Raison-Peyron et al., 2001). In one case, anaphylaxis was possibly caused by streptomycin residues (Tinkelman, and Bock, 1984). Angioneurotic edema and tightness in the chest may also be caused by penicillin residues in meat (Schwartz and Sher, 1984). Antibiotics can encourage the spread of antibiotic resistance in bacteria, making treatment of human infection more difficult. For this reason it has been recommended that antibiotics used in human medicine should not be used in animals. Widespread use of antimicrobials for disease

control and health maintenance in animals has been paralleled by an increase in bacterial resistance in those animals. Resistant bacteria then spread among groups of animals, including fish, or to the local environment (i.e., local soil, air, and water) through the spreading of manure or through contaminated foods to humans. Although correct cooking procedure kills bacteria, contamination can occur through improper handling before cooking. Many of the antimicrobial-resistant *Escherichia coli (E.coli)* strains that cause urinary tract and bloodstream infections in humans appear likely to have originated from contaminated retail meat (Carlet et al., 2012).

Antibiotic residues in milk that is used to produce fermented products can interfere with the fermentation process by affecting desired lactic acid bacteria. Normally this is just a technical problem resulting in financial loss, but,when it occurs, pathogens present in the milk may grow and pose a health hazard later. For these reasons many countries have regulations prohibiting the sale of milk from cattle being treated for mastitis, and milk is routinely tested for the presence of antibiotic residues (Nisha, 2008).

Disruption of normal human flora in the intestine is another harmful effect of drug residues in human food. The bacteria that usually live in the intestine act as a barrier to prevent incoming pathogenic bacteria from becoming established and causing disease. Antibiotics might reduce total numbers of these benign bacteria or selectively kill some important species (Myllyniemi et al., 2000).

2.1.4 ANIMALS AND ANTIBIOTICS

Antibiotics were first used in veterinary medicine for the treatment of mastitis in dairy cows shortly after they were developed (Foley et al., 1946). The need for antibiotics varies with the

species of animal and by the many different cultures throughout the world which influence the pattern of trade and reasons for keeping animals. Antibiotics are not only used for treatment of animals, but also for growth promoting purposes; the breakdown of antibiotic use is probably around 99% for food producing animals and 1% for pet animals, but the exact figure is not known.

Livestock production in developed countries has changed over the last 30 years. The overall trend in developed regions has been to keep a greater number of animals on fewer farms but in larger units. The increased movement of animals within and between countries and the relaxation of border checks has also lead to disease outbreaks. The changing consumer demands as well as scientific advances have been a major influence on agriculture during the second half of the twentieth century. Disease is inevitable in individual animals or groups of animals that may also be carriers of pathogens with varying excretion rates. Examples of animal diseases requiring the most extensive use of antimicrobials for therapy or prophylaxis are respiratory and enteric diseases, especially of pigs and cattle, and mastitis in dairy cattle (Johnston, 1998).

While the treatment of bacterial disease in man and in pet animals is invariably directed to the individual patient, the treatment of food producing animals, especially pigs and poultry, is frequently on a group or herd basis (Walton, 1983).

In a number of species, production is in large, confined rearing systems, the majority of which are indoors. With many animals and birds in close proximity any disease present will therefore spread rapidly. On the other hand, animals housed in intensive units, which if they have been well designed and are run to a high level of biosecurity, can be at less risk of exposure to pathogens compared with free-range animals. Pathogens in animals can have serious

implications for both animal and human health. In the animal population, disease can cause death of the animal or some level of morbidity, which would result in a reduced production. Death of animals requires their replacement with the inevitable cost, but may also mean loss of an important genetic line or of a much-loved animal. There will always be situations when, despite the very best husbandry and correct use of appropriate preventive measures, disease will occur.

For many years vaccines have been used to reduce disease in animals. In sheep, the use of effective multivalent clostridial vaccines is long established and more recently vaccines against the two most common causes of abortion, *Chlamydia psittaci* and *Toxoplasma gondii*, have become available and have further reduced the need for antibiotic use. There are also the well used and effective combined rotavirus and *Escherichia coli* K99 vaccine for calves, an *E. coli* vaccine for pigs prepared from selected commonly occurring strains and the leptospiral vaccines for dogs and cattle. The introduction of a new vaccine for furunculosis in the Atlantic salmon provided one of the most dramatic demonstrations possible of the considerable reduction in antibiotic use (ACMSF, 1999).

Disease control in animals is multi-faceted and if the concerns about antibiotic resistance are to be addressed, the traditional 'fire-brigade' responses to sick animals and the routine use of antibiotic growth promoters are no longer acceptable (Weirup, 1998).

However effective an antibiotic may be, for the treatment to be successful there must be an accurate diagnosis of the disease, the use of the correct antibiotic for that disease and careful observance of all instructions for use (EC, 1999; SMAC, 1998).

In professional hands with diligent attention to Good Veterinary Practice, antibiotics have a vital role to play in control of bacterial disease in animals. At all times while dealing with the health of single animals, herds or flocks, due note must be taken of the implications of any action on the health of humans. The most satisfactory method of medicating groups of animals or birds is often by medication of the feed or the water. The decision as to which vector (of the medicine) should be used depends on a number of factors but in most cases, for very good reasons, it will be by in-feed medication. The term mass medication has been used to describe the medication of groups of animals with antibiotics incorporated in the feed. This is often confused with the use of sub-therapeutic doses of antibiotics for growth promotion purposes. Following the report of the Swann Committee in 1969, there were significant changes to the practice of incorporating antibiotics in animal feed (Swann Report, 1969).

The issues for the public are frequently related to the possibility of residues in foods resulting from such use and there may also be confusion with concerns about antibiotic resistance. However, as one of the possible causes of residues in animals is cross-contamination of feeds in the feed mill, this cross-contamination is equally important when considering antibiotic resistance. Therefore, regular compliance monitoring is essential for the effectiveness of the controls within the feed mill. For medicines which require a prescription before incorporation in the animal feed, there is at least involvement by the veterinary service before the prescription is issued. For other medications, such as growth promoters, there is no requirement and there is usually no involvement of the veterinary service in the decision to use them. To have antibiotics used in a farm without prior knowledge of the veterinary service conflicts with the principles of prudent use of antibiotics and may compromise the efficacy of therapy.

Within the animal industry there are a number of problems and concerns that impact on antibiotic resistance. One of the greatest concerns is associated with the number and the quantity of antibiotics used in food–animal production. Reduction in antibiotic usage is achieved by implementing all the elements of preventive veterinary medicine and the application of on farm disease control measures. Alternatives to the routine use of antibiotics include improvements in breeding strategies, attention to detail in all aspects of the husbandry and the use of the veterinary service as well as development of new vaccination techniques. On farm, the possible application of the principles of the Hazard Analysis Critical Control concept will make a contribution to reduction in use of antibiotics as well as improved food safety. The controls applied on farm most frequently relate to the use of good farming practices which will include the highest level of stockmanship, control of building design and husbandry along with the use of effective hygiene measures at all stages of production. The use of all-in all-out systems, followed by effective cleaning and disinfection before restocking, with biosecure housing will provide an optimal environment along with appropriate use of available vaccines and the highest standards of management.

The role of the veterinary profession in the control and prevention of disease in animals is critical. All farms should have a veterinary health plan that is based on the results from good surveillance of the disease trends on the farm including antibiotic resistance. This surveillance should also enable emerging antibiotic resistance to be recognized.

To reduce the need for antibiotics, the method of production may have to change. Methods for rapid diagnosis must be developed to enable improved empirical therapy of sick animals. The use of antibiotics as part of the farm veterinary health plan is the way forward and must be an

essential component of any Farm Quality Assurance Scheme. The development of a 'formulary' approach, as described for medical practitioners, giving the prescribing veterinary surgeon a suggested first level, second level or even third level of choice, must be investigated. One of the problems is in the decision making tree, of which antibiotic to use when the need has been established. The choice of antibiotic depends on a number of factors including the patient(s) and the on farm factors. It is important to start treatment early in the disease and therefore unrealistic in many cases to expect culture and sensitivity tests to be complete before starting antibiotic treatment. How often is there a temptation to use one of the 'more modern' or 'newly released' antibiotics rather than one of the old and established antibiotics which is still likely to be effective? Part of the problem may be the fear of being the subject of litigation if the animal does not get better or even dies; thus the temptation is to go for a more certain cure. What role does the advertising of POMs have in persuading a veterinary surgeon to choose a particular antibiotic? The advertisement that implies or suggests that the animals will get better if drug is used may also send a message to the client that 'your vet was wrong not to choose it'. Use of first choice antibiotics which are the preferred option for a certain condition, followed by a second choice antibiotic chosen by a responsible clinician (if there is good reason not to use the first choice antibiotic), in no way undermines the decision making process for the responsible veterinarian. There is also the option to use an antibiotic after sensitivity testing results are known. The education of farmers and veterinary surgeons is essential, as is the adherence to codes of practice such as the one produced by the British Veterinary Association on the use of medicines (BVA, 2000).

Any code of practice should be based on scientific knowledge and should include concrete guidelines for choice and use of antimicrobials taking into account the consequences for both

animal and human. The teaching of the undergraduate doctor and veterinary surgeon has in the past not been directed at solving clinical problems (Davey et al., 1993).

In the medical and veterinary faculties, the teaching of pharmacology has been conducted in different sections of the course with a clear separation, possibly of a number of years, between the teaching of the pharmacokinetic and pharmacodynamics of antibiotics and their use in the clinical training. The whole subject area should be taught in an integrated manner where the antibiotic resistance teacher shares the teaching platform with the clinician, microbiologist and pharmacologist. Access to effective antibiotics is essential for animal health and welfare reasons, but they must only be used when required. As in humans, disease is inevitable either in the single animal or in groups of animals (Johnston, 1998; Walton, 1983).

Control of antibiotic resistance is essential to protect the ability to treat disease in animals and in man. This was identified by the Report of the Standing Medical Advisory Committee in the UK (SMAC, 1998), Report of the Scientific Steering Committee of the EU (EC, 1999) and the Report of the Advisory Committee on the Microbiological Safety of Food (ACMSF, 1999).

There is in addition, an urgent need to establish rational bases for the use of antimicrobial drugs. This can only be based on current knowledge of resistance development and transfer along with the mechanisms of action of antibiotics. The prudent use of antibiotics involves measures that restrict use. This restriction of use is not sufficient despite being appropriate and relevant. There is an urgent need to establish bases for the use of antimicrobial drugs that must be from knowledge of the mechanisms of resistance development and transfer and the mechanisms of action of antimicrobial drugs. The actual effect of antibiotic use, for therapy and for growth promotion has not been vigorously studied in order that the effect of use on antibiotic on

resistance pattern can be defined. This is lacking at the most basic level of knowledge, such as for example, studying the effect of feeding of antibiotics to a group of pigs and then noting the emergence of resistance, if at all, over time and successive groups in the same house. The recent expert reports highlight the need for effective monitoring which should be extended to 'healthy' animals for salmonellae, campylobacter, *E. coli* O157, and other *E.coli* (EC, 1999; SMAC, 1998; Swann Report, 1969).

There is also a need to establish the consumption of different antibiotics according to the species of animals in which they are used. Meaningful information on the amount of antibiotics used by the veterinary profession in the treatment of animals and used in medicated feed, as well as the quantity of growth promoters is currently difficult to obtain (ACMSF, 1999; EC, 1999).

The main concern regarding the use of antibiotics in animals must be the emergence of antibiotic-resistant bacteria that could infect humans either directly or by the transfer of resistant genes to human pathogens. It is perhaps unfortunate that most of the recommendations from the Swann Working Group were not implemented at that time. By full implementation of the Swann Report there may well have been less need for concern on antibiotic use in animals at the start of the new Millennium (Swann Report, 1969).

It is of the greatest importance that in both veterinary and in human medicine that the problems associated with the prescription and use of antibiotics is addressed. Having both the medical and veterinary professions on the same platform at meetings to discuss the subject of antibiotic resistance is one of the important steps in dealing with the problem.

2.2 CLASSIFICATION OF ANTIBIOTICS

Beta-Lactams: Penicillin G, Ampicillin, Amoxicillin, Cloxacillin, Dicloxacillin

Tetracyclines: Chlortetracycline, Oxytetracycline, Tetracycline

Sulphanamides (and trimethoprim): Sulphathiazol, Sulphamethazine, Sulphadoxine, Sulphisoxazole

Aminoglycosides: DH-Streptomycin, Neomycin, Streptomycin, Gentamycin, Tobramycin, Amikacin

Macrolides: Erythromycin, Tylosin

Quinolones: Ciprofloxacin (a fluoroquinolone)

Cyclic Peptides: Vancomycin, Streptogramins, Polymyxins

Lincosamides: Clindamycin

Oxazolidinoes: Linezolid (Zyvox)

Miscellaneous Antibiotics: Chloramphenicol, Dapson, Malachite green

2.2.1 β-LACTAMS ANTIBIOTICS

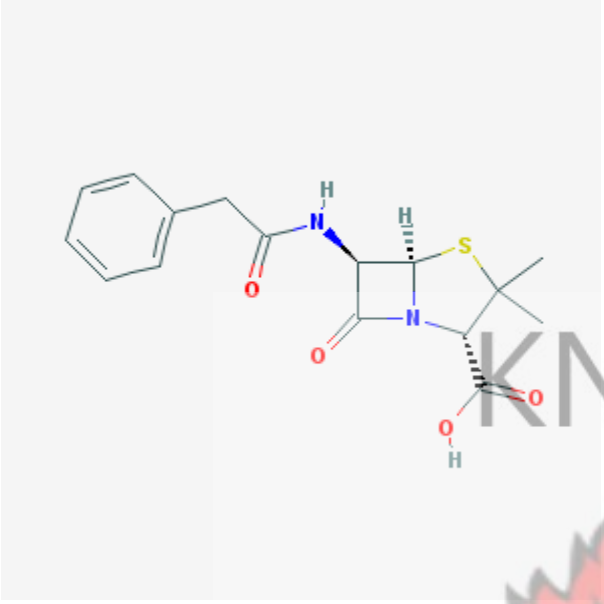


Figure 2.2 Penicillin G

β-Lactams are probably the most widely used class of antibiotics in veterinary medicine for the treatment of bacterial infections in animal husbandry and bovine milk production. There are MRLs for all food producing species ranging from 4 µg/l for ampicillin in milk to 300 µg/kg for oxacillin, cloxacillin and dicloxacillin in bovine tissues like muscle fat, liver and kidney (OJEC L224, 2004).

β-Lactam antibiotics basically consist of two classes of thermally labile compounds, penicillins and cephalosporins. Both classes contain a bulky side-chain attached to 6-aminopenicillanic acid and 7-aminocephalosporanic acid nuclei, respectively. (Fagerquist and Lightfield, 2003).

The presence of an unstable four-member ring in the β-lactam structures makes these compounds prone to degradation by heat and in the presence of alcohols. Penicillins are also readily isomerized in an acidic environment. Because of these characteristics, several precautions

concerning pH and temperature have to be taken in each step of the sample-preparation procedure to avoid analyte degradation.

Especially, at low concentrations degradation can be significant. The penicillin G concentration in milk of 7.5 µg/l decreased by 18% in 16 h at room temperature. The pH of the extraction buffer is important: optimum stability was obtained at pH 6.7 for animal tissues and at pH 4.6 for milk, and degradation was less at sub-ambient temperatures. β-Lactams are extracted from milk and animal tissues (liver and kidney are the target organs for penicillins) with salt buffers. The aqueous extract is concentrated and cleaned by C_{18}-SPE or WCX-SPE. The LC separation and detection is mostly based on ion pairing LC with a UV detector or, sometimes after derivatization, by GC-FID. The use of these conventional detection techniques is often complicated due to interfering matrix components. As has already been mentioned for aminoglycosides, the use of LC–MS can solve these selectivity problems. Several studies describe methods available today for the selective confirmatory analysis of β-lactam antibiotics in milk at the MRL level by LC–MS, Liquid Chromatography triple quadruple Mass Spectrometry (LC–QqQ-MS) or Liquid Chromatography Ion Trap Multiple Stage Mass Spectrometry (LC–ITMS*n*). Analyte extraction in combination with tandem MS detection was based on a single liquid extraction with, for example, acetonitrile followed by Ultra filtration (UF); in the case of single-stage MS detection, various Liquid-Liquid Extraction (LLE) steps were used like addition of acetonitrile (to prevent analyte binding to proteins) followed by LLE with dichloromethane, hexane–acetonitrile, water, phosphate buffer (pH 7) and again dichloromethane. The RPLC separation was performed on C_{18}-bonded silica with an acetonitrile/water gradient containing an ion-pairing reagent, for example di-*n*-butylamine

acetate (DBAA). An LC–IT-MS*n* method for the determination of β-lactams in kidney was reported by Fagerquist and Lightfield (Fagerquist and Lightfield, 2003).

After extraction of the analytes with acetonitrile and water, clean-up of the extracts was by C_{18}-SPE, with subsequent RPLC on a C_{18} column; the eluent was a methanol–water gradient with 0.1% formic acid. The authors concluded that IT-MS*n* is very useful for identification purposes, but they were unable to obtain reproducible quantitative results. This problem, which is probably due to ion suppression, often occurs when the final extract still contains too many matrix components. Additional clean-up has to be introduced to solve this problem. LC–UV_{220} was used to screen food for various penicillins using a specific combination of SPE columns for sample clean-up. They used a salt buffer for extraction and the extract was cleaned by C_{18}-SPE and, next, by a purification of the eluate on a QMA silica-based strong-anion-exchange cartridge. Separation was by RPLC on a C_{18} column (Stationary Phase) with acetonitrile–0.02M phosphate buffer pH 6.2 (43:57, v/v) mobile phase, containing 12 mM cetyltrimethylammonium chloride. For bovine liver spiked at levels of 0.1 mg/kg the recoveries for the six penicillins were 83–96%.Limit of Detections (LODs) of the penicillins in bovine liver and kidney were in the MRL range, viz. 0.02–0.05 mg/kg. The authors also reported a confirmatory Liquid Chromatography Electro spray Ionization Triple Quadruple Mass Spectrometry (LC–ESI(−)QqQ MS) method.

