DETERMINATION OF OXYTETRACYCLINE RESIDUES IN MUSCLES OF CAMEL (*Camelus dromedarius)* SLAUGHTERED IN SOKOTO MAIN ABATTOIR

BY

ONWUEGBUNAM, CASIMIR UCHENDU
(ADM. NO: 11210803181)

MASTER OF VETERINARY PUBLIC HEALTH (MVPH)
DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

DECEMBER, 2016

USMANU DANFODIYO UNIVERSITY, SOKOTO

(POSTGRADUATE SCHOOL)

DETERMINATION OF OXYTETRACYCLINE RESIDUES IN MUSCLES OF CAMEL (*Camelus dromedarius)* SLAUGHTERED IN SOKOTO MAIN ABATTOIR

A Dissertation

Submitted to the

Postgraduate School,

USMANU DANFODIYO UNIVERSITY, SOKOTO, NIGERIA

In Partial Fulfillment of the Requirements
For the Award of the Degree of

MASTER OF VETERINARY PUBLIC HEALTH (MVPH)

BY

ONWUEGBUNAM, CASIMIR UCHENDU

(ADM. NO: 11210803181)

DEPARTMENT OF PUBLIC HEALTH AND PREVENTIVE MEDICINE

DECEMBER, 2016

DEDICATION

I dedicate this research work to the Almighty God, Who means everything to me and to my beloved parents, Chief Dominic Okeychukwu Onwuegbunam and Lolo Agnes Obiageli Onwuegbunam.

CERTIFICATION

This dissertation by ONWUEGBUNAM, Casimir Uchendu has met requirements for the award of degree of Master of Veterinary Public Health (MVPH) of the Usmanu Danfodiyo University, Sokoto and is approved for its contribution to knowledge.

_____	_____
Prof. O.O Faleke	**Prof. A.A. Magaji**
Major Supervisor	**Co-Supervisor I**
_____	_____
Prof. A.I. Daneji	**Prof. A.U Junaidu**
Co-Supervisor II	**Head of Department**

_____

External Examiner

ACKNOWLEDGEMENTS

All thanks and profound gratitude goes to God Almighty Who gave me life, health and strength to pursue my Masters Degree these years in the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria and to undertake this work to a fruitful end.

My many thanks and appreciation to my supervisors Prof. O.O Faleke (Major Supervisor), Prof. A.A Magaji (Co-Supervisor I), Prof. A.I Daneji (Co-Supervisor II) whose inspiration, corrections and suggestions helped to bring this work to this standard. I also want to specially thank the Head of Department Prof. A.U Junaidu for his support to this work.

I cannot sufficiently thank and express my sincere gratitude to my parents Chief Dominic Okeychukwu Onwuegbunam and Lolo Agnes Obiageli Onwuegbunam and my siblings for their immense and tireless moral and spiritual support.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Gluteal and neck muscle tissues from slaughtered camels from Sokoto main abattoir collected over a period of three weeks, were analyzed using Lateral-flow immunoassay (LFIA) technique. Out of the 180 meat samples obtained from 90 camels during the study, 25 (13.89%) comprising of 16 (64%) gluteal muscle sample and 9 (36%) neck muscle sample had detectable level of oxytetracycline residue. Twenty two (22) of the overall total number of camels examined were female, out of which 5 (22.73%) had detectable level of oxytetracycline residue in their muscle, and 68 were male out of which 12 (17.65%) had detectable level of oxytetracycline residue in their muscle. The incidence of oxytetracycline residues observed in this study could be as a result of the indiscriminate use and misuse of veterinary drugs as commonly practiced among livestock producers and marketers without observing withdrawal period prior to slaughter, and may probably reflect camel meat as a source of sustained consumer exposure to drug residues. Regulatory authorities should therefore ensure compliance with good agricultural practices including withdrawal period of drugs used for treatment of food animals, while livestock producers should also be educated on responsible use of drugs in food animals. Routine drug residues surveillance and monitoring programs in meat and other edible livestock products should be established in the country to ensure food safety.

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. They can be classified according to their effects as either bactericidal or bacteriostatic and also according to their range of efficacy as narrow or broad in spectrum.

The use of antibiotics in animals shortly followed their use in humans for the purpose of disease prevention and treatment (Gustafson, 1993). Today, antimicrobial drugs are used to control, prevent, and treat infection and to enhance animal growth and feed efficiency (Tollefson and Miller, 2000).

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 feeds 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), and allergy (e.g., penicillin) (Nisha, 2008.).

Oxytetracycline (OTC), *[4S-4a,4a_,5a,5a_,6b,12a_)]-4(dimethylamino)-4, 4a, 5, 5a, 6, 11, 12a-octahydro-3, 5 ,6, 10 ,12, 12a-hexahydoxy-6-methyl-1, 11-dioxo-2-naphtacenecarboxamide* is a broadspectrum antimicrobial agent that is active against bacteria and some chlamydiae, rickettsiae and protozoa. It is widely used in veterinary medicine because of its wide spectrum and advantageous pharmacokinetic features (Mestorino *et al*., 2007). It has been reported that tetracyclines (TC) are the most predominantly prescribed antibiotics in Africa, and of all antibiotic-associated residues they represent 41% of cases, followed by β-lactams at 18% (Wageh *et al.*, 2013). In food-producing animals, tetracyclines may be administrated orally in food or drinking water, parenterally, or through intramammary infusion. Oxytetracycline is widely distributed into body tissues and can be found in high concentration in the excretory organs especially the liver and in the bile (Prescott and Baggot, 1993).

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 TC residues and 20% of them had residue levels above the WHO allowable limit (Muriuki *et al.*, 2001). In a study carried out in 2006 in Hanoi, 5.5% of all meat samples were positive for TC residues and the maximum residual limits (MRLs) were exceeded in 0.69% of the samples (Nhiem *et al.*, 2006). 21.7% of all samples and 5% of kidney and liver samples from slaughterhouses in Tabriz in Iran contained TC residues above the MRLs set by the WHO (Mesgari *et al.*, 2009).

Ehsani's report on broiler meat study in Ahvaz, Iran showed that 60% of the samples had contamination and tetracycline residue was significantly higher than European legal concentration (100ug/Kg) in 10% of samples (Ehsani *et al.*, 2010).

Dipeolu (2002) reported 15.6% positive TC residues in goat meat samples from southwestern Nigeria. Olatoye and Ehinmowo (2009) reported that out of a total of 180 anaylsed samples of meat and other edible portions of slaughtered cattle from Akure metropolitan abattoir in Ondo state, 98 (54.44%) had detectable levels of oxytetracycline residues from which 62(34.44%) had oxytetracycline residues at violative levels above the WHO/FAO maximum residue limits (MRLs).

Camels are large mammals that live in dry area, and they have padded feet. Scientifically, Camels are classified under the Kingdom: Animalia, Phylum: Chordata, Class: Mammalia, Order: Artiodactyla, Family: Camelidae, Genus: Camelus and Species: *Camelus dromedaries* (one-humped camel) or *Camelus bacterianus* (two-humped camel). The dromedary or one-humped camels are distributed in the desert of Northern Africa, Arabia and the Middle East, while a feral population is found in Australlia (www.ultimateungulate.com).

The population of camel in Nigeria is predominant in the Northern States, particularly in Borno, Kano, Sokoto, Katsina, Kebbi, Jigawa and Yobe State (Mohammed, 2000).

1.1 Statement of the Problem

In Nigeria like most developing countries, antibiotics are used in animals indiscriminately for the prevention and treatment of bacterial infection (Dina and Arowolo 1991; Kabir *et al.*, 2004).

An official monitoring programme on drug residues in anmal products is lacking in Nigeria (Kabir *et al.*, 1999). Therefore, this could be an important factor that would

lead to drug misuse and subsequently drug residues in animal products (eggs, milk and meat).

Human health problem that could arise from the consumption of unacceptable levels of oxtetracycline residues in meat include gastrointestinal disturbances, hypersensitivity, bone and teeth problems in children and development of bacterial resistance (Woodward 1991; Czeizel *et al.,* 1998; Larkin *et al*., 2004).

1.2 Aim

This work sought to determine the residue level of oxytetracycline in camels slaughtered in Sokoto main abattoir.

1.3 Objectives

- To determine the presence or absence of oxyteracycline residue in meat from slaughtered camel (*Camelus dromedarius*) using lateral-flow immunoassay (LFIA) technique.
- To compare the proportion of positive samples between gluteal and neck muscle samples.

1.4 Justification

- In Sokoto, camel meat is becoming an acceptable food for the public (Mustapha and Olusiyi, 1993; Abubakar and Maigandi, 1994; Agaie *et al.,* 1997); however, no work has been done to ascertain the wholesomeness of camel meat in relation to the presence oxytetracycline residues.

- To provide information on the prevalence of oxytetracycline residue in meat of camel slaughtered in the study area and provide base line data for further research.

CHAPTER TWO

2.0 Review of Literature

2.1 Background Information

Antimicrobials are classified according to their chemical structure. Each, class is characterised by a typical core structure, and the various members of the class are differentiated by the addition or removal of secondary chemical structures from the core structure (Kennedy *et al*., 1998; Guardabassi and Dalsgaard, 2004). They can also be classified as broad or narrow spectrum, depending on the range of bacterial species against which they are active, or as bacteriostatic or bactericidal on the basis of their mechanism of action. An antimicrobial that exhibits a large dilution difference between inhibitory and cidal effects is considered to be a bacteriostatic drug. One that kills the bacterium at or near the same drug concentration that inhibits its growth is considered to be a bactericidal drug (Prescott, 2000a).

Mechanisms of action of antimicrobials fall into four categories: inhibition of cell wall synthesis, damage to cell membrane function, inhibition of nucleic acid synthesis or function, and inhibition of protein synthesis. The aim of antimicrobial therapy is to rapidly produce and then to maintain an effective concentration of drug at the site of infection for sufficient time to allow host-specific and non-specific defenses to eradicate the pathogen (Prescott, 2000a; Prescott and Walker, 2000).

The most commonly used antimicrobials in food-producing animals are the ß-lactams, tetracyclines, aminoglycosides, lincosamides, macrolides, pleuromutilins and sulfonamides. Antimicrobials are administered to animals by injections (intravenously, intramuscularly, or subcutaneously), orally in feed or water, topically on the skin and by intramammary and intrauterine infusions (Mitchell *et al*., 1998). Theoretically, all of

these routes may lead to residues appearing in foods of animal origin such as milk, meat and eggs (Johnston, 1998).

ß-lactams e.g. pennicillin G, amoxicillin, ampicillin, oxacillin, and cephalosporins; quinolones, e.g. ciprofloxacins, and most aminoglycosides are bactericidal, while tetracyclines and macrolides are bacteriostatic. Sulphonamides alone are bacteriostatic agents, but when combined with trimethoprim, a bactericidal effect occurs.

Knowledge of the antimicrobial spectrum of different antimicrobial substances as well as of the pharmacokinetics and pharmacodynamics of the species requiring treatment is of paramount importance for the outcome of the treatment (MAF, 2003).

Among the various indications, parenterally administered penicillin G has been used for the treatment of mastitis, arthritis and respiratory infections (Prescott, 2000b; Ranheim *et al*., 2002), and first generation cephalosporins for the treatment of mastitis (Hornish and Kotarski, 2002). OTC is used e.g. for the treatment of respiratory and gastrointestinal infections (Haagsma and Mengelers, 1989; Riviere and Spoo, 1995), fluoroquinolones for the treatment of infections of the respiratory, gastrointestinal, and urinary tracts (Vancutsem *et al*., 1990; Kaartinen *et al*., 1995) and macrolides to treat respiratory and enteric infections (Anadón and Reeve-Johnson, 1999; Draisci *et al*., 2001). Aminoglycosides are used mainly in the treatment of infections caused by aerobic, gram-negative bacteria (Isoherranen and Soback, 1999). Sulphonamides and trimethoprim are used e.g. for the treatment of respiratory and alimentary tract infections (Boison *et al*., 1996).