Some what surprisingly, although QqQ-MS is much more sensitive, the LODs were the same for UV and QqQ-MS detection. Two further studies on the determination of β-lactams, both penicillins and cephalosporins, in milk should be mentioned. They discuss LC–ESI(+)QqQ-MS and LC–ESI(+)IT-MS*n* methods which yield low LODs, and also pay attention to the selection of proper pH and temperature conditions during extraction. Two frequently selected MS–MS fragment ions of the β-lactams are *m*/*z* 160, formed due to the cleavage of the β-lactam ring

(carbonyl group) and *m*/*z* 114, formed by a further loss of the carboxylic acid group, COOH. Subsequently, amoxicillin and ampicillin are difficult to analyse due to their amphoteric nature. Generally ESI(−) is the most sensitive ionisation mode for the present class of compounds, but it can only be used when the amphoteric β-lactams are not included; when these compounds are included, the ESI(+) mode is preferable.

2.2.2 TETRACYCLINES

Tetracycline antibiotics (TCAs) are broad-spectrum antibiotics against gram-positive as well as gram-negative bacteria. They are also used for promoting growth in cattle and poultry.

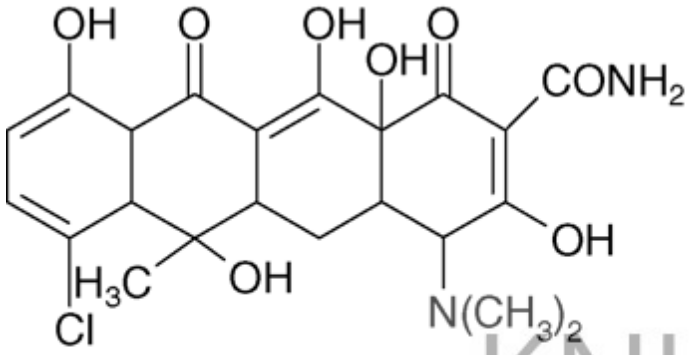
The basic structure of TCAs is a hydronaphthacene skeleton containing four fused rings. The various TCAs mainly differ in their substitution patterns at the C5, C6 and C7 positions (Fig. 2.3). Of the eight commercially available TCAs, chlortetracycline (CTC), oxytetracycline (OTC), tetracyline (TC) and doxytetracycline (DOX) are most commonly applied to food-producing animals. Their MRLs range from 100µg/kg for muscle to 600 µg/kg for kidney (OJEC L224, 2004).

Due to the presence of two ketone groups in positions 1 and 11, TCAs can readily chelate to metal ions. They can also interact with silanol groups during LC separation on a silica based stationary phase, even if this phase is end-capped; this causes severe tailing of TCA peaks. Many authors eliminate this problem by adding chelating agents, such as oxalic acid and EDTA salts, to the eluent. However, the presence of non-volatile agents in the LC eluent prevents the use of ESI MS for detection because of the rapid contamination of the sample cone orifice. Moreover, both oxalic acid and EDTA cause a drastic reduction of the ion signal intensities of TCAs. In combination with MS, volatile buffer solutions like ammonium acetate or formic acid have to be

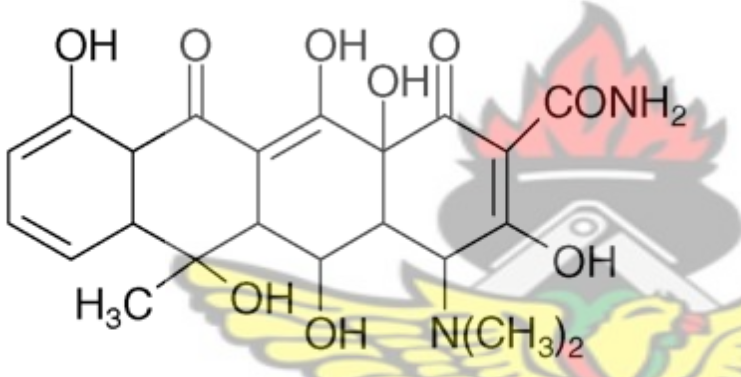
used, although this has a negative effect on the peak shape and separation. Another problem is that CTC and DOX peaks frequently show excessive fronting. The type of column used and the LC conditions, particularly the column temperature, play a main role here. It has been reported that CTC and DOX rapidly isomerizes to give 4-*epi*-tetracyclines in aqueous solutions at pH 2–6. In addition, keto tautomers are readily formed in aqueous solutions. The products of both tautomerization and epimerization are eluted well before the parents, OTC and DOX. This phenomenon, rarely mentioned in the literature, complicates quantification of CTC and DOX. In a recent review on the LC analysis of TCAs in food, the authors discuss the above problems of chelate formation, silanol interactions and epimerization, and also gave a very complete overview of all available LC–UV and LC–FLD techniques for TCA analysis.

They finally concluded that by using the chelating ability of TCAs, very selective extraction can be obtained and that the addition of EDTA or oxalic acid during separation helps to prevent undesired secondary interactions. Most analytical methods are based on the extraction of TCAs from tissues with EDTA–McIlvaine buffer (citric acid with disodium hydrogen phosphate). For tissues and milk an additional clean-up on C_{18}-SPE or HLB SPE is required prior to RPLC on a C_{18} column with a water–acetonitrile or –methanol gradient mobile phase. Depending on the detection technique used, EDTA–phosphate buffers (UV detection), or volatile ammonium

acetate buffers or formic acid (MS detection) are used.



Chlortetracycline



Oxytetracycline

Figure 2.3 Typical Structures of Tetracyclines

An example of the more traditional LC–UV analysis of TCAs in bone meal, used for the production of feedstuff, is given below. After LPE of the analytes from feed by means of sodium succinate buffer (pH 4), RPLC on a C_{18} column gave LODs of around 1 µg/kg, demonstrating that the method can be used for the monitoring of feed (concentrations detected were 1000–2000 µg/kg). A rapid procedure for the extraction of OTC and its 4-epimer from calf tissues involves LPE with sodium succinate buffer (pH 4) combined with clean-up by HLB-SPE and LC−IT-MS*n*.

This simple approach gave LODs of <50 µg/kg for this specific TCA and its epimer. As regards environmental concerns, TCAs are known to show strong sorption and are therefore expected to remain in the soil or to be transported through to surface water via particulate matter after excretion.

Revert´e et al., (2003) used LC–ESI(+)MS to determine TCAs and quinolones in wastewater; Lindsey et al., (2001) used the same technique for sulfonamides and tetracylines in surface and groundwater. In the latter study, the neutral loss' of 35 Da – i.e., the loss of ammonia plus water – was used for the selective detection of TCAs. Both groups could detect TCAs down to 10 ng/l. Since suspected concentrations in surface and groundwater are 1–500 ng/l, there is still room for more sensitive analytical methods for these antibiotics in rivers and lakes, especially in their sediments. Adequate methods and monitoring results for these compartments are scarce.

2.2.3 CHLORAMPHENICOL

Chloramphenicol (CAP) is a broad-spectrum antibiotic active against a variety of pathogens. Although CAP was, previously, widely used in veterinary and human medicine, reports of plastic anaemia in humans arising from its use led to its ban in the US and EU in 1994. Thiamphenicol and florfenicol, which have structures similar to CAP (Fig.3) were permitted as substitutes (Corsia and Nazzari, 2002).

MRLs for thiamphenicol are 50 µg/kg for bovine and chicken tissues, and for florfenicol, 100 µg/kg for muscle to 3000 µg/kg for bovine liver. Due to the ban of CAP, very sensitive detection methods have been developed. Recently, the MRPLs of CAP for meat, eggs, milk, urine, aquaculture products and honey were all set at 0.3 µg/kg (OJEU L71, 2003). As regards analysis,

an organic solvent, predominantly ethyl acetate, or an aqueous phosphate buffer is used as extraction solvent for CAP from biological matrices. Next, the primary extract is cleaned by a variety of LLE and/or SPE steps. GC in combination with chemical ionisation (CI)-MS provides excellent analyte detectability down to 0.1 µg/kg in muscle tissues; the results for urine are less good due to matrix interferences. GC–MS in the electron impact (EI) mode is slightly less sensitive but has the distinct advantage of yielding spectra which can be searched in electronic libraries. The main drawback of using GC–MS for CAP analysis is the need for derivatization in order to improve its chromatographic properties.

Gantveng et al., (2003) described a GC–EI-MS method for CAP in urine. After hydrolysis, washing with ethyl acetate and clean-up by C_{18} SPE, the analyte was derivatized with a mixture of N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and 10 vol.% Trimethylchlorosilane (TMCS). A 30m×0.25mm i.d., 0.25µm film thickness HP- 5MS column was used. The Limit of Concentration LOC in 'dirty' urine was 2 µg/l.

One recent alternative uses GC–ECD after selective extraction of CAP from muscle by means of MSPD and subsequent conversion into the trimethylsilyl derivative. Although the method is rapid and uses only a few ml of organic solvent, the LODs of 2–4 µg/kg found for cattle, pig and horse muscle tissue do not permit CAP monitoring at the MRPL level of 0.3µg/kg. Until recently, the interest in LC–tandem MS as a confirmatory method for CAP was limited because of the availability of GC–MS procedures. As is well-known, LC–MS does not require derivatization and, today, CAP detectability in sophisticated LC–MS procedures approaches that of GC–MS.

In 2003, interest in the determination of CAP in shrimps suddenly increased due to a number of non-compliant results in the Netherlands and Germany. As a consequence, several new LC–MS procedures were developed. Liquid Chromatography Atmospheric Pressure Chemical Ionization Triple Quadruple Mass Spectrometry (LC APCI(−)QqQ-MS) offered sensitivity and selectivity superior to that of GC–MS. Even in urine, the LOD was 0.02 µg/kg as against 2 µg/kg for GC–MS.

LC–tandem MS method for CAP in meat and seafood. After ethyl acetate extraction and clean-up on silica-SPE, the analysis was on a C_{18} column with a water–acetonitrile eluent.

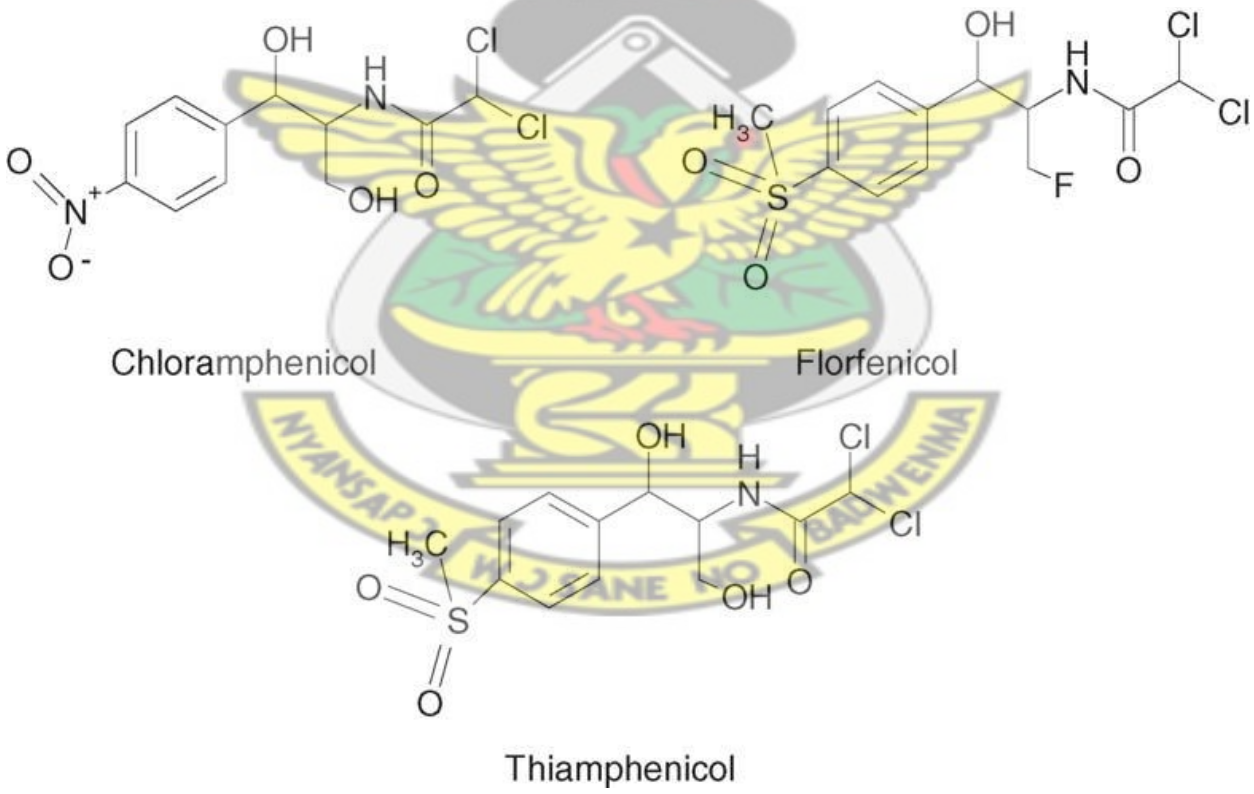


Figure 2.4 Structures of amphenicols

The use of ESI(−)QqQ-MS enabled highly precise quantification of CAP down to 0.05 µg/kg in fish and shrimps. The overall absolute recovery of ^{14}C-labelled CAP spiked at 2.5 µg/kg into a blank chicken meat was 60±5% (n = 4). Ramos et al. (Ramos et al., 2003) used LC–ESI(−)-MS for the determination of CAP in shrimps. After phosphate extraction and C_{18}-SPE cleanup, an additional LLE with ethyl acetate was performed with, next, a conventional RPLC separation; the Limit of Quantification (LOQ) was 0.2 µg/kg.

Van de Riet et al. (Van de Riet et al., 2004) used a LC–ESI(−)-MS to determine chloramphenicol, thiamphenicol and florfenicol in farmed aquatic species. After PLE with acetone, the extracts were partitioned with dichloromethane, the aqueous layer was removed and the organic layer evaporated to dryness. The residue was dissolved in dilute acid and defatted with hexane, and the aqueous layer prepared for LC analysis on a C18 column with a water–acetonitrile gradient. Recoveries were 71–107%; LODs were 0.1µg/kg for florfenicol and chloramphenicol, and 0.3µg/kg for thiamphenicol.

2.3 ANTIBIOTICS IN ANIMAL HUSBANDRY

Since the early 1950s, antimicrobial agents have been used in livestock farming to treat infections and improve growth and feed efficiency. It is estimated that in Europe, before the ban of growth promoters, especially pigs and poultry were treated with the majority of antibiotics administered in agricultural livestock production, while other species received only one percent of prescriptions (Ungemach, 2000).

The amounts of antibiotics used in 1 year can only be calculated roughly: in 1999, 13,288 tonnes of antibiotics were used in the EU and Switzerland, of which 29% were used in veterinary medicine, 6% as growth promoters and 65% were used in human medicine (FEDESA, 2001).

However, since the prohibition of growth promoters in 2006, an actual decline in antibiotics used in agricultural has been recorded. In the USA, the percentage of antibiotics used in livestock farming was 70% out of a total of approximately 16,200 tonnes (Union of Concerned Scientists, 2001).

Disease decreases animal performance in livestock production. The intention of the use of antibiotics is to limit progression of disease in the population. Following the National Committee for Clinical Laboratory Standards (2002), herd or flock antibiotic use can be described by the terms of therapy, control, prevention and growth promotion. Therapy includes all antibiotic treatment of animals showing frank clinical disease. If antimicrobial treatment is administered to a herd or flock in which morbidity and/or mortality has exceeded baseline norms, it is defined as a control, whereas prevention is the use of antibiotics in animals considered to be at risk, but before showing the onset of disease or identification of causal agents. The administration of an antimicrobial over a period of time, usually as a feed additive, to growing animals is defined as growth promotion, resulting in improved physiological performance.

Since 2006, all growth promoters have been banned from European agriculture by Regulation No 1831/2003 (European Parliament and Council, 2003) and therefore have not been taken into consideration in the following reflections. For cattle and swine, individual antibiotic treatment may be practical, but for poultry, antibiotics are applied orally with feed or water. For example, in cattle, antimicrobials are mainly used for treatment of mastitis in cows and of respiratory infections in calves. In pigs, they are administered to treat gastrointestinal disorders in the weaning period or pneumonia later in life. Many of these antibiotic products are closely related to antibiotics used in human medicine, including β-lactams, tetracyclines, sulphonamides with or without trimethoprim, macrolides, lincosamides and quinolones. An overview of current data on

animal antibiotics world-wide, especially tylosin, tetracycline and sulphonamides, has been given by Sarmah et al. (2006).

Antibiotics are not completely eliminated in animal organisms, as they are bioactive substances, acting highly effectively at low doses and excreted after a short time of residence. Antibiotics are optimised with regard to their pharmacokinetics in the organisms: organic accumulation is, as in other pharmaceutics, objectionable and thus, they are excreted as parent compounds or metabolites (Kümmerer et al., 2000; Thiele-Bruhn, 2003).

Excretion rates are dependent on the substance, the mode of application, the excreting species and time after administration, but it has been shown that rates vary between 40 and 90% for tetracyclines and sulphonamides (Berger et al., 1986; Haller et al., 2001; Halling-Sørensen, 2001).

Sulphamethoxazole degrades to about 85%, whereas other substances are relatively inert in the body, e.g. the degradation rate for amoxicillin is between only 10 and 20% (Hirsch et al., 1999). Excretion rates of antibiotics are summarised by Jjemba (2002) and Zuccato et al. (2001).

If intracorporal degradation takes place, it is often proceeded in the faeces, but if antibiotics are not metabolised, recalcitrants persist in the environment (Kümmerer et al., 2000). Additionally, antibiotic metabolites can be transformed back to their parent compound after excretion. For instance, some antibiotics are transformed to conjugates such as acetylated metabolites, becoming inactive and analytically camouflaged, but in manure the acetyl group can be cleaved, releasing the original active ingredient (Christian et al., 2003).

For fluorchinolones and sulphonamides, the adsorption to faeces, being rich in organic matter, is strong (Marengo et al., 1997). Even by aeration of manure and increasing temperatures (Winckler and Grafe, 2001), these substances are not transformed and consecutively, distributed in the environment in an unaltered state. Possible entry paths of veterinary antibiotics are displayed in the following.

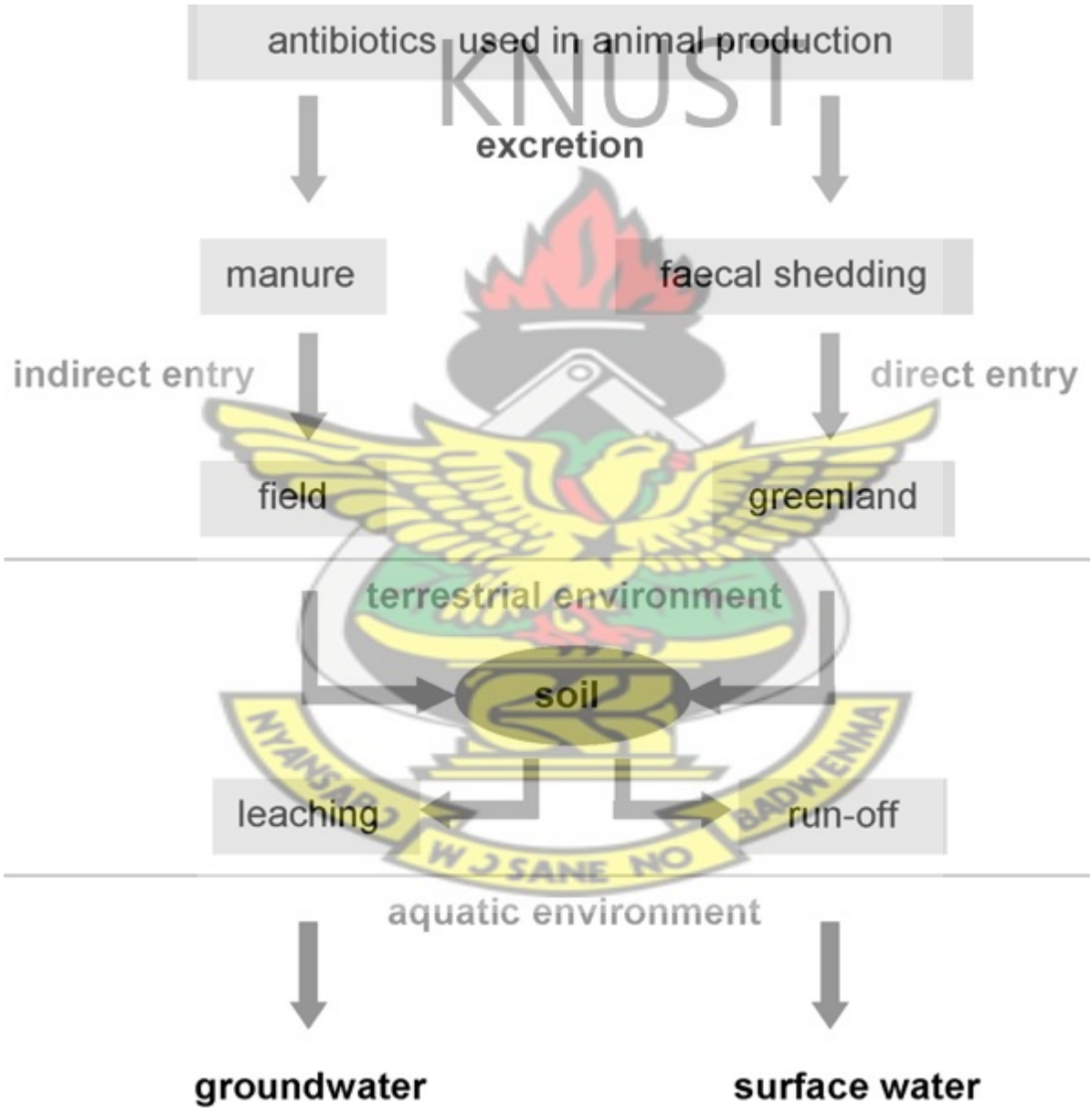


Figure 2.5 Veterinary antibiotics in the environment: anticipated exposure pathways (Kemper, 2008)

2.3.1 ANTIBIOTICS IN SOIL

Once in the environment, antibiotics depends on the physico–chemical properties, prevailing climatic conditions, soil types and a variety of other environmental factors. The fate and behaviour of antibiotics in soil have been recognized as one of the emerging issues in environmental chemistry. Antibiotics used for veterinary purposes are excreted by the animals and end up in soils via grazing livestock or manure used as agricultural fertiliser (Jørgensen and Halling-Sørensen, 2000). The loads of antibiotics shed by manuring have been estimated up to kilograms per hectare (Winckler and Grafe, 2000). Other minor agricultural entries into the environment may originate from antibiotics in the dust from the exhaust air of stable ventilation, as reported by Hamscher et al. (2003).

Often, antibiotics are released into the environment only slightly transformed, or even unchanged and conjugated to polar molecules. Chemical and physical behaviour in the soil depends on the molecular structure of the pharmaceutical. An overview of the fate of antibiotic compounds in soils and the sorption coefficients in soils, sediment and slurry was also published by Thiele-Bruhn (2003).

With regard to their various structural classes, antibiotics are ionised, amphoteric and for this reason, adsorption to soils take place. Due to these physico–chemical properties, such as their molecular structure, size, shape, solubility and hydrophobicity, the sorption and fixation of these substances in soils differ considerably. Many substances are polar, partially water soluble and therefore strongly retarded in soils. For example, the transport of tetracyclines seems to be restricted to fast preferential and macropore flow or to be facilitated by cotransport with mobile

colloids such as dissolved organic matter (Thiele-Bruhn, 2003). Most antibiotics are adsorbed very fast. Their antibiotic potency is mostly decreased by sorption and fixation, but that does not necessarily mean a complete elimination of the antimicrobial activity (Sengeløv et al., 2003). However, experimental studies on the antimicrobial activity of soil-bound tetracycline and tylosin have shown that even though these compounds are tightly adsorbed by clay particles, they remain active, showing antimicrobial effects that may influence the selection of antibiotic resistant bacteria in the terrestrial environment (Chander et al., 2005).

A detailed overview of the sorption of veterinary pharmaceutical in soils is represented by Tolls (2001). If the application of contaminated manure on soil exceeds the degradation rate of antibiotics, an accumulation of this compound has to be expected. In general, the concentration of antibiotics in certain soil layers is termed as terracumulation (Rooklidge, 2004). These sorbed compounds pose a reservoir of pollutants that can be mobilized in soils and further contaminate ground water by leaching or by erosion to surface waters (Pedersen et al., 2003). To date, only a few studies on mobility and transport of antibiotics in soil exist. Alder et al. (2001) reported diffuse contamination of surface water after antibiotic leaching from agricultural soils. Examinations of ground water and leachate from fields with intensive livestock production and manuring detected none or only antibiotics in small numbers (Hirsch et al., 1999; Kemper et al., 2007).

Column experiments showed, depending on the soil type, the retention of the adsorbing tylosin at different depths, while olaquindox, only weakly adsorbing, leached through the columns and oxytetracycline was not transported at all into deeper soil segments as this compound is strongly adsorbed to soil (Rabølle and Spliid, 2000). For oxytetracyclines and tylosin, distribution

coefficients in manure are smaller than in soils (Loke et al., 2002). A lowering effect of manure was also detected for sulphachloropyridazine: the distribution coefficient decreases with an increasing proportion of manure in soils, mainly due to the pH effect of alkalinic manure (Boxall et al., 2002).

For tetracyclines, a detection at soil depths of up to 30 cm over long time periods was described by Hamscher et al. (2002). This data demonstrated that tetracyclines not only occur in significant amounts in soil after fertilisation with liquid manure, but also persist and accumulate in the environment. This strong binding to soil-organic matter is based on the ability of the tetracyclines to form complexes with double-charged cations, such as calcium, which occur in high concentrations in soil (Samuelsen et al., 1992). The decomposition of antibiotics is driven by many factors. Photodegradation, as described for fluorchinolones, tetracyclines and sulphonamides (Sengeløv et al., 2003), does not play a major role since the influence of light is reduced when antibiotics are protected in sludge or slurry. Degradation in soil is mainly brought about by microbial activity, especially enzymatic reactions, transforming the parent compound via hydroxylation and oxidative decarboxylation (Al-Ahmad et al., 1999). Even though these reactions are reversible, antibiotics usually further degrade in manure and soil (Ingerslev and Halling-Sørensen, 2000).

Biodegradation in soil increases when manure or sludge with high numbers of microorganisms is added (Ingerslev and Halling-Sørensen, 2001). The soil serves as a vast reservoir for micro-organisms. High numbers of bacteria, ranging from 10^6 g^{-1} in agricultural soils to as high as 10^9 g^{-1} in forest soils, are important in maintaining mineral immobilisation and decomposition processes (Nwosu, 2001). Antibiotics are affected in two ways: on the one hand, the microbial

community can be severely disturbed by antibiotic activities, on the other hand, these environmental bacteria can acquire and provide gene-encoding resistance. Some soil micro-organisms possess a natural tolerance towards antibiotics (Esiobu et al., 2002). Especially Pseudomonas spp. often have an intrinsic resistance to antimicrobials (Sengeløv et al., 2003).

In other bacteria, antimicrobial-specific resistance develops after the application of antibiotics. For instance, soil micro-organisms became resistant after the application of tetracycline-contaminated manure (Frund et al., 2000). Regarding this promotion of resistance, an overview of the acquisition of genes coding for resistance in soil bacteria is presented by Seveno et al. (2002). Furthermore, in most cases, resistance is not provoked in the soil, but directly in the faeces and then spread to the soil by manuring. In Sweden and the Netherlands, the analysis of numerous faecal samples revealed a high prevalence of bacteria resistant against various antibiotics (Van den Bogaard et al., 2000).

After manure fertilisation, tetracycline-resistant bacteria increased in soil as well as in ground water, but declined again after the cessation of slurry application to a level of non-slurry fertilised soil within eight months (Sengeløv et al., 2003). The ability to take up DNA from the environment occurs quite commonly among environmental isolates, as shown in a review of the role of natural transformation in the transfer of bacterial resistance (Lorenz and Wackernagel, 1994). Mobile genetic elements, transferring resistance from non-pathogenic to pathogenic bacteria, especially the important bacterial IncQ plasmid, were detected in microbial communities of environmental habitats and pig manure (Smalla et al., 2000; Smalla and Sobecky, 2002). The transfer of antibiotic resistance determinants is elucidated by Nwosu (2001).

2.3.2 ANTIBIOTICS IN WATER

The demand for the Earth's limited supplies of freshwater, especially against the background of a continued exponential growth in human population, has resulted in the claim for the protection of the integrity of water resources (Kolpin et al., 2002).

In recent years, the occurrence and fate of antibiotics in the aquatic environment have been subjects to many investigations carried out in several countries. More than 30 antibiotic substances have been found in sewage influent and effluent samples, in surface waters and even ground and drinking water.

Regarding antibiotics in animal husbandry, administered drugs, their metabolites or degradation products reach the aquatic environment by the application of manure or slurry to areas used agriculturally, or by pasture-reared animals excreting directly on the land, followed by surface run-off, driftage or leaching in deeper layers of earth. In this way, soils can act as a source of antibiotic contamination of the aquatic environment (Alder et al., 2001).

Most of the antibiotics are partially water-soluble and therefore about 90% of one dose can be excreted in urine and up to 75% in animal faeces (Halling-Sørensen, 2001). According to Sarmah et al. (2006), occurrence of antibiotic residues in streams, lakes or other aquatic environment in the US is not unlikely, when given an estimate of 100,000 million kg of faeces and urine being produced annually by the 60 million hogs raised in the US and given that the use of their waste as fertiliser is a common practice. Besides this, animal-used antimicrobial compounds can also be released into the environment via aquaculture. Residuals and resistant bacteria from

aquaculture operations have been reported during recent years (Teuber, 1999; Olsen et al., 2001; Rice, 2001).

The qualitative and quantitative influence of antibiotics on the resident marine bacterial community was summarised by Nygaard et al. (1992). The effects of sub-inhibitory concentrations against non-marine aquatic bacteria are mainly unknown, but the impact of various antibiotics remaining active against bacteria living in wastewater has been documented (Kümmerer, 2003).

In wastewater and sewage treatment plants, resistant and multi-resistant bacteria have been detected, possibly entering the food chain directly via sewage sludge used as fertiliser or wastewater serving for irrigation (Guardabassi et al., 1998; Witte, 1998; Feuerpfeil et al., 1999; Kümmerer, 2003).

Antibiotic effects on organisms living in the aquatic environment such as algae and daphnids (Daphnia magna) have been reported at concentrations between 5 and 100 mg/l (Holten-Lützøft et al., 1999; Wollenberger et al., 2000). Few studies have dealt with the impact of antibiotic use on populations of bacteria, especially indicator bacteria such as enterococci, in natural waters (Wiggins et al., 1999; Goni-Urriza et al., 2000). Under test conditions in aquatic systems, most of the examined antibiotic compounds have been persistent, while only few have been partially biodegraded (Al-Ahmad et al., 1999; Kümmerer et al., 2000). To figure out the occurrence of antimicrobial drugs in surface waters and effluents from municipal sewage treatment plants, several studies have been conducted place in Germany (Christian et al., 2003), Switzerland (Alder et al., 2001; Golet et al., 2001), and the US (Lindsey et al., 2001; Kolpin et al., 2002).

Penicillins and tetracyclines are not usually expected to be found in the aquatic environment due to the easy hydrolysation of penicillins and the precipitation and accumulation of tetracyclines, as described above. The structure of β-lactams such as penicillin, benzylpenicillin or cloxacillin, consisting of the β-lactam-ring, contribute to the poor stability of this group in the environment: the ring can be opened by β-lactamase, a widespread enzyme in bacteria, or by chemical hydrolysis. Thus, intact penicillins are usually not found in the environment (Myllyniemi et al., 2000). Neither tetracyclines nor tylosin were detected in any water sample by Hamscher et al. (2002).

Further confirmation of these findings is supported by Lindsey et al. (2001) and Zhu et al. (2001). However, these substances have been detected in low levels in U.S. surface water samples (Kolpin et al., 2002) and in higher levels in overland flow water (Krapac et al., 2005).

In Northwest Germany, a study was conducted sampling a series from surface waters, detecting a wide range of antibiotics in all samples (Christian et al., 2003): sulphonamides, macrolides and lincosamides were analysed frequently, whereas β-lactams were rarely found. Tetracyclines were not detected because of their strong adsorption to organic matter. The presence of tetracycline-resistant bacterial isolates in lagoons and groundwater underlying two swine production facilities was published by Chee-Sanford et al. (2001).

MacKie et al. (2006) detected both tetracycline residues and tetracyclineresistance genes in groundwater impacted by swine production facilities. However, antibiotic input by agricultural use is the minor origin of antimicrobials in the aquatic environment. Most of the analysed substances originated from discharge of sewage into rivers, only for a couple of samples could an influence of animal husbandry on the occurrence of antibiotics in surface waters be assumed.

The major part of antibiotic input is carried out by human administration via hospital effluents or municipal wastewater, as reviewed by Kümmerer (2001a).

2.4 USAGE

2.4.1 AFRICA

Data on the consumption of antibiotics by food-producing animals in African countries are lacking. However, Mitema et al. (2001) assessed antimicrobial consumption in Kenya by collating data between 1995 and 1999 from the official record of the Pharmacy and Poisons Board of the Ministry of Health. Their study revealed that approximately 14600 kg of active antimicrobials are used in animal food production in Kenya, of which, tetracyclines and sulphonamides + trimethoprim account for nearly 78% of the use (56% and 22%, respectively). The authors further concluded that no antibiotics were used as growth promoters in Kenya, although speculation suggests some soluble tetracyclines and sulphonamides soluble powders or solutions are used as growth promoters. In other African countries such as the United Republic of Tanzania and Uganda, veterinary antimicrobials are easily accessible and under low levels of control from government authorities (WHO, 2001).