In Europe, more than 65% of the antimicrobials prescribed for veterinary therapeutic use were tetracyclines, whereas in Finland the ß-lactams were the most widely used group (FEDESA, 2004; NAM, 2004).

Feed additives are substances which improve livestock and poultry production. In the EU all substances used for growth promotion are licensed on the basis of the European Council Directive 70/524/EEC (EEC, 1970). Today, only four growth promoting antimicrobials are permitted in the EU for use in animal feeding stuffs. These substances, avilamycin, flavophospholipol, salinomycin and monensin, are to be phased out by 2006. In contrast to veterinary medicines, the zootechnical feed additives do not have MRLs, except for those which have a dual authorisation (McEvoy, 2002). In the United States food animal production uses large amounts of subtherapeutic doses of antimicrobials for disease control (Lathers, 2001).

2.1.2 The Origin of Antimicrobial Residues in Meat

The most likely cause of violative drug residues is the failure to observe withdrawal times (Paige and Kent, 1987; Van Dresser and Wilke, 1989; Guest and Paige, 1991; Paige, 1994). Improper maintenance of treatment records or failure to identify treated animals adequately may lead to their omission (Sundlof, 1989).

Faecal recycling, where the drug excreted in faeces of treated animals contaminates the feed of untreated animals, can be the cause of residues of certain antimicrobial groups sucg as the tetracyclines (Bevill, 1984; McCaughey *et al*., 1990). Housing of unmedicated pigs in boxes where pigs had previously been treated orally with sulfamethazine resulted in residues in urine, kidney and diaphragm (Mc Caughey *et al*., 1990; Elliott *et al*., 1994; Kietzmann *et al*., 1995).

Violative drug residues can also occur as a result of improper use of a licensed product or through the illegal use of an unlicensed substance. Extra label dosages and use of drugs which have not been approved for the species in question may lead to violative residues (Papich *et al*., 1993; Kaneene and Miller, 1997; Higgins *et al*., 1999). Residues can also occur in calves fed milk and/or colostrum from cows receiving antimicrobials (Guest and Paige, 1991).

In most countries ß-lactams are widely applied in mastitis therapy and are consequently the major reason for failures to satisfy at least dairy control requirements for inhibitory substances (Sternesjö and Johnsson, 1998).

The disease status of an animal and the way in which drugs are administered influence the potential for residues. Disease may affect the pharmacokinetics of the drugs, and/or their metabolism; the presence of infection and/or inflammation may cause the drug to accumulate in affected tissues (Kaneene and Miller, 1997). Subcutaneous and intramuscular administrations increase the potential for residues at the injection sites (Kaneene and Miller, 1997; Berends *et al*., 2001). Secondary drug concentration peaks in plasma have been detected after subcutaneous injections of benzathine and procaine penicillin, possibly reflecting a certain degree of inflammation at the injection site (Ranheim *et al*., 2002).

Contamination of animal feeding stuffs with a variety of compounds also occurs. The significance of this contamination depends on the pharmacodynamics of the compound and the species affected (McEvoy, 2002). Contamination of feeding stuffs seems to be an important source of unintended application of antimicrobials. In a survey carried out in Northern Ireland, antimicrobials were detected in 44% of feeds declared by the manufacturers to be free of medication (Lynas *et al*., 1998). Residual quantities of

medicated meal may be retained at various points along the production line, contaminating subsequent batches of meal as they are processed (Kennedy *et al*., 2000). Data from a sulfamethazine residue programme suggested that 25% of violations were due to inadequate cleaning of feed mixers (Guest and Paige, 1991).

2.1.3 Effects of Antimicrobial Residues

Antimicrobial residues in foods of animal origin may cause several health problems. In addition to toxic effects, effects on intestinal microbiota and the immune system are important (Gorbach, 1993; Waltner-Toews and McEwen, 1994; Perrin-Guyomard *et al*., 2001).

2.1.7 Effects of Low Doses of Antimicrobials on Human Gut Microbiota

The microbiota in the human gastrointestinal tract form an extremely complex, yet relatively stable, ecological community, containing more than 400 bacterial species (Carman *et al*., 1993). The concentration of anaerobic microbiota is 1011-1012 CFU g-1 faeces, and the concentration of aerobic microbiota much lower-less than 0.1% of the normal microbiota consists of aerobes (Vollaard and Claesner, 1994). In addition to the resident dominant anaerobic microbiota, the microbiota consists of a subdominant microbiota, a resident minority microbiota and a variable microbiota composed of bacteria which may be present for a variable period of time (Boisseau, 1993).

Colonisation resistance means the natural defence by normal microbiota against colonisation and translocation by exogenous potentially pathogenic microbes or against the overgrowth of indigenous opportunistics (Van Der Waaij *et al*., 1971; Barza *et al*., 1987; Corpet, 1993). Administration of antimicrobial agents may cause disturbances in these functions (Nord and Edlund, 1990). To what extent disturbances in the ecological balance between host and microorganisms occur depends on the spectrum of the

antimicrobial agent, the dose, pharmacokinetic and pharmacodynamic properties, and in-vivo inactivation of the agent (Sullivan *et al*., 2001).

Four microbiological endpoints have been identified that could be of public health concern: modification of the metabolic activity of microbiota, changes in bacterial populations, selection of resistant bacteria, and perturbation of the barrier effect (Boisseau, 1993; Gorbach, 1993; Sundlof *et al*., 2000; Perrin- Guyomard *et al*., 2001). In cases of reduced colonisation resistance not only are the minimal infectious or colonisation doses of pathogenic or resistant bacteria considerably lower, but animals also excrete these bacteria in higher numbers and over a longer period of time compared to animals with an intact colonisation resistance (Van Den Bogaard and Stobberingh, 1999).

Some data have been reported on antimicrobial susceptibility and emergence of resistant bacteria with low doses of antimicrobials (Rollins *et al*., 1975; Mamber and Katz, 1985; Corpet, 1987; Tancrede and Barakat, 1989).

Similarly, increased susceptibility to *Salmonella* infection with low antimicrobial doses has been described. Tetracyclines may, in relatively low doses, have some impact on the faecal anaerobic microbiota of humans (WHO, 1991; Waltner-Toews and McEwen, 1994). A close relationship between tetracycline, streptomycin, gentamicin and chloramphenicol residues and the resistance of bacteria isolated from the samples was found, suggesting that the presence of low levels of antimicrobials might exert a positive pressure towards the selection and expression of resistance in bacteria colonizing animal tissues (Vázquez-Moreno *et al*., 1990).

2.1.8 Hypersensitivity Reactions

Drug hypersensitivity is defined as an immune-mediated response to a drug agent in a sensitized patient, and drug allergy is restricted to a reaction mediated by IgE (Riedl and Casillas, 2003). Drugs are foreign molecules, but their molecular weights are usually too small to be immunogenic. For drugs to be immunogenic, they must act as haptens, which must combine with carrier proteins to be immunogenic and elicit antibody formation (Dewdney *et al*., 1991).

Immunologic reactions may manifest from life-threatening anaphylactic reactions to milder reactions, such as rashes. Drug-induced allergic reactions may occur acutely (within 60 min of challenge), subacutely (1-24 h), or as latent responses (1 day to several weeks). The acute and some subacute disorders are often due to Type I (IgE)-mediated reactions and, more rarely, due to IgG antibodies (Type II). Immune complex disorders (Type III) are much rarer in this context. Type IV (cell mediated) responses develop more slowly. The principal types of disorder are: Type I: anaphylactic shock, asthma and angioneurotic oedema; type II: haemolytic anaemia and agranulocytosis; type III: serum sickness and allergic vasculitis, and type IV: allergic dermatitis (Dayan, 1993; Riedl and Casillas, 2003).

Anaphylaxis exposure rapidly leads to severe acute bronchostriction, often risking a degree of asphyxia, marked hypotension, possibly oedema at the site of challenge, and severe general illness (Dayan, 1993).

Antimicrobial drug residues in animal tissues may cause hypersensitivity reactions in humans. An allergic reaction may be triggered by antimicrobial residues in a previously sensitised individual. In relation to primary sensitisation, 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. The duration of exposure is also short (Dewdney *et al*., 1991; Sundlof *et al*., 2000). Notwithstanding their non-toxic nature, ß-lactams appear to be responsible for most of the reported human allergic reactions to antimicrobials (WHO, 1991; Sundlof, 1994; Fein *et al*., 1995). Aminoglycosides, sulphonamides and tetracyclines may also cause allergic reactions (Paige *et al*., 1997). Certain macrolides may in exceptional cases be responsible for liver injuries, 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 (Tscheuschner, 1972; Kanny *et al*., 1994; Raison-Peyron *et al*., 2001). In one human case anaphylaxis was possibly caused by streptomycin residues (Tinkelman and Bock, 1984), and angioneurotic oedema and tightness of chest by penicillin residues in meat (Schwartz and Sher, 1984). Failure to associate minor hypersensitivity reactions, e.g. urticaria, with exposure to allergenic residues may be one reason for the lack of reported cases, although it may also be due to a genuine dearth of reactions (Woodward, 1991).

2.1.9 Other Harmful Effects

Hazards of chloramphenicol observed in association with clinical use in humans include dose-related, reversible suppression of the bone marrow, gray baby syndrome, which is a circulatory collapse in children less than 30 days on high doses, and irreversible, idiosyncratic, non-dose related aplastic anemia (Schmid, 1983; WHO, 1988; Waltner-Toews and McEwen, 1994). Aplastic anemia can occur in susceptible individuals exposed to concentrations of chloramphenicol that might remain as residues

in edible tissues of chloramphenicol-treated animals (Settepani, 1984). Aminoglycosides can produce damage in urinary, vestibular and auditory functions (Clark, 1977; Shaikh and Allen, 1985).

2.2 Safety Evaluation of Antimicrobial Drug Residues

To assess the safety of ingested antimicrobial residues, national and international committees evaluate data on chemical, pharmacological, toxicological and antimicrobial, properties of the drugs derived from studies of experimental animals and observations in humans (Fink-Gremmels and Van Miert, 1994; Woodward, 1998).

In the safety evaluation of veterinary drugs, tests undertaken to demonstrate the safety of the substance are performed in order to determine a no observed (adverse) effect level (NO(A)EL). This level is the basis for calculating an acceptable daily intake (ADI). After an ADI has been determined, maximum residue limits (MRLs) are determined for various food commodities so that overall residue intake remains below the set ADI in a standard food basket. Finally, to insure that drug residues have declined to a safe concentration in various tissues, a specified period of drug withdrawal is set for a veterinary medicinal product.

In the European Union (EU) maximum residue limits (MRLs) must be established for all pharmacologically active substances for the concerned animal species and relevant tissues or products. These MRLs are the basis for the determination of limits of detection (LODs) of various analytical methods used in residue surveillance.

2.2.4 Acceptable Daily Intake (ADI)

The ADI is an estimate of the residue, expressed on a body weight basis that can be ingested daily over a lifetime without any appreciable health risk (EC, 2001). The

ADI approach was originally developed to take account of effects based on classical toxicology, and it was applied to the results of standard toxicity studies. These studies were used to derive a NO (A) EL. The ADI was calculated by dividing this by a suitable safety factor, usually 100, which assumes that humans are 10 times more sensitive than animals and that within the human population there is a 10-fold range of sensitivity (IPCS, 1987; Woodward, 1998).