2.4.2 THE USA

In the United States information on the total annual production and use of pharmaceuticals including antibiotics is generally not available. Thus, estimates on the annual production and usage of antibiotics for human health and agriculture are controversial (Mellon et al., 2001; AHI, 2002). A recent report by Isaacson and Torrence (2002) based on a colloquium held by the

American Academy of Microbiology in Santa Fe, New Mexico, outlined the confusion estimating the amount of antibiotics produced and changes in their usage.

Antibiotics are routinely used at therapeutic levels in livestock operations to treat disease and at sub-therapeutic levels (<0.2 g kg^{-1}) to increase feed efficiency and improve growth rate (Kiser, 1976; Cohen, 1998). According to the UCS (Union of Concerned Scientists), in their report Hogging it, of the estimated 16 million kg of antimicrobial compounds used annually in the US, approximately 70% are used for non-therapeutic purposes (UCS, 2001). Antibiotics used in animal feeding in the US have increased from nearly 91000 kg in 1950 to 9.3 million kg in 1999 (AHI, 2002), which is a slight increase from the 1998 total of 8.1 million kg. Of the 9.3 million kg of antibiotics used, about 8 million kg were used for treatment and prevention of disease and only 1.3 million kg were used for improving feed efficiency and enhancing growth. This increase from 1998 to 1999 is largely attributed to greater use of ionophores and arsenicals, which increased 1.1 million kg from 1998 to 1999 (AHI, 2002). While arsenicals and ionophores are classes of pharmaceuticals not used in human medicines, there are some important pharmaceuticals that are used in both animal and human medicines. Fig. 2.6 shows the reported pharmaceuticals in the US in 1999 by the AHI.

A USDA survey (1996) indicated that about 93% of all grower/finisher pigs in the US received antibiotics in their diets at some time during the grower/finisher period. According to Swine'95 study (NAHMS, 1996), pork producers

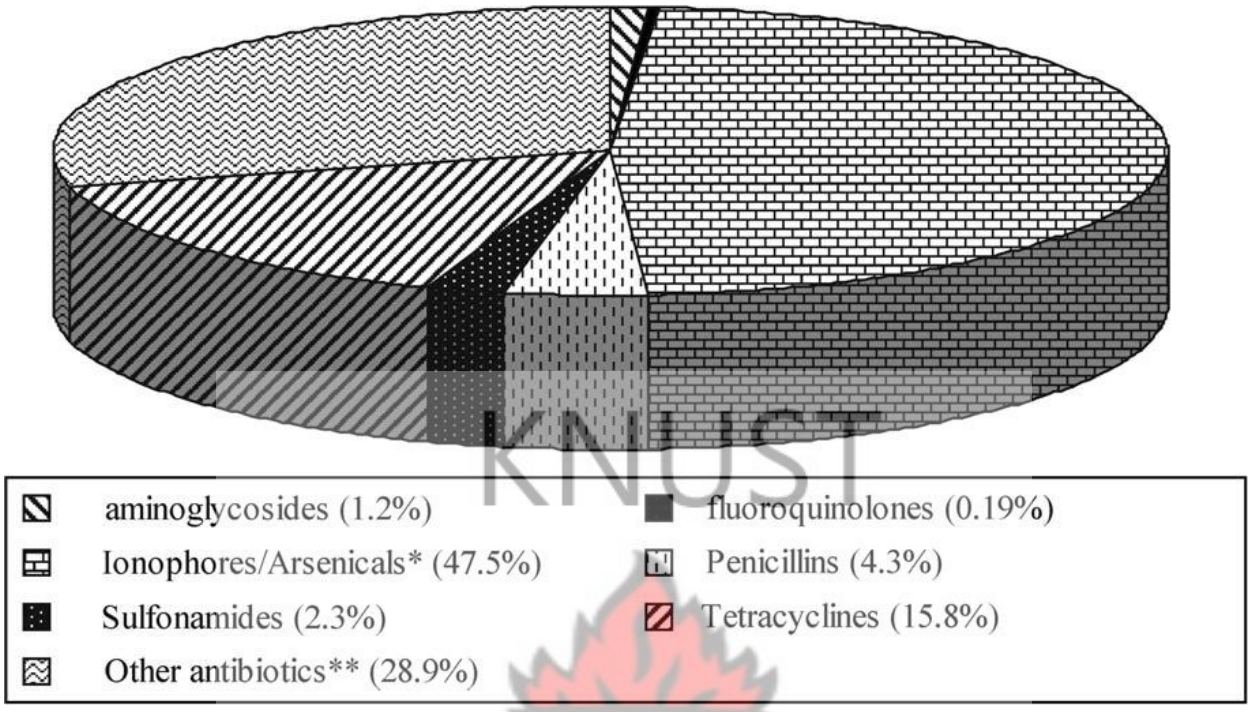


Figure 2.6 Antibiotics use reported in millions of kilograms by AHI (Animal Health Institute) survey in 1999. Amounts shown in parentheses indicate percentages of total antibiotics (* denotes the antibiotics developed for animal production and not related to traditional antibiotics, ** includes cephalosporins, macrolides, lincosamides, polypeptides, streptogramins, and other minor compounds). (Ajit et al., 2006)

used feed antibiotics much more commonly than antibiotics administered in water. They found that 91% of all operations used antibiotics in feed for disease prevention during the grower/finisher phase of production. Regionally, use of antibiotics in feed varied from 80.0% in the Southeast to 95.1% in the Midwest. The three most frequently used antibiotics in swine productions dentified in 1996 in the US were tylosin (30.4%), chlortetracycline (40%), and bacitracin (52.1%). These compounds were fed to swine for 2–2 and half months during their production cycle. The values in brackets in the preceding sentence indicate the percentages of producers using antibiotics for disease prevention in grower/finisher rations.

A 1998 survey conducted by the Animal Health Institute (AHI) reported there were 109 million cattle, 7.5 billion chickens, 92 million swine, and 292 million turkeys in the US (AHI, 2002) and

a 2002 survey conducted by the National Agricultural Statistics Service (NASS) reported 104 million cattle, 8.6 billion chickens, 60 million swine, and 275 million turkeys in the US (NASS, 2002). The annual production of food-producing animals also leads to a large volume of agricultural waste. The USDA estimated that in 1997 meat-producing animals excreted approximately 1400 billion kg of waste (Horrigan et al., 2002). A significant portion of food-producing animals in the US is raised in confined animal-feeding operations (CAFOs). CAFOs are livestock-raising operations, such as hog, cattle, dairy, and poultry farms, where animals are kept and raised in confined situations. Under the Clean Water Act, CAFOs are defined as point sources of pollution and are therefore subject to National Pollutant Discharge Elimination System (NDPES) permit regulations. Under these regulations, CAFOs are defined as facilities with 1000 animal unit (AU). According to one report there are currently more than 6600 AFOs in the US that have >1000 animals and are classified as CAFOs (USDA, USEPA, 1998). In the case of matured hogs, the number is between 1250 and 2550, with each animal weighing nearly 25 kg in body weight under the EPAs two- and three-tier structures. CAFOs are a rapidly growing sector of the US agricultural economy. An estimated 376000 livestock operations confine animals in the US, generating approximately 128 billion pounds of manure each year (USEPA, 2000). CAFOs are the largest of these livestock operations and are regulated under the Clean Water Act. Given that the total production of antibiotics in the US now stands at more than 22 million kg annually, with about one-half being used for agriculture (Levy, 1998), and considering a significant fraction of animals are produced in CAFOs, the usage of antibiotics in CAFOs is in the order of millions of kilograms each year. The majority of swine CAFOs and cattle feedlots store their liquid and solid waste in large lagoons or in concrete pits. The waste lagoons can cover several acres and hold millions of liters of liquid and solid manure. The

majority of these operations depend on anaerobic digestion. In general, the liquid manure is pumped from the lagoons and applied to agricultural fields as fertilizer. In the US, most states now require that the lagoons are lined to prevent or minimize leakage and the levels of these waste-storage structures must also be maintained to ensure that the dams are not breached. Thus, there are times when manure may be applied that do not coincide with agricultural crop needs. In most poultry operations the litter is stored in piles to compost. The composted litter can be applied to fields at rates up to 3 tons per acre. The amount of time that the litter is composted is variable. Most of the animal waste from CAFOs is applied to fields within 10 miles of where the manure was generated. Thus, in many cases the degree of application exceeds the capacity of the soil with respect to nutrients (Kellogg et al., 2000).

In addition the veterinary pharmaceuticals contained in the waste may be applied to soil, that has not fully processed the manure from the last application. A report from the USDA (1996) indicates nearly 98% of swine operations with 300 or more hogs dispose manure on land owned or privately rented by the operation. The duration of conservation and subsequent field application standards depend on the national legislative regulations in the US. Most hog CAFOs use one of three waste handling systems: flush under slats, pit recharge, or deep under-house pits. Flush housing uses fresh water or recycled lagoon water to remove manure from sloped floor gutters or shallow pits. The flushed manure is stored in lagoons or tanks along with any precipitation or runoff that may come into contact with the manure. Flushing occurs several times a day. Pit recharge systems are shallow pits under slatted floors with 15–25 cm of pre-charge water. The liquid manure is pumped or gravity fed to a lagoon approximately once a week. Deep pit systems start with several centimeters of water, and the manure is stored under the house until it is pumped out for field application on the order of twice a year. Most large

operations have 90–365 days storage, and the deep pit system uses less water, creating slurry that has higher nutrient concentrations than the liquid manure systems. This type of slurry system is more common in Midwestern states and the cooler climates in the US (USEPA, 2000). Given an estimate of 100000 million kg of faeces and urine being produced annually by the 60 million hogs raised in the US (Meadows, 1999), and given the land application of animal waste as a source of fertilizer in agricultural sector is a common practice, occurrence of antibiotic residues in streams, lakes or other aquatic environment is not unlikely.

2.4.3 THE UK/EUROPEAN UNION (EU)

In the UK, certain classes of antibiotics are incorporated into the feed of animals in order to improve their growth rates. According to the Veterinary Medicine Directorate (VMD, 2001), the antibiotics are sold as prescription-only medicines (POMs), general sales list (GSL) medicines and pharmacy and merchant list (PMLs) medicines. Tetracyclines are the most widely used antibacterial compounds, followed by sulphonamides, β-lactams, macrolides, aminoglycosides, fluoroquinolones and others. Sulphonamides are the second most widely used veterinary antibiotics in the UK, accounting for nearly 21% of total sales (Ungemach, 2000). From 1993 to 1998, sales of antimicrobial growth promoters in the UK remained largely static.

However, after 1998 there was a 69% decrease in sales, and at present, out of 448000 kg of antimicrobials, 28000 kg are used as growth promoters in animals. Although usage data on individual antimicrobial compounds used as growth promoters in the UK are limited, according to International Medicinal Statistics (IMS), 7.5 kg of monensin was used in 2000. It is likely that this number is an underestimate of the total sales of growth promoters, as most of the products

are classified as zootechnical feed additive or pharmacy and merchant only list medicine (EA, 2001).

Other compounds identified as potentially major use growth promoters in the UK include flavophospolipol and salinomycin sodium. The use of antibiotics as growth promoters in the European Union is subject to Directive 70/524/EEC, covering additives in feeding stuffs and also includes a requirement that at the level permitted in animal feed does not adversely affect human, animal health, or the receiving environment (EU Directive 70/524/EEC, 1970). Total amounts of antibiotics used for animal health in EU member states are available from respective national authorities. While usage data have been made available only in Sweden, Denmark and Finland and to a lesser extent – the Netherlands, little or no information on usage and trends of antibiotics sales is available from countries such as Austria, Belgium, France, Germany, Greece, Ireland, Italy, Luxemburg, Portugal, Spain and the UK (EMEA, 1999). Antibiotics together with other compounds (anthelmintics or parasiticides) are the most important groups of veterinary pharmaceuticals, both with a market volume of more than 200 million Euros alone in 1999 (Tolls, 2001).

It has been reported that of the total usage of 5 million kg of antibiotics, 3.5 million kg are used for therapeutic purposes (Kay and Boxall, 2000), while the remaining 1.5 million kg are used as feed additive for growth promotion (Alder et al., 2000). Sweden, the first to ban the use of antimicrobial growth promoters in 1986, claimed numbers of antibiotic resistant bacteria remained lower than its neighbours and other countries during the period 1986–1995 (Wierup, 2001). Following the bans on growth promoters by Sweden in 1986, Denmark banned the use of avoparcin as growth promoter in 1995. In the following years, virginiamycin, tylosin, bacitracin,

spiramycin, carbadox and olaquindox were banned as growth promoters in the EU. Following the official ban on the growth promoter virginiamycin in January 1998 by the EU, the Danish food-animal industries decided to voluntarily discontinue all further use of antimicrobial growth promoters in broilers, slaughter pigs and cattle in February and March 1998 (DANMAP, 2000). This resulted in a dramatic decrease in antibiotic use and by 2000, the use of growth promoters in Danish food animals were nil. The report of the DANMAP (Danish Integrated Antimicrobial Resistance Monitoring and Research Program) is available in English at www.svs.dk/uk/Organization/Frm_org.htm. Germany also banned the use of avoparcin as growth promoters in animals in 1996. According to a recent report published in the American Association of Swine Veterinarian's electronic newsletter in December 2005, the use of all growth promoters in pigs will be banned in the EU from 1st January 2006 (Burch, 2006). These growth promoters include avilamycin, flavophospholipol and the ionophores monensin for cattle and salinomycin for pigs in addition to previously banned growth promoters.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY AREA

The geographical coordinates of Kumasi are 6° 41' 0" North, 1° 37' 0" West.

The Kumasi Metropolitan area has an approximate area of 254 square kilometres and it is located between latitudes $6^0$35" and $6^0$4"N and longitudes $1^0$30" and $1^0$35" E. It shares boundaries with the Kwabre District to the north, Atwima Kwanwoma and Atwima Nwabiagya District to the west, Ejisu-Juaben Municipality to the east and Bosomtwe District to the south.

3.2 COLLECTION OF MEAT SAMPLES

Beef and mutton were bought between October and November 2013 from three different markets in the Kumasi metropolis and transported to the laboratory in ice chest. In total sixty samples were collected or bought from the three different markets over a period of six weeks. The markets are Central market, Asafo market, Abattoir or Mayanka market. At each market ten samples of beef and mutton each were bought using the systematic and random sampling methods. All samples were stored in the deep freezer at -19^{o}C until time for analysis.

3.3 SAMPLE PREPARATION/EXTRACTION

The raw meat (beef and mutton) was ground in a blender and then 5 g of this was taken and put into a 50 ml capped polypropylene centrifuge tube. 15 ml of acetonitrile/double distilled water in the ratio 15:2 was then added. It was homogenized completely using a homogenizer for one minute. After this it was centrifuged at 4,000 rpm at 4^{o}C for 5 minutes. The supernatant was decanted and stored. 10 ml acetonitrile/double distilled water in the ratio 15:2 was added to

residue and mixed thoroughly with a spatula to resuspend the ground meat. The homogenization was repeated for one minute and centrifuged at 4,000 rpm at 4^{o}C for 5 minutes. The supernatant was again decanted and stored. The procedure was repeated and the supernatants combined to the previous two supernatants.

Due to the sensitivity of the experiment the combined supernatants was allowed to air dry (37^{o}C) instead of evaporating with heated source. Approximately 6 ml of solvent remained in the flask to which phosphate buffer of pH 8.5 was added to make the volume 20 ml. After this it was filtered and 10 ml of the sample extract was loaded onto the cartridge.

The cartridge was previously conditioned with 3 ml methanol and equilibrated with 3 ml 0.1% formic acid in water. After the loading of the sample it was washed with 2 ml 0.1% formic acid in water and 2 ml phosphate buffer of pH 8.5 in water. This was allowed to dry with 3 ml acetonitrile. Finally it was dried, reconstituted in mobile phase and vortexed. This was then transferred to the autosampler vial for HPLC analysis.

3.4 PREPARATION OF 0.01 M PHOSPHATE BUFFER

A mass of 18.0 g anhydrous Na_2HPO_4 was weighed and placed into a 1 L volumetric flask and dissolved completely in deionized water. It was diluted to volume and filled to the mark. 10 % dilution of this was made. The pH was 7.4 and was adjusted to 8.5 with 0.1 M NaOH.

3.5 HPLC CONDITIONS

Flow Rate: 1.4 ml/min

Column Used: C_{18}

Wavelength: 360 nm

Mobile Phase A: 0.2% Formic acid

B: Acetonitrile

C: Methanol

In the ratio, (A: B: C) = (60:20:20)

Oven Temperature: 35^{o}C

Type of Elution: Isocratic

3.6 CHEMICALS/REAGENTS

HPLC grade acetonitrile (Fisher Scientific, UK)

HPLC grade methanol (Fisher Scientific, UK)

Double distilled and deionized water

HPLC grade n-hexane (Fisher Scientific, UK)

Anhydrous Potassium dihydrogen phosphate (KH_2PO_4) HPLC grade (Fizmerk Chemicals India)

HPLC grade Phosphoric acid/Formic acid (Fisher Scientific UK)

Disodium hydrogen phosphate (Na_2HPO_4) HPLC grade (Fizmerk Chemicals India)

All other chemical reagents were of analytical grade.

3.7 PURIFICATION

10 ml of the extract was loaded onto the conditioned and equilibrated cartridge. The cartridge was washed with 0.1% formic acid in water and then pH 8.5 potassium phosphate buffer. Finally, the sample was eluted with 3 ml acetonitrile. The sample was filtered with a 13mm, 45µm PTFE syringe filter (Agilent p/n 5185-5836). The eluent was dried. 10 ml of residue was re-suspended in mobile phase. The sample was vortexed for 2 minutes and then transferred to a 2 ml autosampler vial (Agilent p/n 5182-0864).

3.7.1 SPE PROCEDURE

3ml methanol/ACN was conditioned

↓

3ml 0.1% formic acid in water was equilibrated

↓

10ml of 5g sample extract (20ml final volume) was loaded

↓

2ml 0.1% formic acid in water was washed

↓

2ml phosphate buffer pH 8.5 in water was washed

↓It was dried with 3ml ACN for 3 minutes

It was dried, reconstituted in mobile phase and vortexed

3.8 HEALTH RISK ASSESSMENT ASSOCIATED WITH THE CONSUMPTION OF BEEF AND MUTTON IN KUMASI METROPOLIS

The health risk posed by the consumption of beef and mutton from the three markets mentioned above by the antibiotics under studied (Chloramphenicol, Oxytetracycline and Penicillin G) was assessed.

The level of concern (LOC) is a threshold concentration of a chemical above which a hazard to human health may exist, was calculated as the ratio of Tolerable Daily Intake (TDI) to the Rate of Meat Consumption (RMC). Risk quotient (RQ) was calculated as the ratio between concentration of antibiotic in the beef and mutton to the level of concern (LOC) for the antibiotics.

$$\text{Level of Concern (LOC)} = \frac{Tolerable\ Daily\ Intake\ (TDI)}{Rate\ of\ meat\ consumption\ (RMC)} \quad \text{- Equation 1}$$

$$\text{Risk Quotient (RQ)} = \frac{Concentration\ of\ antibiotic\ in\ meat}{Level\ of\ concern\ (LOC)} \quad \text{-Equation 2}$$

The data on national rate of meat consumption (RMC) was calculated from the Empirical Economic Letters which estimates the daily food supply from beef and mutton in Ghana to be 0.065 kg/person/day or 65 g/person/day (Osei-Asare, 2014).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 CHLORAMPHENICOL RESIDUE IN BEEF AND MUTTON

Out of a total of 30 beef samples analysed during this study, 24 (80%) comprising of 8 (33.3%) from Central Market, 8 (33.3%) from Asafo Market and 8 (33.3%) from Central Abattoir Market had detectable levels of chloramphenicol (CAP) residues, while the rest of 6 samples (20%) did not show any CAP residue. Antibiotic penicillin G was not detected in any of the samples. The mean beef residue levels of chloramphenicol were 217.92±167.83 mg/kg, 213.19±76.86 mg/kg, 164.36±51.25 mg/kg in Central Market, Asafo Market and Central Abattoir Market, respectively, while the ranges of chloramphenicol were 93.58 to 508.10 mg/kg, 84.64 to 349.53 mg/kg, 81.42 to 254.29 mg/kg in Central, Asafo and Central Abattoir Market, respectively (Table 4.1). For the 30 mutton samples analysed, 25 (83.33%) comprising of 9 (36%) from Central Market, 8 (32%) from Asafo Market and 8 (32%) from Central Abattoir Market had detectable levels of chloramphenicol, while the remaining 5 samples (16.67%) had no detectable residues. All the positive beef and mutton samples recorded chloramphenicol levels above the maximum residue limits (0.3 µg/kg). Mutton samples yielded higher concentration result of the residue than beef samples at Central Abattoir Market for chloramphenicol samples (Figure 4.1). The mean mutton residue levels of chloramphenicol were 259.63±199.53 mg/kg, 154.16±60.62 mg/kg, 270.22±172.42 mg/kg in Central, Asafo and Central Abattoir Markets, respectively. The ranges for chloramphenicol residue levels from the individual markets were: 73.38 mg/kg to 301.21 mg/kg in Central Market; 100.00 mg/kg to 281.99 mg/kg in Asafo Market and 92.68 mg/kg to 574.63 mg/kg in Central Abattoir Market for mutton residues. Mean chloramphenicol residue levels in mutton samples from the three markets were not significantly different ($p>0.05$) using

one-way ANOVA, however, chloramphenicol residue levels in beef samples were significantly different ($p<0.05$) as shown in Table 4.3. The mean, range and numbers of the samples (beef and mutton) positive for chloramphenicol residues are shown in Tables 4.1 and 4.2.

Table 4.1: The mean (mg/kg), range (mg/kg), and number of samples (beef and mutton) positive for chloramphenicol residues

Slaughter houses		Tissue types	
		Beef	Mutton
Central Market	Positive	8/10 (80%)	9/10 (90%)
	Mean	217.92 $mgkg^{-1}$	259.63 $mgkg^{-1}$
	Range	95.58-508.10 $mgkg^{-1}$	73.38-301.21 $mgkg^{-1}$
	Standard deviation	167.83 $mgkg^{-1}$	199.53 $mgkg^{-1}$
Asafo Market	Positive	8/10 (80%)	8/10 (80%)
	Mean	213.19 $mgkg^{-1}$	154.16 $mgkg^{-1}$
	Range	84.64-349.53 $mgkg^{-1}$	100.00-281.99 $mgkg^{-1}$
	Standard deviation	76.86 $mgkg^{-1}$	60.62 $mgkg^{-1}$
Central Abattoir	Positive	8/10 (80%)	8/10 (80%)
	Mean	164.36 $mgkg^{-1}$	270.22 $mgkg^{-1}$
	Range	81.42-254.29 $mgkg^{-1}$	92.68-574.63 $mgkg^{-1}$
	Standard deviation	51.25 $mgkg^{-1}$	172.42 $mgkg^{-1}$

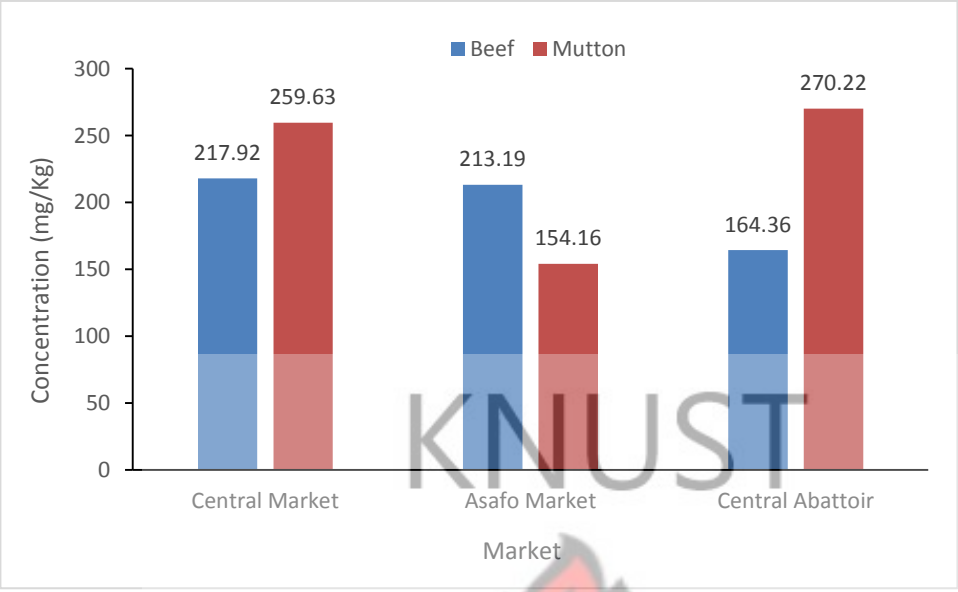


Figure 4.1: Mean detectable concentrations (mg/kg) of chloramphenicol in beef and mutton samples

4.2 OXYTETRACYCLINE RESIDUES IN BEEF AND MUTTON

Out of the 30 beef samples analysed during this study, 15 (50%) comprising of 5 (33.33%) from Central Market, 5 (33.33%) from Asafo market and 5 (33.33%) from Central Abattoir had detectable levels of oxytetracycline residues while remaining 15 samples (50%) had no detectable residues. The recommended maximum residue limits (MRLs) for oxytetracycline are 200 µg/kg, 600 µg/kg and 1200 µg/kg in muscle (beef), liver and kidney respectively (FAO, 1999). Out of the positive samples, 15 samples which showed residues of oxytetracycline above maximum residue limits (MRLs) were 4 (26.67%) in beef samples from Central Abattoir. At Central and Asafo Markets no samples recorded residue above the maximum residue limits. The mean beef residues of oxytetracycline are 86.18±17.20 mg/kg, 87.17±13.07 mg/kg, 480.25±238.83 mg/kg for Central, Asafo and Central Abattoir Markets, respectively. The ranges

of oxytetracycline in the beef tissue are 68.70-110 mg/kg, 70.72-100 mg/kg and 70.12-658.22 mg/kg in Central, Asafo and Central Abattoir Markets, respectively.

For the 30 mutton samples analysed, 15 (50%) comprising of 5 (33.33%) each from Central Market, Asafo market and Central Abattoir had detectable levels of oxytetracycline residues while remaining 15 samples (50%) had no detectable residues. Out of the positive samples, 5 (33.33%) had oxytetracycline residue at violative levels while 10 (66.67%) had residue levels below the maximum residue limits (MRLs) for oxytetracycline. The mean Oxytetracycline residues in mutton are 181.13±53.33 mg/kg, 239.70±141.65 mg/kg and 105.08±57.68 mg/kg for Central, Asafo and Central Abattoir Markets, respectively. The ranges of oxytetracycline in the mutton tissue are 110.35-240.99mg/kg, 87.41-400.45mg/kg and 75.88-190.88mg/kg for Central, Asafo and Central Abattoir Markets, respectively. Beef samples had higher concentration of residue than mutton samples at Central Abattoir Market for oxytetracycline (Figure 4.2). There were significant difference ($p<0.05$) in the mean oxytetracyline residue levels in beef samples from the three markets (Table 4.3). Mean oxytetracyline residue levels in mutton samples from the three markets were not significantly different ($p>0.05$).

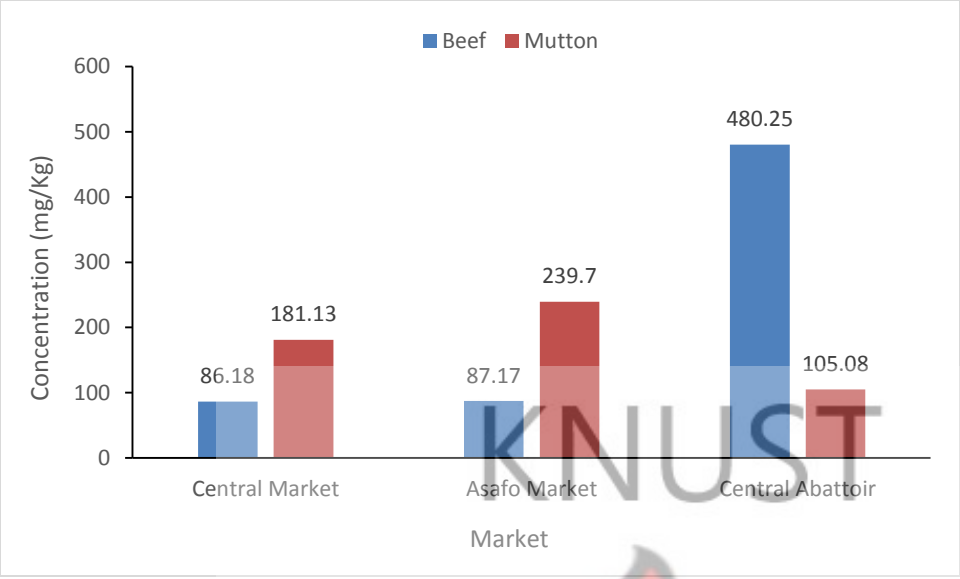


Figure 4.2: Mean detectable concentrations (mg/kg) of oxytetracycline in beef and mutton samples

Table 4.2: The mean (mg/kg), range (mg/kg), and number of samples (beef and mutton) positive for oxytetracycline residues

Slaughter houses		Tissue types	
		Beef	Mutton
Central Market	Positive	5/10 (50%)	5/10 (50%)
	Mean	86.18 $mgkg^{-1}$	181.13 $mgkg^{-1}$
	Range	68.70-110.00 $mgkg^{-1}$	110.35-240.99 $mgkg^{-1}$
	Standard deviation	17.20 $mgkg^{-1}$	53.33 $mgkg^{-1}$
Asafo Market	Positive	5/10 (50%)	5/10 (50%)
	Mean	87.17 $mgkg^{-1}$	239.70 $mgkg^{-1}$
	Range	70.72-100.00 $mgkg^{-1}$	87.41-400.45 $mgkg^{-1}$
	Standard deviation	13.07 $mgkg^{-1}$	141.65 $mgkg^{-1}$
Central Abattoir	Positive	5/10 (50%)	5/10 (50%)
	Mean	480.25 $mgkg^{-1}$	105.08 $mgkg^{-1}$
	Range	70.12-658.22 $mgkg^{-1}$	75.88-190.88 $mgkg^{-1}$
	Standard deviation	238.83 $mgkg^{-1}$	57.68 $mgkg^{-1}$

The study further revealed that out of the 15 positive samples for oxytetracycline residue in mutton 2 (40%) and 3 (60%) and 2 (40%) in Central Market and Asafo Market had residues of oxytetracycline above MRLs. At Central Abattoir Market no samples were above the maximum residue limits.

Table 4.3: Correlation between the mean detectable concentrations (mg/kg) of oxytetracycline and chloramphenicol residues in beef and mutton samples

	Beef (chlo.)	Beef (oxy.)	Mutton (chlo.)	Mutton (oxy.)
Beef (chlo.)	1.000			
Beef (oxy.)	-0.997*	1.000		
Mutton (chlo.)	-0.502	0.568	1.000	
Mutton (oxy.)	0.864	-0.900	-0.870	1.000

* Correlation is significant at the 0.05 level (2-tailed)
oxy. – oxytetracycline; chlo. – chloramphenicol

Oxytetracyline and chloramphenicol have been used as an important class of antibiotics in animal food. As such, they have also been a source of concern for residue monitoring authorities around the world. In response to this concern the World Health Organization and the Food and Agriculture Organization (FAO, 1999) joint committee on residues of some veterinary drugs in animals and foods recommend maximum residue limits for various drugs in edible tissue of food animals. About 26.67% and 33.33% in beef and mutton samples, respectively contain residue levels above recommended standard. Organs of metabolism and excretion of the drugs are delicacies to some meat consumers putting greater risk of accumulation of residues in this group of consumers. The total prevalence (50%) obtained in this study is higher than the 16.11% in cattle (Dipeolu and Alonge, 2002) and 33.1% obtained in broilers (Kabir *et al*., 2004) in Nigeria and higher than 45.6% oxytetracycline residues reported from slaughtered cattle in Kenya by

Muriuki *et al*., (2001). This could also be a result of the higher sensitivity and specificity of HPLC in quantitation of drug residues than the microbial inhibition techniques used by the previous authors in Nigeria. The high prevalence (50%) of this study may be an indication of widespread misuse of veterinary drugs by food animal producers across Kumasi, since these animals were sourced from different parts of the city. This may be due to the fact that greater proportion of cattle reared in Ghana are done by the nomadic herdsman who have access to veterinary drugs and always purchased drugs over the counter for administration to their animal without veterinary prescription and supervision. This is also an indication of lack of adequate veterinary public health regulatory control in the country. This impose great risks and hazards to human health that could result in allergy, cancer, embryo toxicity and antibiotic resistance effects on the consumers. Literature (Kabir *et al*., 1999; Aliu *et al*., 2001) reported the socio-economic implications of drugs and chemical residues in carcasses as resulting in physico-chemical changes in meat lead to condemnation and severe economic losses by the stakeholders. This is also a critical factor in international meat trade that can deprive the country earnings from meat products on the international market.

4.1 HUMAN CONSUMPTION LEVELS

In order to determine the point at which a hazardous substance presents a health risk, food safety experts have developed the concepts of the maximum residue limit (MRL). This is the amount of residue considered to have no significant and toxicological risk for human health. MRLs are based on “acceptable daily intakes”, which in turn are typically based on “no observable adverse effects” levels derived from and in vitro trials.

When antibiotics present in beef and mutton exceed the MRLs there is a health risk associated with the consumption of such meat. Due to the absence of available data on health criteria for these antibiotics (Chloramphenicol, Oxytetracycline and Penicillin G) in Ghana the World Health Organization and the Food and Agriculture Organization (WHO/FAO, 1999) joint committee on residues of some veterinary drugs in animals and foods data were used and also the committee for veterinary medicinal product. The acceptable daily intake of Oxytetracycline is 0.03 µg/kg. The LOC which is a threshold concentration of a chemical above which a hazard to human health may occur were evaluated and compared to the maximum concentrations obtained for the three antibiotics with the exception of Penicillin G whose concentrations were not detected in the meat.

TDI- Tolerable or Acceptable Daily Intake (in µg/kg) for Chloramphenicol 0.300 µg/kg and for Oxytetracycline 0.0300 µg/kg

The - Rate of meat consumption (RMC) in Ghana was calculated from the Empirical Economic Letters which estimates the daily food supply for beef and mutton to be 65g/person/day.

LOC - Level of concern (µg/g) calculated from the TDI and RMC –shown by equation 1

RQ - Risk quotients calculated from the means and LOC – shown by equation 2

Table 4.4: Risk analysis of the mean concentration of antibiotics present in beef samples from Central, Asafo and Central Abattoir Markets.