2.2.5 Maximum Residue Limit (MRL)

According to Council Regulation 2377/90 (EEC, 1990) maximum residue limit means the maximum concentration of residue resulting from the use of a veterinary medicinal product which may be legally permitted or recognized as acceptable in or on a food, allocated to individual food commodities. It is based on the type and amount of residue considered to be without any toxicological hazard for human health as expressed by the ADI, or on the basis of a temporary ADI that utilizes an additional safety factor.

In calculating an MRL, the ADI, the residue depletion patterns of a compound in the edible tissues of a particular food-producing animal and the theoretical food intakes are taken into account. Possible persistence of residues in organs or at the injection sites are also considered (Fitzpatrick *et al*., 1995; EC, 2001). Once the process of safety evaluation is complete and MRLs have been derived for a particular substance, consideration is given to the likely level of residue which may be expected to remain after the use of the substance in accordance with good veterinary practice, and to the availability of analytical detection methods suitable for use for routine monitoring purposes. The MRLs may be further reduced to take account of these factors (EC, 2001).

2.2.6 Withdrawal Period

To ensure that drug residues have declined to a safe concentration following the use of drugs in animals, a specified period of drug withdrawal must be observed prior to providing any products for human consumption. It is the time which passes between the last dose given to the animal and the time when the concentration of residues in the tissues: muscle, liver, kidney, skin/fat or products milk, eggs, honey is lower than or equal to the MRL (Cholas, 1976; Nouws and Ziv, 1978; Jackson, 1980).

The CVMP recommends the use of a statistical method in the assessment of a withdrawal period (CVMP, 1995) whenever possible, and particularly for products containing new chemical entities. Linear regression technique is recommended. A withdrawal period is determined at the time when the upper one-sided tolerance limit with a given confidence is below the MRL. For old chemical entities data are often insufficient to assess the withdrawal time by a statistical method. A simpler method consists of declaring the withdrawal time as the time in which the residues in all tissues of all observed animals have fallen below the respective MRLs (Concordet and Toutain, 1997). Bayesian methods have also been proposed to derive inferences on the parameters of interest (Fisch, 2000). However, establishment of accurate pre-slaughter withdrawal times is hardly possible with irritative drug formulations which are administered intramuscularly or subcutaneously (Nouws, 1984; Nouws *et al*., 1990).

2.3 Control of Antimicrobial Residues in Meat

There is no doubt that neither humans nor animals can live without antibiotics. However, the misuse of antibiotics may result in health hazards in both human and animal population. Thus, the reduction of antibiotic use constitutes a challenge for the

world. The following steps could be considered in order to ahieve a reduction in antibiotics usage.

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

- The use of alternatives to antibiotics, such as plant-derived antimicrobial substances and probiotics, may represent a promising option; vaccination against some bacterial diseases may be of great value in the near future.

- The reduction of unnecessary antibiotic use in animals in captivity should be pursued, as should antibiotic use for the management of viral disease in animals; the reduction of prophylactic antibiotic use should also be considered.

- Strict national legislation must be passed around the world to avoid the unnecessary use of antibiotics. In 2006, the European Union banned the use of antibiotics for the purpose of livestock health maintenance (Carlet *et al.,* 2012).

- National monitoring of antibiotic residues in foods and updating of the maximum permissible limits of these residues for each country should be undertaken.

- Antibiotics use in feed additives should be stopped.

- Avoid using antibiotics in the veterinary field without a veterinarian's prescription.

- Strict observation of antibiotic cessation times should be made; the avoidance of antibiotics lacking clearly documented pharmacokinetic and pharmacodynamic properties must be considered.

- The heat treatment of meat, milk, and eggs may inactivate antibiotic contaminants in feedstuffs.

- The freezing of animal-derived foods may also contribute to the reduction of some antibiotic contamination.

2.4 Administration, Distribution, and Metabolism of Tetracycline and Its Occurrence as Tissue Residue.

2.4.1 Tetracyclines

Drugs of the tetracycline group are amphoteric, forming salts with both acids and bases. They are used as parent compounds (e.g., oxytetracycline dihydrate) or as salts (e.g., oxytetracycline hydrochloride). Their lipid solubilities range from moderate (oxytetracycline and chlortetracycline) to high (doxycycline and minocycline), so that they are able to traverse cell membranes moderately or readily. The former two drugs are natural tetracyclines, while the latter two are semi-synthetic.

After oral dosing, the bioavailability of tetracyclines varies between drugs, being lowest for oxytetracycline and chlortetracycline and highest for doxycycline, but for all drugs except doxycycline, it is relatively low.

This is of importance from both therapeutic and residue perspectives, because low bioavailability is associated with a high degree of inter-animal variability in both amount absorbed and the resulting plasma concentration–time profile. This can be expected to lead to high variability between animals in residue depletion.

After absorption, the tetracyclines are partially bound to plasma protein. Reported values in farm animal species are 46–51% (chlortetracycline), 28–41% (tetracycline), 21–76% (oxytetracycline), and 84–92% (doxycycline) Papich *et al.*, 2009. For the latter

drug, high protein binding raises questions concerning effective dosage. The recommended dosage for pigs in drinking water is 10 mg/kg, which provides AUC24 h/MIC ratios that are claimed to be effective for several respiratory tract pathogens (Prats *et al.*, 2005). However, Toutain and coworkers, cited by Lees *et al.*, 2006 taking an AUC24 h/MIC breakpoint of 24 h (i.e., an average plasma concentration over the dosing interval equal to the MIC) and using population pharmacokinetic data, predicted for systemic effect a dosage of 20 mg/kg, based on total plasma concentration. Allowing for 90% protein binding, the predicted effective dose would be 200 mg/kg, which is totally unrealistic.

2.4.2 Pharmacokinetics, Distribution, Bioavailability, and Relationship to Antibiotic Residues

Tetracyclines are used extensively in food-producing species. Thus, chlortetracycline, oxytetracycline, and doxycycline are formulated for use as both in-feed and/or inwater products, in poultry, pigs, fish, and cattle for some or all of the following purposes: growth promotion, prophylaxis, metaphylaxis, and therapy. Another major use, particularly of oxytetracycline in parenteral formulations, is for therapy of a range of diseases, including calf and piglet pneumonias. Parenteral solution formulations of various strengths, ranging from 5% to 30% and containing a range of organic solvents, such as propylene glycol, 2- pyrrolidone, and *N*-methylpyrollidone, are in widespread use. When used in higher strengths (≥10%), the formulations create a depot, from which slow absorption occurs, at intramuscular injection sites. After intramuscular administration, a fraction of the dose that remains in solution is rapidly absorbed to achieve maximum plasma concentrations within 1–2h. However, as the organic solvents in the formulation are dispersed and absorbed, a larger fraction of the administered oxytetracycline precipitates. This provides a depot for subsequent slow absorption

phases, giving rise to flip-flop pharmacokinetics and also an acute inflammatory reaction.

There have been many studies in calves and pigs confirming the retardation effect (prolonged absorption) of high-dose, high-strength solutions of oxytetracycline. Craigmill *et al*. (2004) reported an analysis for 41 datasets from 25 published articles on oxytetracycline in cattle. Their metaanalysis for a dose of 20 mg/kg intramuscularly indicated mean values of 5.61 μg/ml *(C*max*)* and 21.6 h *(T*1/2*).* The advantages of these formulations relate to convenience and economy (single injection) plus animal welfare (avoiding multiple injections) and maintenance of plasma concentrations equal to or greater than MICs of sensitive organisms for periods of 48–96 h. Nouws studied both irritation at injection sites and persistence of oxytetracycline for relatively long periods after intramuscular dosing with 10 of the then available formulations (Nouws *et al.,* 1984).

As tetracyclines have moderate to high lipophilic properties, the poor bioavailability associated with oral administration is somewhat surprising. Papich and Riviere suggest that causes may be multifactorial (Papich *et al.,* 2009). As zwitterions, they are mainly ionized at pH within GIT liquor.

Moreover, feed reduces bioavailability, and tetracyclines chelate with polyvalent cations. Oxytetracycline absorption has been shown, experimentally, to be reduced by feed, dairy products, Ca^{2+}, Mg^{2+}, Al^{3+}, and Fe^{2+} ions and antacids. Even though doxycycline has a similar structure, affinity for metals is different from that of oxytetracycline with greater affinity for zinc and less for calcium.

Supplementation of feed for piglets with zinc can drastically reduce bioavailability of doxycycline.

Moderate variability in absorption of oxytetracycline from different intramuscular injection sites in calves was reported by Nouws and Vree (Nouws *et al.*, 1983). Bioavailability values of 79%, 86%, and 89% were obtained for injection into the buttock, neck, and shoulder, respectively. The same group reported variable bioavailability and residue profiles with 10 formulations of oxytetracycline in pigs (Nouws *et al.*, 1984) and 5 formulations in calves, sheep, and pigs (Nouws JFM *et al.*, 1990).

Despite moderate to high degrees of binding to plasma protein, tetracyclines are generally well distributed to most tissues. Volumes of distribution are generally similar to body water volume (0.6–0.7l/kg). Distribution volumes in excess of this are probably indicative of higher concentrations in intra- than extracellular fluid or binding to specific tissues, including bone. Doxycycline and minocycline traverse cell membranes better than do chlortetracycline and oxytetracycline, and doxycycline in particular concentrates intracellularly.

Systemic clearance of tetracyclines is similar to or higher than GFR. Up to 60%, depending on individual drugs, is eliminated by glomerular ultrafiltration and approximately 40% of administered dose is excreted in feces, but percentages are dependent on drug and route of administration. Bile: plasma concentration ratios may be as high as 20: 1. For doxycycline, biliary excretion exceeds urinary excretion. Tetracyclines are also metabolized to inactive compounds, except for doxycycline, for which no metabolites have been detected in calves and pigs. In addition to possible metabolism, residue analysis, especially of chlortetracycline, can be hindered by the fact that chlortetracycline is subjected not only to epimerization but also to keto-enol tautomerism, resulting in ketoenol tautomers in the chromatogram, which influence the

quantification of residues (Cherlet *et al.*, 2006). In the EU, MRLs for tetracyclines are expressed as the sum of parent compound plus the 4-epimer.

Terminal half-life varies with species, individual drug, and formulation. With the exceptions of retard, in-feed, and in-water formulations, the half-life in most species is sufficient to justify dosing once or twice daily. There are, however, exceptions; half-lives of oxytetracycline after intravenous dosing of 0.7 h (turkey) and 81.5 h (rainbow trout) have been reported. 77 As with all AMDs, there is the possibility of altered pharmacokinetics, as a consequence of disease, but the nature and direction of the change are not readily predictable. Pijpers *et al.*, 1991 reported an increase in half-life of oxytetracycline after oral dosing in pigs with pneumonia (14.1h) compared to healthy pigs (5.9h), with both values higher than half-life after intravenous dosing (3.7h), described by (Mevius *et al.*, 1986).

Bound residues of tetracyclines may occur in bones of slaughtered animals for months after treatment. Theoretically, these could reach the food chain via contaminated (mechanically deboned) meat or meat and bonemeal. The accumulation of tetracyclines in tissues is illustrated by the findings of Toutain and Raynaud, 1983 for oxytetracycline in calves. Concentrations of oxytetracycline were relatively high in liver and kidney compared to the extrapolated zero-time concentration for serum (4.2mg/l). The time required for residues to deplete to 0.1mg/l in serum was 143 hr, considerably shorter than the time required for residues to deplete to 0.1mg/kg in liver and kidney, but similar to the depletion time for muscle. The data nicely illustrate the importance of tissue elimination half-life in determining decrease to the 0.1 mg/kg concentration; despite an almost three-fold higher initial concentration in kidney compared to liver, the longer half-life for liver leads to a longer time to depletion to 0.1mg/kg for liver.