Antibiotics	LOC (ug/g)	RQ (Central Market)	RQ (Asafo Market)	RQ (Central Abattoir Market)	TDI (ug/kg)
Chloramphenicol	4.62	0.0472	0.0461	0.0356	0.3
Oxytetracyclinw	0.462	0.1865	0.1887	1.0395	0.03

Table 4.5: Risk analysis of the mean concentrations of antibiotics present in mutton samples from Central, Asafo and Central Abattoir Markets

Antibiotics	LOC (ug/g)	RQ(Central Market)	RQ(Asafo Market)	RQ(Central Abattoir Market)
Chloramphenicol	4.62	0.0562	0.0334	0.0585
Oxytetracycline	0.462	0.3920	0.5188	0.2274

4.2 HUMAN HEALTH IMPLICATIONS FROM THE CONSUMPTION OF BEEF AND MUTTON FROM THE CENTRAL, ASAFO AND CENTRAL ABATTOIR MARKETS

Calculations of the risk associated with the consumption of beef and mutton from the three markets, Central, Asafo, and Central Abattoir was carried out to ascertain whether it poses a threat to human consumers. It is worthy to note that the evaluation of risk quotient provided a convenient way of examining the antibiotics. For cases where the RQ<1, the antibiotics involved are unlikely to cause harm to human consumers (Fung et al., 2004). The RQ's for all the samples were below 1 (thus RQ<1) with the exception of oxytetracycline in beef from the Central Abattoir Market which was just above 1 (thus RQ>1) hence the consumption of this meat is likely to cause harm to its consumers and those below 1 are unlikely to cause harm to its consumers.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

This study has shown that oxytetracycline and chloramphenicol residues of 50% and 90% prevalence respectively are present in portions of beef and mutton meant for human consumption in Kumasi metropolis. Most of the samples contained drug residues above the MRLs recommended standard. . The mean beef residues of oxytetracycline are 86.18±17.20 mg/kg, 87.17±13.07 mg/kg, 480.25±238.83 mg/kg for Central, Asafo and Central Abattoir Markets, respectively. The mean Oxytetracycline residues in mutton are 181.13±53.33 mg/kg, 239.70±141.65 mg/kg and 105.08±57.68 mg/kg for Central, Asafo and Central Abattoir Markets, respectively. The mean beef residue levels of chloramphenicol are 217.92±167.83 mg/kg, 213.19±76.86 mg/kg, 164.36±51.25 mg/kg for Central Market, Asafo Market and Central Abattoir Market, respectively. The mean mutton residue levels of chloramphenicol were 259.63±199.53 mg/kg, 154.16±60.62 mg/kg and 270.22±172.42 mg/kg for Central, Asafo and Central Abattoir Markets, respectively. The mean amounts of Oxytetracyline and chloramphenicol residues can accomplish drug resistance in consumers and perhaps in some cases, can have digestive and allergic effects. It could also change organoleptic specifications in some meat samples. Therefore, edible tissues of cattle and sheep in Kumasi do not have desired conditions because of the presence of Oxytetracyline and chloramphenicol residues more than the MRLs. Oxytetracyline and chloramphenicol residues are routinely misused in Ghanaian livestock and are deposited within their tissues at significant levels rendering most of the meat unsafe and unwholesome for human consumption.

5.2 RECOMMENDATION

1. Livestock producers in the country should be given extension education on good agricultural practices and responsible use of antibiotics in food animals; including correct diseases diagnosis, adequate dosage and observance of withdrawal periods of drugs used for treatment of food animals.
2. Regulatory authorities should also ensure proper meat inspection and drug residues surveillance program should be established in the country to ensure food safety.
3. Further studies are necessary to evaluate other drug residues of food originated from animal such as liver, chicken meat, egg and milk to evaluate the hazardous of these residues in relation with daily intakes and other related factors.
4. Effect of cooking on residual antibiotics.

REFERENCES

Abassi, M.M., Nemati, M., Babaei, H., Ansarin, M., and Nourdadgar, A. (2012). "Solid-Phase Extraction and Simultaneous Determination of Tetracycline Residues in Edible Cattle Tissues Using and HPLC-FL Method." *Iranian J. Pharma. Res*, Article 10, Volume 11, Number 3: Page 781-787

Abd El-Aty, A. M., Goudah, A., and Abo El Sooud, K. (2001). "Pharmacokinetics, intramuscular bioavailability and tissue residue profiles of ceftazidime in a rabbit model". "Cited in" *Dtsch Tierarztl Wochenschr*, 108(4): 168-71.

Addo, K. K., Mensah, G. I., Aning, K. G., Nartey, N., Nipah, G. K., Bonsu, C., Akyeh, M. L., and Smits, H. L. (2011). "Microbiological quality and antibiotic residues in informally marketed raw cow milk within the coastal savannah zone of Ghana". *Trop. Med. Int. Health*, 16(2): 227-32.

AHI, (2002). Animal Health Institute. Available from: <http://www.ahi.org/>.

Ajit, K.S., Meyer, T.M., and Boxal, B.A.A. (2006). "A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment." *Chemosphere 65*: 725-759

Al-Ahmad, A., Daschner, F.D., and Kümmerer, K. (1999). "Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G, and sulfamethoxazole and inhibition of waste water bacteria". *Arch. Environ. Contam. Toxicol.* 37, 158–163.

Alder, A.C., McArdell, C.S., Giger, W., Golet, E.M., Molnar, E., and Nipales, N.S. (2000). Determination of antibiotics in Swiss wastewater and in surface water. Presented at Antibiotics in the Environment, Cranefield, UK, 2 February 2000.

Alder, A.C., McArdell, C.S., Golet, E.M., Ibric, S., Molnar, E., Nipales, N.S., and Giger, W. (2001). Occurrence and Fate of Flouroquinolone, Macrolide, and Sulfanamide Antibiotics During Wastewater Treatment and in Ambient Waters in Switzerland. Symposium Series 791, American Chemical Society, Washington, DC, pp. 56–69.

Aliu, Y. O., Mammah, M., and Abdullahi, U. S. (2001). "Optimal Livestock Production in Nigeria; value of medicinal products and food safety consideration". A lecture delivered at the IITA Ibadan Tuesday August 1st 2001.

Al-Mazeedi, H.M., Abbas, A.B., Alomirah, H.F., Al-Jouhar, W.Y., Al-Mufty, S.A., Ezzelregal, M.M., and Al-Owaish, R.A. (2010). "Screening for tetracycline residues in food products of animal origin in the State of Kuwait using Charm II radioimmunoassay and LC/MS/MS methods". *Food Addit. Contam. Part A* 27: 291-301.

Anadon, A., Bringas, P., Martinez- Larranaga, M. R. and Diaz, M. J. (1994). "Bioavailability, pharmacokinetics and residues of chloramphenicol in the chicken". *J. Vet. Pharmacol. Ther.*, 17: 52-58.

Aning, K. G., Donkor, E. S., Omore, A., Nurah, G. K., Osafo, E. L. K., and Staal, S. (2007). Risk of exposure to marketed milk with antimicrobial drug residues in Ghana. *The Open Food Science Journal*, 1, 1-5.

Babapour, A., Azani, L., and Fartashmehr, J. (2012). " Overview of Antibiotic Residues in Beef and Mutton in Ardebil, North West of Iran." *World Applied Sciences Journal 19 (10)*: 1417-1422

Benet, J.J., and Belleemain,V.(2005). Responding to consumer demands for safe food: A major role for veterinarians in the 21st Century. *Minneapolis: World Veterinary Congress.*

Berger, K., Petersen, B., and Buning-Pfaue, H. (1986). Persistence of drugs occurring in liquid manure in the food chain. *Archiv. Lebensmittelhygiene* 37, 99–102.

Bester, B. H., and Lombard, S. H. (1979). The effect of the dye-marking of mastitis remedies on the incidence of antibiotic residues in Pretoria's market milk supplies. *J. S. Afr. Vet. Assoc.,* 50(3): 151-3.

Bigby, M., Jick, S., and Arndt, K. (1986).Drug-induced cutaneous lesion reactions.A report from the Boston Collaborative drug surveillance program on 15,438 inpatients (1975-1982).*Journal of the American Medical Association*, 256: 3358-3363

Botsoglou, N.A., and Fletouris D.J. (2001). Drug Residues in Foods. *Marcel Dekker, New York* 27-116.

Boxall, A., Blackwell, P., Cavallo, R., Kay, P., and Tolls, J. (2002). The sorption and transport of sulphonamide antibiotic in soil systems. *Toxicol. Lett*. 131, 19–28.

Burch, D. (2006). Anticipated effects of the withdrawal of antibiotic growth promoters (AGPs) from pigs in the European Union on 1st January 2006. *<http://www.octagon-services.co.uk/articles/withdrawalAGP.htm>, p. 7*

BVA Guidelines on the Prudent Use of Antimicrobials. *British Veterinary Association, London,* 2000.

Carlet, J., Jarlier, V., Harbarth, S., Voss, A., Goossens, H., and Didier Pittet, D. (2012). Ready for a world without antibiotics? The Pensières Antibiotic Resistance Call to Action. *Antimicrob. Resist. Infect. Control*, 1:11.

Cars, O., Hogberg, L. D., Murray, M., Nordberg, O., Sivaraman, S., Lundborg, C. S., So, A. D., and Tomson, G. (2008). Meeting the challenge of antibiotic resistance. *B. M. J.*, 337: 14-38.

Chander, Y., Kumar, K., Goyal, S.M., and Gupta, S.C. (2005). Antibacterial activity of soil-bound antibiotics. *J. Environ. Qual.* 34, 1952–1957.

Chee-Sanford, J., Aminov, R., Krapac, I., Garrigues-Jeanjean, N., and Mackie, R. (2001). Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. *Appl. Environ. Microbiol.* 67, 1494–1502.

Chen, C., and Gu, X. (1995): Determination of tetracycline residues in bovine milk, serum and urine by capillary electrophoresis. *J. AOAC. International*, 76:1369–1377.

Christian, T., Schneider, R., Farber, H.A., Skutlarek, D., Meyer, M.T., and Goldbach, H.E. (2003). Determination of antibiotic residues in manure, soil, and surface waters. *Acta Hydrochim. Hydrobiol.* 31, 36–44.

Cinquina, A.L., Logo, F., Anastasi, G., Gianetti, L., and Cozzani, R. (2003). Validation of a high- performance liquid chromatography method for the determination of oxytetracycline, tetracycline, chlortetracycline and doxycycline in bovine milk and muscle. *J. Chromatogr. A* 987: 227-233.

Codex Alimentarius Commission (CAC) (1998): Reports of the sessions of the codex committees on residues of veterinary drugs in Foods. Washington, D. C., USA.

Cohen, M. (1998). Antibiotic use. In: Harrison, P.F., Lederberg, J. (Eds.), Antimicrobial Resistance: Issues and Options. Division of Health Sciences Policy, Institute of Medicine, National Academy Press, Washington, DC, p. 41.

Commission of the European Communities, (2005). Report for 2005 on the results of residue monitoring in food of animal origin in the Member States.consultation. Geneva, Switzerland, *WHO/FNU/FOS/95.3*, Pp13-17.

consultation. Geneva, Switzerland, WHO/FNU/FOS/95.3, Pp13-17.

DANMAP (Danish Integrated Antimicrobial Resistance Monitoring and Research Programme), (2000). Consumption and antimicrobial agents and occurrence of antimicrobial resistance in

bacteria from food animals, foods and humans in Denmark. Bager, F., Emborg, H.D., (Eds.). Available from: <*http://www.svs.dk/uk/Organisation/Frm_org.htm*>.

Darwish, S.W., Eldaly, A.E., El-Abbasy, T.M., Ikenaka, Y., Nakayama, S., and Ishizuka, M. (2013). "Antibiotic residues in food: the African Scenario." *Japanese Journal of Veterinary Research 61 (supplement)*: S13-S22

Davey P., Hudson S., Ridgeway A., and Reeves, D. (1993). A survey of undergraduate and continuing medical education about antimicrobial chemotherapy in the United Kingdom. *Br J Clin Pharmac* ;36:511–9.

Dayan, A.D. (1993). Allergy to antimicrobial residues in food: assessment of the risk to man. *Vet. Microbiol.* 35: 213-226.

Delgado, C., Rosegrant, M., Steinfeld, H., Ehui, S., and Courbois, C. (1999). Livestock to 2020: The next food revolution. Food, Agriculture and the Environment discussion paper 28. Washington, D.C: International Food Policy Research Institute, Food and Agriculture Organization of the United Nations, and the International Livestock Research Institute

Dewdney, J. M., Maes, L., Raynaud, J. P., Blanc, F., Scheid, J. P., Jackson, T., Lens, S., and Verschueren, C. (1991). Risk assessment of antibiotic residues of β-lactams and macrolides in food products with regard to their immuno-allergic potential. *Food Chem. Toxic.*, 29: 477-483.

Dipeolu, M.A., and Alonge, D.O. (2002). "Residues of streptomycin antibiotic in meat sold for human consumption in some states of south-western Nigeria". *Arch. Zootec.*, 51: 477-480.

Diploelo, M.A. (2002). Residues of tetracycline antibiotic in marketed goats and pigs in Lagos and Ogun States Nigeria. *Trop. J. Anim. Sci.* 5: 47-51.

Donkor, S.E., Newman, J.M., Dayie, T.K.D.N., Bannerman, E., Olu-Taiwo, M., and Tay, C.K.S. (2011). "Investigation into the risk of exposure to antibiotic residues contaminating meat and egg in Ghana." *Food control 22*: 869-873

Donoho, A. L. (1978): Biochemical studies on the fate of monensin in animals and in environment. *J. Anim. Sci*., 58:1528–1539.

Draisci, R., delli Quadri, F., Achene, L., Volpe, G., Palleschi, L. and Palleschi, G. (2001). A new electrochemical enzyme linked immunosorbent assay for the screening of macrolide antibiotic residues in bovine meat. *Analyst, 126*: 1942-1946.

Ehsani, A., Fazlara, A., Maktabi, S., and Najafzadeh, H. (2010). Measurement of tetracycline residue in consumed broiler meats in Ahvaz city by HPLC. *J. Vet. Med. Lab.* 2: 119-129

Ekuttan, C. E., Kang'ethe, E. K., Kimani, V. N., and Randolph, T. F. (2007). Investigation on the prevalence of antimicrobial residues in milk obtained from urban small holder dairy and non-dairy farming households in Dagoretti Division, Nairobi, Kenya. *East Afr. Med. J.*, 84(11): 87-91.

Eltayb, A., Barakat, S., Marrone, G., Shaddad, S., and Stålsby Lundborg, C. (2012). Antibiotic use and resistance in animal farming: a quantitative and qualitative study on knowledge and practices among farmers in Khartoum, Sudan. *Zoonoses Public Health*, 59(5): 330-8.

EMEA (European Agency for the Evaluation of Medicinal Products), (1999). Antibiotic resistance in the European Union Associated with therapeutic use of veterinary medicines. Report and qualitative risk assessment by the Committee for Veterinary Medicinal products, London, 14 July 1999. *EMEA/CVMP/342/99-Final*, p. 33.

Environment Agency (EA), (2001). Pesticides 1999/2000: A summary of monitoring of the aquatic environment in England and Wales, National Centre for Ecotoxicology and Hazardous Substances, Environment Agency, Wallingford, UK. environment. *J. Anim. Sci*., 58:1528–1539.

Esiobu, N., Armenta, L., and Ike, J. (2002). Antibiotic resistance in soil and water environments. *In. J. Environ. Health Res.* 12, 133–144.

EU Directive 70/524/EEC, (1970). Consolidated text produced by the CONSLEG System of the Office for Official publishing of the European Commission (EC), 2003. Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on Additives for Use in Animal Nutrition. European Communities. *CONSLEG:1970L0524-31/03/2003*, p. 38.

EU Directive 70/524/EEC, 1970. Consolidated text produced by the CONSLEG System of the Office for Official publishing of the European Communities. *CONSLEG:1970L0524* 31/03/2003, p. 38.

Ezenduka, E. V., Oboegbulem, S. I., Nwanta, J. A., and Onunkwo, J. I. (2011). Prevalence of antimicrobial residues in raw table eggs from farms and retail outlets in Enugu State, Nigeria. *Trop. Anim. Health Prod*., 43(3): 557-9.

Fagerquist, C.K., and Lightfield, A.R. (2003). Rapid Commun. Mass Spectrom. 17 ; 660.

FAO, (1999). "Residues of some veterinary drugs in animals and foods". *Food and nutrition paper* 41(3): 97-119.

Fein, S. B., Lin, J., and Levy, A. S. (1995). Food borne illness: perception, experience, and preventive behavior in the United States. *J. Food Prot*., 58: 1405-1411.

Feinman, S. E., and Matheson, J. C. (1978): Draft environmental impact statement: Sub therapeutic antibacterial agents in animal feeds. Food and drug administration department of health, education and welfare report, Food and Drug Administration, Washington, D.C., Pp 372.

Feuerpfeil, I., Lopez-Pila, J., Schmidt, R., Schneider, E., and Szewzyk, R.. (1999). Antibiotikaresistente Bakterien und Antibiotika in der Umwelt. “Cited in” Bundesgesundheitsbl, Gesundheitsforsch, Gesundheitsschutz 42, 37–50.

Foley E.G., Lee S.W., and Hartley N.J.(1946). The effect of penicillin on staphylococci and streptococci commonly associated with bovine mastitis. *J Food Technol* ;8:129–33.

Food and Agriculture Organization (FAO)/World Health Organization (WHO) (1995)

Frund, H.C., Schlosser, A., and Westendarp, H. (2000). Effects of tetracycline on the soil microflora determined with microtiter plates and respiration measurement. “Cited in” *Mitteilgn. Dtsch. Bodenkundl. Gesellsch*. 93, 244–247.