Similar data were reported for doxycycline in broiler chickens (Anad´on *et al.,* 1994). After dosing orally at 20 mg/kg for 4 days, 1- and 5-day residue concentrations (mg/kg) were as follows: kidney 1.92 and 0.17, liver 1.93 and 0.12, and muscle 1.18 and 0.06, respectively. Other tetracyclines, including tigecycline, recommended for human but not veterinary use.

2.4.3 Prevalence

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 TC residues and 20% of them had residue levels above the WHO standard (Muriuki *et al.,* 2001). Dipeolu reported 15.6% positive TC residues of goat meat samples from two states in Nigeria (Dipeolu, 2002). In Nigeria, Olatoye and Ehinmowo, 2009 reported that Out of a total of 180 samples of meat and other edible portions of slaughtered cattle from Akure metropolitan abattoir analyzed, 98 (54.44%) of the total samples had detectable levels of oxytetracycline residues from which 62(34.44%) had oxytetracycline residues at violative levels above the WHO/FAO maximum residue limits (MRLs). In a study carried out in 2006 in Hanoi, 5.5% of all meat samples were positive for TC 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 TC 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, (Tabriz, Iran) contained TC residues above the MRLs set by the WHO (Mesgari *et al.,* 2009). In addition, Ehsani *et al.* (2010) and colleagues were reported the results of a study on broiler meats in Ahvaz, Iran. They showed that 60% of

the samples had contamination and tetracycline residue was significantly higher than European legal concentration (100ug/Kg) in 10% of samples.

2.5 Detection and Identification of Antimicrobial Residues

A screening method is the first-hand analysis of the sample to establish the presence or absence of residues (Aerts *et al.*, 1995). It should be a low-cost and high-sample throughput method, optimised to prevent false-negative results and to have an acceptable number of false-positive results. In order to prevent false-negative results, it should be positive for all samples that contain residues at MRL; preferably at 50% of the MRL (Heitzman, 1994; Korsrud *et al.*, 1995 and 1998).

Microbiological methods are suitable for large scale screening because of their convenience and broad spectrum characteristics (Aerts, 1995; Haasnoot *et al.*, 1999). In the search for rapid methods for determining the interaction of antimicrobial agents and organisms, intermediate and end products of bacterial metabolism, as well as the interaction of the organism with various energy sources have been examined (Amsterdam, 1996). Today, most microbiological methods used in antimicrobial residue analytics are based on agar diffusion. Some methods based on growth inhibition in liquid media, but only a few tests based on alternative microbiological methods, have been described.

Microbiological tests are unspecific, indicating only the presence of an inhibiting agent. Physico-chemical methods are specific and quantitative but they may be time consuming if the identity of the antimicrobial being sought is not known before work begins. Therefore, a post-screening test is needed for the preliminary characterisation of the residue (Lott *et al*., 1985; Aureli *et al*., 1996; Ferrini *et al.*, 1997).

2.5.1 Test Matrix

Residue testing can be performed before or after slaughter. The majority of antimicrobial screening procedures use post-slaughter matrices, but pre-slaughter matrices such as serum and urine have been used, too. Microbial inhibition tests are generally conducted directly on the sample itself without any preliminary isolation steps (Boison and Mac Neil, 1995). The sample can be applied directly to the medium, in stainless steel cylinders or on filter paper discs impregnated with liquid sample (Mitchell *et al*., 1998).

Pharmacokinetics describes how the drug behaves in the body, i.e. absorption, distribution, metabolism and elimination, as reflected in the time course of drug concentrations in plasma or tissue (Greko, 2003). In order to select a suitable test matrix, pharmacokinetic data on the antimicrobials are needed. Drug residues may exist bound to carrier proteins or to cellular macromolecules and yet still be biologically active if acted upon by enzymes in the gastrointestinal tract (Lindsay, 1983). Bioassays do not detect compounds bound to proteins. Therefore, for certain compounds the detection sensitivity of diffusion tests is strongly influenced by chemical pretreatments, such as protein denaturization (Boison and Mac Neil, 1995; Isoherranen and Soback, 1999).

If organ tissue, renal pelvis fluid, urine etc. is used as the test matrix, the test result is expected to predict residue concentrations in muscle tissue. The reason for the use of other test matrices than muscle tissue is that higher residue concentrations are usually found in those tissues.

2.6.2 Kidney Tissue

The highest free drug concentrations of most antimicrobials are found in kidney and renal pelvis (Nouws, 1981; Aerts *et al*., 1995). A high incidence of false positive

reactions (Engel *et al.*, 1983) and varying ratios of residue concentrations in kidney and muscle of healthy and diseased animals (Nouws and Ziv, 1977; Nouws, 1981) complicate the use of kidney tissue as the test matrix.

After intramuscular administration of OTC to cattle, the injection sites and kidneys contained the highest residue concentrations; the tetracyclines concentrated also in the liver (Pakkala *et al.*, 1976; Timoney, 1979; Guillot etal., 1989; Mawhinney *et al*., 1996). OTC concentrations in edible tissues could be predicted from kidney cortex concentrations, whereas renal pen G concentrations did not correlate with muscular drug concentrations (Nouws and Ziv, 1977; Black and Gentry, 1984). Aminoglycosides are usually found in tissues in low concentrations and unbound, except in the renal cortex where they tend to concentrate (Teske *et al.*, 1972; Sande and Mandell, 1980; Wilson *et al*., 1991; Isoherranen and Soback, 1999). Accumulation is unpredictable (Haasnoot *et al*., 1999). Because of the slow reversibility of tissue binding, complete excretion is greatly delayed (Bowen, 1979).