Fung, C.N., Lam, J.C.W., Zheng, G.J., Connell, D.W., Monirith, I., Tanabe, S., Richardson, B.J., and Lam, P.K.S. (2004). “Mussel-based monitoring of trace metal and organic contaminants along the east coast of China using perna viridis and mytilus edulis.” *Environmental Pollution, 127 (2)*: 203-216

Golet, E.M., Alder, A.C., Hartmann, A., Ternes, T.A., and Giger, W. (2001). Trace determination of fluoroquinolone antibacterial agents in solid-phase extraction urban wastewater by liquid chromatography with fluorescence detection. *Anal. Chem.* 73, 3632–3638.

Goni-Urriza, M., Capdepuy, M., Arpin, C., Raymond, N., Caumette, P., and Quentin, C. (2000). Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and Aeromonas spp. *Appl. Environ. Microbiol*. 66, 125–132.

Goudah, A., Sher Shah, S., Shin, H. C., Shim, J. H., and Abd El-Aty, A. M. (2007). Pharmacokinetics and mammary residual depletion of erythromycin in healthy lactating ewes. *J. Vet. Med. A Physiol. Pathol. Clin. Med.,* 54(10): 607-11.

Guardabassi, L., Petersen, A., Olsen, J.E., and Dalsgaard, A. (1998). Antibiotic resistance in Acinetobacter spp. isolated from sewers receiving waste effluent from a hospital and a pharmaceutical plant. *Appl. Environ. Microbiol.* 64, 3499– 3502.

Guest, G. B., and Paige, J. C. (1991). The magnitude of the tissue residue problem with regard to consumer needs. *J. Am.Vet. Med. Assoc*., 198: 805-808

Gustafson, R. 1993.Historical perspectives on regulatory issues of antimicrobial resistance. *Vet. Hum. Toxicol.*, 35: 2-5.

Haapapuro, E. R., Barnard, N. D., and Simon, M. (1997): Review-Animal waste used as livestock feed: dangers to human health. *Prev. Med*., 26: 599–602.

Haller, M.Y., Muller, S.R., McArdell, C.S., Alder, A.C., and Suter, M. (2001). Quantification of veterinary antibiotics (sulphonamids and trimethoprim) in animal manure by liquid chromatography–mass spectrometry. *J. Chromatogr. A* 952, 111–120.

Halling-Sørensen, B. (2001). Inhibition of aerobic growth and nitrification of bacteria in sewage sludge by antibacterial agents. *Arch. Environ. Contam. Toxicol*. 40 .

Hamscher, G., Sczesny, S., Hoper, H., and Nau, H. (2002). Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. *Anal. Chem*. 74, 1509–1518.

Harbarth, S., and Samore, M. H. 2005. Antimicrobial resistance determinants and future control. *Emerg. Infect. Dis*., 11: 794-801.

Hardman, J.G., and Limbird, L.E. (2007). Goodman and Gilman's the Pharmacological Basis of Therapeutics. McGraw-Hill, New York 1239-1245.*health, education and welfare report, Food and Drug Administration, Washington, D.C*., Pp 372.

Henzelin, A. B., Perroud, M. C. S., Le Breton, M. H., Hammel, Y. A., Germain, I., Bebius, A., (2007). Contaminants and residues in food: strategies (if any) to screen and analyse veterinary drug residues in food from animal origin. In 5th International Frensenius Conference, Frankfurt.

Hirsch, R., Ternes, T., Haberer, K., and Kratz, K.L. (1999). Occurrence of antibiotics in the aquatic environment. *Sci. Total Environ*. 225, 109–118.

Holten-Lutzøft, H.C., Halling-Sørensen, B., and Jorgensen, S.E. (1999). Algae toxicity of antibacterial agents applied in Danish fish farming. *Arch. Environ. Contam. Toxicol*. 36, 1–6.

Horrigan, L., Lawrence, R.S., and Walker, P. (2002). How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environ. Health Perspect*. 110, 445–456.

Ibrahim, A. I., Junaidu, A. U., and Garba, M. K. (2010). Multiple antibiotic residues in meat from slaughtered cattle in Nigeria. Internet *J. Vet. Med*., 8: 1-DOI: 10.5580/1fcd.

Ingerslev, F., and Halling-Sørensen, B. (2000). Biodegradability properties of sulphonamids in activated sludge. *Environ. Toxicol. Chem*. 19, 2467–2473.

Ingerslev, F., and Halling-Sørensen, B. (2001). Biodegradability of metronidazole, olaquindox, and tylosin and formation of tylosin degradation products in aerobic soil-manureslurries. *Endotoxicol. Environ. Saf*. 48, 311–320.

Isaacson, R.E., and Torrence, M.E. (2002). The role of antibiotics in agriculture. *A report from American Academy of Microbiology,Washington, DC,* 15 pp.

Ivona, K. and Mate, D. (2002): Evaluation of the sensitivity of individual test organisms to residual concentrations of selected types of drugs. *Slov. Vet. Res.,* 9: 78-82.

Jjemba, P.K. (2002). The potential impact of veterinary and human therapeutic agents in manure and biosolids on plants grown on arable land: *a review. Agric. Ecosyst. Environ*. 93, 267–278.

Johnston A.M. (1998). Use of antimicrobial drugs in veterinary practice. *B. M..J* . 317: 665–7.

Johnston, M.A. (2001). "Animals and Antibiotics." *International Journal of Antimicrobial Agents 18*: 291-294

Jørgensen, S.E., and Halling-Sørensen, B. (2000). Drugs in the environment. *Chemosphere 40*, 691–699.

Kabir, J., Umoh, J. U., and Umoh, V. J. (1999). "Public health awareness and health concern for veterinary drug residues in meat in Nigeria". *Health and Hygiene*, 20, 20–24.

Kabir, J., Umoh, V. J., Audu-Okoh, E., Umoh, J. U. and Kwaga, J. K. P. (2004). "Veterinary drug use in poultry farms and determination of antimicrobial drug residues in commercial eggs and slaughtered chicken in Kaduna State, Nigeria". *Food Control*, 15: 99-105.

Kang'ethe, E. K., Aboge, G. O., Arimi, S. M., Kanja, L. W., Omore, A. O., and McDermott, J. J. (2005). Investigation of risk of consuming marketed milk with antimicrobial residues in Kenya. *Food Control*, 16, 349-355.

Kanny, G. Puygrenier, J., Beaudoin, E., and Moneret-Vautrin, D. A. (1994). Choc anaphylactique alimentaire: implication des résidus de pénicilline. *Allerg. Immunol*., 26: 181-183.

Kay, P., and Boxall, A.B.A. (2000). Environmental Risk Assessment of Veterinary Medicines in Slurry. *SSLRC Contract JF* 611OZ, Cranfield University, 2000.

Kellogg, R.L., Lander, C.H., Moffitt, D.C., and Gollehon, N. (2000). Manure Nutrients Relative to the capacity of Cropland and Pastureland to Assimilate Nutrients: spatial and Temporal Trends for the United States, US Department of Agriculture, NPS00-0579.

Kemper, N. (2008). "Veterinary antibiotics in the aquatic and terestrial environment." *Ecological Indicators 8*: 1-13

Kemper, N., Farber, H., Skutlarek, D., and Krieter, J. (2007). Determination of antibiotic residues in leachate of conventional and organic dairy farms. In: Proceedings of the XIIIth International Congress in Animal Hygiene, Tartu, Estland, June 17–21, 2007.

Khattab, W. O., Elderea, H. B., Salem, E. G., and Gomaa, N. F. (2010). Transmission of Administered Amoxicillin Drug Residues from Laying Chicken to their Commercial Eggs. Egypt *Public Health Assoc*., 85(5-6): 297-316.

Kiser, J.S. (1976). A perspective on the use of antibiotics in animal feeds. *J. Anim. Sci.* 42, 1058–1072.

Koenen-Dierick, L., L. Okerman, L. De Zutter, J.M., Degroodt, J.V., Hoof and S.A., Srebrnik, (1995). One-plate microbiological screening testing in kidney tissue and meat; an alternative to the EEC four plate method? *Food Additives and Contaminants*, 12(1): 77-82.

Kolpin, D., Furlong, E., Meyer, M., Thurman, E., Zaugg, S., Barber, L., and Buxton, H. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. *Environ. Sci. Technol*. 36, 1202–1211

Korsud, G. O., Naylor, J. M., MacNeil J. D., and Yates, W. D. (1987). Persistence of chloramphenicol residues in calf tissues. *Can. J. Vet. Res*., 51: 316-318.

Krapac, I.G., Koike, S., Meyer, M.T., Snow, D.D., Chou, S.F.J., Mackie, R.I., Roy, W.R., and Chee-Sandford, J.C. (2005). Long-term monitoring of the occurrence of antibiotic residues and antibiotic resistance in groundwater near swine confinement facilities. *Report of the CSREES Project 2001*- 35102-10774.

Kümmerer, K. (2003). Significance of antibiotics in the environment. *J. Antimicrob. Chemother*. 52, 5–7.

Kümmerer, K., 2001a. Drugs in the environment: emission of rugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources—a review. *Chemosphere 45*, 957–969.

Kümmerer, K., Al-Ahmad, A., and Mersch-Sundermann, V. (2000). Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test. *Chemosphere 40*, 701–707.

Kurwijila, L. R., Omore, A., Staal, S., and Mdoe, N. S. Y. (2006). Investigation of the risk of exposure to antimicrobial residues present in marketed milk in Tanzania. *Journal of Food Protection*, 69(10), 2487-2492.

Lee, H. J., Lee, M. H. and Ruy, P. D. (2001). Public health risks: chemical and antibiotic residues. Asian-Aust. *J. Anim. Sci*., 14: 402-413.

Lee, M. H., Lee, H. J., and Ryo, P. D. (2000). Public health risks: chemical and antibiotic residues-review. *Asian-Aust. J. Ani. Sci.*, 14, 402-413.

Levy, S.B. (1998). The challenge of antibiotic resistance. *Sci. Am.* 278, 46–53.

Lindsey, M.E., Meyer, M., and Thurman, E.M. (2001). Analysis of trace levels of sulphonamide and tetracycline antimicrobials, in groundwater and surface water using solid-phase extraction and liquid chromatography/mass spectrometry. *Anal. Chem.* 73, 4640–4646.

Loke, L., Hallig-Sørensen, B., and Tjørnelund, J. (2002). Determination of the distribution coefficient (log Kd) of oxytetracycline, tylosin A, olaquindox and metronidazole in manure. *Chemosphere 48*, 351–361.

Lorenz, M.G., and Wackernagel, W. (1994). Bacterial gene transfer by natural genetic transformation in the environment. *Microbiol. Rev*. 58, 563–602.

MacKie, R.I., Koike, S., Krapac, I., Chee-Sanford, J., Maxwell, S., and Aminov, R.I. (2006). Tetracycline residues and tetracycline resistance genes in groundwater impacted by swine production facilities. *Anim. Biotechnol*. 17, 157–176.

MacNeil, J.D., (2005). The Joint food and Agriculture Organization of the United Nations/World health Organization Expert Committee on food additives and its role in the Evaluation of the safety of veterinary drug residues in foods. *The Am. Ass. Pharma. Sci. J.*, 7(2): 274-280

Marengo, J., Kok, R., OBrien, K., Velagaleti, R., and Stamm, J. (1997). Aerobic biodegredation of 14C-sarafloxacin hydrochloride in soil. *Environ. Toxicol. Chem.* 16, 462–471.

Mateu, E., and Martin, M. (2001).Why is anti-microbial resistance a veterinary problem as well? *J. Vet. Med. B Infect. Dis. Vet. Public Health* 48: 569-581.

McCaughey, W. J., Elliott, C. T., and Crooks, S. R. H. (1990). Carry-over of sulphadimidine in the faeces and urine of pigs fed medicated feed. *Vet. Rec*., 126: 351-354

McEvoy, J. D. G. (2002). Contamination of animal feeding stuffs as a cause of residues in food: a review of regulatory aspects, incidence and control. *Anal. Chim. Acta*, 473: 3-26.

Meadows, R. (1999). Livestock agency. *Environ. Health Perspect*. 103, 1096–1100.

Mellon, M., Benbrook, C., and Benbrook, K.L. (2001). Hogging it – Estimates of antimicrobial abuse in livestock. Union of Concerned Scientists, January 2000. UCS Publications, Cambridge, MA, USA, 110p.

Mesgari, Abbasi, M., Rashidi, M.R., Javadi, A., Bannazadeh, M., Mirmahdavi, S., and Zabihi, M. (2009). Levels of tetracycline residues in cattle meat, liver and kidney from a slaughter house in Tabriz, Iran. *Turkish J. Vet. Anim. Sci*. 33: 345-349.

Microbial antibiotic resistance in relation to food safety. Report of the Advisory Committee on the Microbiological Safety of food, The Stationary Office, London, 1999.

Miller, G. Y., Algozin, K. A., McNamara, P. E., and Bush, E. J. (2003). Productivity and economic impacts of feed-grade antibiotic use in pork production. *J. Agr. Appl. Econ*., 12: 469 482.

Mitchell, J. L., Giffits, M., W., Mcewen, S. A., Mcnab, W. B. and Yee, A. J. (2002): Antimicrobial drug residues in milk and meat: Causes, concerns, prevalence, regulations, tests and test performance. *J. F. Protection, 61*: 742–756.

Mitema, E. S., Kikuvi, G. M., Wegener, H. C., and Stohr, K. (2001). An assessment of antimicrobial consumption in food producing animals in Kenya. *J. Vet. Pharmacol. Ther*., 24(6): 385-90.

Moat, W. A. (1988). Inactivation of antibiotics by heating in foods and other substrate- a review. *Journal of Food Protocol*, 51, 491-497.

Morshdy, A. E., El-Atabany, A. I., Hussein M. A., and Wageh Sobhy Darwish W. S. (2013). Oxytetracycline residues in bovine carcasses slaughtered at Mansoura Abattoir, Egypt. Jpn. *J. Vet. Res. In Press*.

Muriuki, F.K., Ogara, W.O., Njeruh, F.M., and Mitema, E.S. (2001). Tetracycline residue levels in cattle meat from Nairobi slaughterhouse in Kenya. *J. Vet. Sci*. 2: 97-101.

Myllyniemi, A. L., Rannikko, R., Lindfors, E., Niemi, A., and Bäckman, C. (2000). Microbiological and chemical detection of incurred penicillin G, oxytetracycline, enrofloxacin and ciprofloxacin residues in bovine and porcine tissues. *Food Addit. Contam*., 17: 991-1000.

NAHMS (National Animal Health Monitoring System), (1996). Antibiotic usage in premarket swine. Factsheet, Veterinary Services (Swine'95 study). *<http://www.aphis.usda.gov/vs/ceah/nahms/swine/swine95/sw95antb.pdf>, p. 2.*

NASS (National Agricultural Statistics Service), (2002). *Available from: http://www.usda.gov/nass*

Navratilova, P., Borkovkova, I., Drackova, M., Janstova, B., and Vorlova, L. (2009). Occurance of tetracycline, chlortetracycline and oxytetracycline residues in raw cow's milk. Czech. *J. Food Sci.* 27: 379-385.

Nhiem, D.V., Paulsen, P., Suriyasathaporn, W., Smulders, F.J., Kyule, M.N., Baumann, M.P., Zessin, K.H., and Ngan, P.H. (2006).Preliminary analysis of tetracycline residues in marketed pork in Hanoi, Vietnam. *Ann. N.Y. Acad. Sci.* 1081: 534-542.

Nisha A. R. (2008). Antibiotics residues—A global health hazard. *Vet. World*, 1(12): 375-377.

Nonga, H. E., Simon, C., Karimuribo, E. D., and Mdegela, R. H. (2010). Assessment of antimicrobial usage and residues in commercial chicken eggs from smallholder poultry keepers in Morogoro municipality, Tanzania. *Zoonoses Public Health*, 57(5): 339-44.

Nwosu, V.C. (2001). Antibiotic resistance with particular reference to soil microorganisms. *Res. Microbiol*. 152, 421–430.

Nygaard, K., Lunestad, B.T., Hektoen, H., Berge, J.A., and Hormazabal, V. (1992). Resistance to oxytetracycline, oxolinic acid and furazolidone in bacteria from marine sediments. Aquaculture 104, 21–36. *Off. Anal. Chem. Int*., 77: 481–485.

Official Journal of the European Communities L224, of 18 August 1990, Council Regulation 2377/90/EC; consolidated version of the Annexes 1 to IV updated up to 22.12.2004 obtained from *www.emea.eu.int*.

Official Journal of the European Union, L71 of 13 March 2003, Commission decision amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin, Brussels, Belgium, 2003.

Oh, Y.H., and Han, H.K. (2006). Pharmacokinetic interaction of tetracycline with non-steroidal anti-inflammatory drugs via organic anion transporters in rats. *Pharmacol. Res*. 53: 75-79.

Olsen, S.J., DeBess, E.E., McGivern, T.E., Marano, N., Eby, T., Mauvais, S., Balan, V.K., Zirnstein, G., Cieslak, P.R., and Angulo, F.J. (2001). A nosocomial outbreak of fluoroquinolon-resistant salmonella infection. N. Engl. *J. Med.* 344 1572–1579.

Olufemi, O. I., and Ehinmowo Adebayo Agboola, E. A. (2009). Oxytetracycline Residues in Edible Tissues of Cattle Slaughtered in Akure, Nigeria. *Internet J. Food Safety*, 11: 62-66.

Omeiza, G. K., Kabir, J., Mamman, M., Ibrahim, H., and Fagbamila, I. O. (2012). Response of Nigerian farmers to a questionnaire on chloramphenicol application in commercial layers. *Vet. Ital.*, 48(1): 87-93.

Opinion of the Scientific Steering Committee on Antimicrobial Resistance. European Commission DG24, 28 May 1999.

Osei-Asare, Y.B., and Eghan, M. (2014). "Meat consumption in Ghana, Evidence from Household Micro-Data." *The Empirical Economic Letters, 13 (2)*: 142-153

Paige, J. C. (1994). Analysis of tissue residues. *FDA Vet*., 9: 4-6.

Paige, J. C., Tollefson, L., and Miller, M. (1997). Public health impact on drug residues in animal tissues. *Vet. Human Toxicol*., 39: 162-169.

Pedersen, J., Yeager, M., and Suffet, I. (2003). Xenobiotic organic compounds in runoff from fields irrigated with treated wastewater. *J. Agric. Food Chem.* 51, 1360–1372.

Posyniak, A., Mitrowska, K., Zmudzki, J., and Niedzielska, J. (2005): Analytical procedure for the determination of chlortetracycline and 4- epi-chlortetracycline in pig kidneys. *J. Chromatography A*., 10: 169–174.

Rabølle, M., and Spliid, N. (2000). Sorption and mobility of metronidazole, olaquindox, oxytetracycline and tylosin in soil. *Chemosphere 40,* 715–722.

Raison-Peyron, N., Messaad, D., Bousquet, J., and Demoly, P. (2001). Anaphylaxis to beef in penicillin-allergic patient. *Allergy, 56*: 796-797.

Ramos, M. A., Arnanda, A., Pozuelo, M. M. and Reuvers, T. (2003). Chloramphenicol residues in food samples, their analysis and stability during storage. *J. Liquid Chromat. Relat. Technol.,* 26: 2535-2449.

Ramos, M., Mu˜noz, P., Aranda, A., Rodriguez, I., Diaz, R., and Blanca, J. (2003). *J. Chromatogr. B* 791 ; 31.

Report of the Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine (Chairman Professor Michael Swann). Cmnd 4190. HMSO, London, 1969.

residues in milk with a luminescence based microbial method: The effects of milk composition and assay performance in relation to an immunoassay and a microbial inhibition assay. *J. F. Protection,* 63: 953–957.

Revert´e, S., Borrull, F., Pocurull, E., and Marc´e, R.M. (2003). *J. Chromatogr. A* 1010 ; 225.

Rice, L.B. (2001). Emergence of vancomycin-resistant enterococci. *Emerg. Infect. Dis. 7,* 183–187.

Rooklidge, S.J. (2004). Environmental antimicrobial contamination from terraccumulation and diffuse pollution pathways. *Sci. Total Environ.* 325, 1–13.

Roundant, B., and Moreitain, J. P. (1990). Sulphonamide residues in milk of dairy cows following intravenous injection. *Food Additives and Contaminants,* 7, 527-533.

Salama, N. A., Abou-Raya, S. H., Shalaby, A. R., Emam, W. H., and Mehaya, F. M. (2011). Incidence of tetracycline residues in chicken meat and liver retailed to consumers. *Food Addit. Contam. B,* 4(2): 88-93.

Samuelsen, O.B., Torsvik, V., and Ervik, A. (1992). Long-range changes in oxytetracycline concentration and bacterial resistance towards oxytetracycline in a fish farm sediment after medication. *Sci. Total Environ.* 114, 25–36.

Sarmah, A.K., Meyer, M.T., and Boxall, A.B.A. (2006). A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere 65*, 725–759

Schwartz, M. H., and Sher, T. H. (1984). Anaphylaxis to penicillin in a frozen dinner. *Ann. Allergy,* 52: 342-343.

Sengeløv, G., Agersø, Y., Hallig-Sørensen, B., Baloda, S.B., Andersen, J.S., and Jensen, L.B. (2003). Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. *Environ. Int. 28,* 587–595.

Senyuva, H., Ozden, T., and Sarica, D.Y.(2000). High-performance liquid chromatographic determination of oxytetracycline residue in cured meat products. *Turkish J. Chem*. 24: 369-400.

Seveno, N.A., Kallifidas, D., Smalla, K., van Elsas, J.D., Collard, J.M., Karagouni, A.D., and Wellington, E.M.H. (2002). Occurrence and reservoirs of antibiotic resistance genes in the environment. *Rev. Med. Microbiol*. 13, 15–27.

Shitandi, A. (2004). Risk factors and control strategies for antibiotic residues in milk at farm level in Kenya. *PhD Thesis*. Sweden: Uppsala University.

Shitandi, A., and Sternesjo, A. (2001). Detection of antimicrobial drug residues in Kenyan milk. *J. Food Safety*, 21: 205-268.

Smalla, K., and Sobecky, P.A. (2002). The prevalence and diversity of mobile genetic elements in bacterial communities of different environmental habitats: insights gained from different methodological approaches. *FEMS Microbiol. Ecol.* 42, 165–175.

Smalla, K., Heuer, H., Gotz, A., Niemeyer, D., Krogerrerecklenfort, E., and Tietze, E. (2000). Exogenous isolation of antibiotic resistance plansmids from piggery manure slurries reveals a high prevalence and diversity of IncQ-like plasmids. *Appl. Environ. Microbiol*. 66, 4854–4862.

Sundlof, S. F. (1989). Drug and chemical residues in livestock. *Clin. Toxicol*., 5: 411- 449.

Sundlof, S. F. (1994). Human risks associated with drug residues in animal derived foods. *J. Agromed.*, 1: 5-22.

Sundlof, S. F., Fernandez, A. H., and Paige, J. C.(2000). Antimicrobial drug residues in food producing animals. In: Prescott, J. F., Baggot,J. D. and Walker, R. D. (eds.) Antimicrobial Therapy in Veterinary Medicine, 3rd ed. Ames, Iowa, USA, pp. 744-759.

Syit, A.D. (2008). "Detection and determination of Oxytetracycline and Penicillin G antibiotic residues levels in bovine bulk milk from Debrezeit and Nazareth dairy farms."Proceedings of the 1st international technology, education and environment conference. (c) *African Society for Scientific Research (ASSR)*: 325-346

Teale, C.J. (2002). Antibiotic resistance and the food chain. J. *Appl. Microbiol.* 92: 85-92.

Tetracyclines in animal feed. Committee on human health risk assessment on using subtherapeutic antibiotics in animal feeds. National Academy Press, Washington, DC.

Teuber, M. (1999). Spread of antibiotic resistance with food borne pathogens. *Cell. Mol. Life Sci*. 56, 755–763.

The Path of Least Resistance. Report of the Standing Medical Advisory Committee Sub-Group on Antimicrobial Resistance, Department of Health, London, 1998.

Thiele-Bruhn, S. (2003). Pharmaceutical antibiotic compounds in soils—*a review. J. Plant Nutr. Soil Sci.* 166, 145–167

Tinkelman, D. G., and Bock, S. A. (1984). Anaphylaxis presumed to be caused by beef containing streptomycin. *Ann. Allergy*, 53: 243-244.

Tollefson, L. and Miller, M. A. (2000). Antibiotic use in food animals: controlling the human health impact. *J. AOAC. Int*., 83: 245-256.

Tolls, J. (2001). Sorption of veterinary pharmaceuticals in soils: a review. *Environ. Sci. Technol. 35, 3397–3406. Toxicol.*, 6: 129–135.

UCS (Union of Concerned Scientist), (2001). Hogging it!: Estimates of antimicrobial abuse in livestock. <*http://www.ucsusa.org/food_and_environment/antibiotic_resistance/index*>*, p. 4.*

Ungemach, F.R. (2000). Fifures on quantities of antibacterials used for different purposes in the EU countries and interpretation. *Acta Vet. Scand. Suppl.* 93, 89–97.

Union of Concerned Scientists, 2001. 70 Percent of All Antibiotics Given to Healthy Livestock. Cambridge, MA, USA (Press release, 8 January).

USDA (United States Department of Agriculture), (1996). Animal and Plant Health Inspection Service. Veterinary Services, National Animal Health Monitoring system (US). Antibiotic Usage in premarket Swine. APHIS Veterinary Services factsheet, Januray 1996, Fort Collins, CO. Centres for Epidemiology and Animal Health, USDA:APHIS:VS.

USDA, USEPA, (1998). Clean water initiative-restoring and protecting America's waters. *http://cleanwater.gov/afo/afo_proceedings.html* (Draft Unified national Strategy for Animal Feeding Operations.)

USEPA (United States Environmental Protection Agency), (2000). National management measures to control non-point pollution from agriculture. Office of Water, Non-point Source Control Branch, Draft report.

Van de Riet, J.M., Potter, R.A., Christie-Fougere, M., and Burns, B.G. (2004). *J. Assoc. Off. Anal. Chem. Intern.* 86 ; 510.

Van den Bogaard, A.E., London, N., and Stobberingh, E.E. (2000). Antimicrobial resistance in pig faecal samples from the Netherlands (five abbatoirs) and Sweden. *J. Antimicrob. Chemother.* 45, 663–671.

VMD (Veterinary Medicines Directorate), (2001). Sales of antimicrobial products used as veterinary medicines and growth promoters in the UK in 1999. Veterinary Medicines Directorate: Addlestone, UK.

Walton J.R. (1983) Antibiotics, animal, meat and milk. *Zbl Vet A* ;30:81–92.

Wassenaar, T. M. (2005). The use of antimicrobial agents in veterinary medicine and implications for human health. *Critical Reviews in Microbiology*, 31, 155-169.

water eluent. *Talanta, 59*: 155–159.

Weirup M. (1998) Preventative methods replace antibiotic growth promoters: ten years experience in Sweden. *APUA Newsl*;16:1–5.

WHO (World Health Organisation), (2001). Monitoring antimicrobial usage in food animals for the protection of human health. Report of WHO consultation, Oslow, Norway, 10–13 September 2001, *WHO/CDS/CSR/EPH/2002.11*, p. 21.

WHO. (1999). Evaluation of Certain Veterinary Drug Residues in Food. WHO Technical Report Series (15th Report of the Joint FAO/WHO Expert Committee on Food Additives). No.888.

Wierup, M. (2001). The Swedish experience of the 1986 year ban on antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and usage of antimicrobials. *Microb. Drug Resist*. 7, 183–190.

Wiggins, B.A., Andrews, R.W., Conway, R.A., Corr, C.L., Dobratz, E.J., Dougherty, D.P., Eppard, J.R., Knupp, S.R., Limjoco, M.C., Mettenburg, J.M., Rinehardt, J.M., Sonsino, J., Torrijos, R.L., and Zimmerman, M.E. (1999). Use of antibiotic resistance analysis to identify nonpoint sources of faecal pollution. *Appl. Environ. Microbiol.* 65, 3483–3486.

Wilson, J., Otsuki, T., and Majumdsar, B. (2003). Balancing food safety and risk: do Drug residue limits affect international trade in beef? *J. Int. Trade Econ. Dev.* 12: 377-402.

Winckler, C., and Grafe, A. (2001). Use of veterinary drugs in intensive animal production: evidence for persistence of tetracyclines in pig slurry. *J. Soils Sed*. 1, 66–70.

Winckler, C., and Grafe, A. (2001). Use of veterinary drugs in intensive animal production: evidence for persistence of tetracyclines in pig slurry. *J. Soils Sed*. 1, 66–70.

Witte, W. (1998). Medical consequences of antibiotic use in agriculture. *Science 279*, 996–997.

Wollenberger, L., Halling-Sørensen, B., and Kusk, K.O. (2000). Acute and chronic toxicity of veterinary antibiotics to Daphnia magna. *Chemosphere 40*, 723–730.

Zhao, F., Zhang, X., and Gan, Y. (2004): Determination of tetracyclines in bovine milk by high performance liquid chromatography with coulometric electrode array system. *J. Chromatography A.*, 15: 109–114.

Zhu, J., Snow, D., Cassada, D.A., Monson, S.J., and Spalding, R.F. (2001). Analysis of oxytetracycline, tetracycline, and chlortetracycline in water using solid phase extraction and liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* 928, 177–186.

Zuccato, E., Bagnati, R., Fioretti, F., Natangelo, M., Calamari, D., and Fanelli, R. (2001). Environmental loads and detection of pharmaceuticals in Italy. In: Kümmerer, K. (Ed.), Pharmaceuticals in the Environment-Sources, Fate, Effects and Risks. Springer, Berlin, pp. 19–27

APPENDIX 1: Concentrations of Chloramphenicol and Oxytetracycline in samples

CHLORAMPHENICOL CONCENTRATIONS IN BEEF AND MUTTON SAMPLES

CENTRAL MARKET

CENTRAL MARKET SAMPLES	CONCENTRATION(mg/kg)
CMD1 BEEF	508.09579
CMD2 BEEF	136.97484
CMD3 BEEF	-
CMD4 BEEF	101.46351
CMD5 BEEF	121.11418
CMD6 BEEF	230.12401
CMD7 BEEF	-
CMD8 BEEF	93.58152
CMD9 BEEF	101.17466
CMD10 BEEF	450.80371
CMD1 MUTTON	598.13646
CMD2 MUTTON	146.33237
CMD3 MUTTON	73.38100
CMD4 MUTTON	96.66362
CMD5 MUTTON	181.03449
CMD6 MUTTON	585.06867
CMD7 MUTTON	301.21274
CMD8 MUTTON	142.08075

CMD9 MUTTON	212.76895
CMD10 MUTTON	-

CMD – CENTRAL MARKET DAY

ASAFO MARKET

ASAFO MARKET SAMPLES	**CONCENTRATION(mg/kg)**
AMD1 BEEF	175.47117
AMD2 BEEF	184.08548
AMD3 BEEF	181.74788
AMD4 BEEF	349.53334
AMD5 BEEF	-
AMD6 BEEF	-
AMD7 BEEF	249.68898
AMD8 BEEF	84.64494
AMD9 BEEF	230.17519
AMD10 BEEF	250.13428
AMD1 MUTTON	-
AMD2 MUTTON	126.69522
AMD3 MUTTON	133.50137
AMD4 MUTTON	281.98683
AMD5 MUTTON	141.18094
AMD6 MUTTON	144.06387
AMD7 MUTTON	102.96912

AMD8 MUTTON	-
AMD9 MUTTON	202.90082
AMD10 MUTTON	100.00284

AMD – ASAFO MARKET DAY

ABATTOIR

ABATTOIR SAMPLES	**CONCENTRATION(mg/kg)**
AD1 BEEF	81.42153
AD2 BEEF	167.89582
AD3 BEEF	176.16079
AD4 BEEF	-
AD5 BEEF	-
AD6 BEEF	112.67586
AD7 BEEF	254.29422
AD8 BEEF	184.08548
AD9 BEEF	178.18627
AD10 BEEF	160.19708
AD1 MUTTON	167.86988
AD2 MUTTON	-
AD3 MUTTON	184.19400
AD4 MUTTON	574.63210
AD5 MUTTON	235.23198
AD6 MUTTON	-

AD7 MUTTON	92.67553
AD8 MUTTON	100.23419
AD9 MUTTON	388.88976
AD10 MUTTON	418.00898

AD – ABATTOIR DAY

OXYTETRACYCLINE CONCENTRATIONS IN BEEF AND MUTTON SAMPLES

CENTRAL MARKET

CENTRAL MARKET SAMPLES	**CONCENTRATION(mg/kg)**
CMD1 BEEF	74.50553
CMD2 BEEF	79.91180
CMD3 BEEF	68.70385
CMD4 BEEF	-
CMD5 BEEF	-
CMD6 BEEF	-
CMD7 BEEF	97.77182
CMD8 BEEF	110.00222
CMD9 BEEF	-
CMD10 BEEF	-
CMD1 MUTTON	110.34751
CMD2 MUTTON	223.73910
CMD3 MUTTON	150.00111

CMD4 MUTTON	-
CMD5 MUTTON	-
CMD6 MUTTON	-
CMD7 MUTTON	180.55156
CMD8 MUTTON	240.98976
CMD9 MUTTON	-
CMD10 MUTTON	-

ASAFO MARKET

ASAFO MARKET SAMPLES	**CONCENTRATION(mg/kg)**
AMD1 BEEF	78.01136
AMD2 BEEF	70.71961
AMD3 BEEF	87.15145
AMD4 BEEF	-
AMD5 BEEF	-
AMD6 BEEF	-
AMD7 BEEF	100.00123
AMD8 BEEF	99.98976
AMD9 BEEF	-
AM10 BEEF	-
AMD1 MUTTON	87.40889
AMD2 MUTTON	103.87619
AMD3 MUTTON	351.65727

AMD4 MUTTON	-
AMD5 MUTTON	-
AMD6 MUTTON	-
AMD7 MUTTON	400.45465
AMD8 MUTTON	255.12346
AMD9 MUTTON	-
AMD10 MUTTON	-

ABATTOIR

ABATTOIR SAMPLES	**CONCENTRATION(mg/kg)**
AD1 BEEF	70.11692
AD2 BEEF	607.68267
AD3 BEEF	658.21648
AD4 BEEF	590.00212
AD5 BEEF	480.23129
AD6 BEEF	-
AD7 BEEF	-
AD8 BEEF	-
AD9 BEEF	-
AD10 BEEF	-
AD1 MUTTON	75.88063
AD2 MUTTON	77.56503
AD3 MUTTON	121.04320

AD4 MUTTON	-
AD5 MUTTON	-
AD6 MUTTON	160.87654
AD7 MUTTON	90.02899
AD8 MUTTON	-
AD9 MUTTON	-
AD10 MUTTON	-