Renal pelvis fluid/urine and plasma

No or minimal postmortal degradation occurs in the renal pelvis fluid compared to the kidney cortex, and the fluid contains less natural substances inhibiting bacterial growth (Nouws *et al*., 1988). A paper disc or cotton swab can be introduced into renal pelvis fluid of a carcass or into urine from live animals. The unbound drug diffuses into a swab or paper disk. The greater the binding constant to protein, the more unlikely it will be for a drug to be ”freely” available in the inter- or extra-cellular fluid (Aerts *et al*., 1995; Boison and Mac Neil, 1995). Urine, unlike plasma, does not normally contain proteins.

However, the high level of antimicrobial concentration in the urine is not always related to the residue state of carcass meat (Nouws, 1981), and in diseased animals no correlation can be established between muscle and urine concentrations. Reduced serum protein binding resulting from hypoalbuminemia or the uremic state will increase the free unbound fraction (Baggott, 1980).

Pen G is found in greater amounts in the urine than in the kidney tissue (Erasmuson *et al.*, 1998). OTC concentrations in edible tissues can be predicted from plasma concentrations in calves and pigs (Meijer *et al*., 1993; Lee *et al*., 2001a), and sulphonamide tissue residue concentrations from porcine urine and bile concentrations (Randecker *et al*., 1987; Fodey *et al*., 1997) and bovine plasma concentrations (Lee *et al*., 2001b). However, in order to use fluid matrices as predictors of sulphonamide concentrations in tissues, the correlation between sulphonamide concentrations in the fluid and the chosen tissue requires extensive experimental proof (Bjurling *et al*., 2000).

Metabolites and their microbiological activity

Most veterinary drugs are metabolised in the body to produce more watersoluble compounds, which are more readily excreted (Aerts *et al*., 1995).

Penicillins are eliminated almost entirely by the kidneys, which results in very high concentrations in the urine, and clearance of cephalosporins is through kidneys in most cases (Prescott, 2000b and 2000c). Pen G is metabolised to some extent by hydrolysis of the β-lactam ring, and the metabolites are microbiologically inactive (Vaden and Riviera, 1995). Most tetracyclines are excreted unchanged in urine and, to a lesser extent, bile (Prescott, 2000d).

OTC is not metabolised to a significant extent in the body. It is eliminated in the urine and faeces (Riviera and Spoo, 1995). High concentrations of fluoroquinolones are

found in organs of excretion (Walker, 2000). Enrofloxacin is de-ethylated to ciprofloxacin as the main metabolite (Küng *et al*., 1992; Kaartinen *et al*., 1995 and 1997; Anadón and Reev-Johnson, 1999), and a remarkable activity is due to the metabolite. Aminoglycosides are eliminated unchanged by renal excretion (Sande and Mandell, 1980; Prescott, 2000e).

Chloramphenicol is excreted mainly in the form of a microbiologically inactive glucuronic acid conjugate (Glazko *et al*., 1949 and 1950). The treatment of tissue samples with ß-glucuronidase lowers the high limits of detection (LODs) (Anadón, 1985) for chloramphenicol (Korkeala and Mäki-Petäys, 1984).

2.5.3 Test Bacteria

The LOD of a microbiological test for a given antimicrobial depends primarily on the innate sensitivity of the test bacterium. Use of spore suspensions in place of vegetative organisms gives very reproducible results (Cooper, 1972).

Bacillus subtilis has been widely used (Huber *et al*., 1969; Bartels *et al*., 1972; Levetzow and Weise, 1974; Fabiansson and Rutegård, 1979; Thamdrup Rosdal *et al*., 1979; Bogaerts and Wolf, 1980; Johnston *et al*., 1981; USDA, 1983; Nouws *et al*., 1988; Koenen-Dierick *et al*., 1995) because of its sensitivity to a wide range of antimicrobials, and because of a commercially available spore suspension.

Bacillus megaterium is the test organism in the Calf Antibiotic and Sulfa Test (CAST) (USDA, 1984). *Bacillus cereus* strains have been used to screen for tetracycline residues (McCracken *et al*., 1976; Bugyei *et al*., 1994; Okerman *et al*., 2001 and 2004). *Bacillus stearothermophilus* has also been widely used (Kundrat, 1968; Bielecka *et al*., 1981; Braham *et al*., 2001). It is the test organism in PremiRTest (DSM Food Specialities, Delft, Netherlands), in the Charm Farm Test (CFT) (Charm Sciences Inc.,

Malden, MA, USA), and in the Brilliant Black Reduction Test Kit (BR Test) (Enterotox Laboratories, Germany; Lloyd and Van Der Merwe, 1987). The problem with *B. stearothermophilus* is its sensitivity to the inhibitory activity of lysozyme (Van Schothorst and Peelen- Knol, 1970), making the bacterium less suitable for the residue detection in kidney tissue.

Non-sporulating bacteria are also used as test organisms. *Micrococcus luteus* (Huber *et al*., 1969; Van Schothorst and Van Leusden, 1972; McCracken *et al*., 1976; Nouws, 1979; Bogaerts and Wolf, 1980; Okerman *et al*., 2001) is especially sensitive to β- lactams and macrolides (Okerman *et al*., 1998). E.g. *M. luteus* 9341, 9341a and 15957 are currently classified as *Kocuria rhizophila.*

Escherichia coli strains (Ellerbroek, 1991; Choi *et al*., 1999; Okerman *et al*., 2001) are used to detect fluoroquinolone residues. A bioluminescent bacterium *Photobacterium phosporeum* has been used for the detection of chloramphenicol and oxolinic acid (Tsai and Kondo, 2001).

The size of inoculum has a direct bearing on the zone of inhibition and needs to be meticulously standardized (Cooper, 1972; Barry, 1976). A low to moderate inoculum improves sensitivity (Renard *et al.*, 1992); however, too low inoculum tends to produce granular and poorly defined zone edges (Davis and Stout, 1971).

2.5.4 Agar Diffusion Tests

Formation of an Inhibition Zone

In agar diffusion tests the agar is inoculated in a standardized manner, and the sample is applied to the agar surface. During the first hours of diffusion, the concentration of antimicrobial within agar medium at the edge of the sample is relatively

high and diminishes sharply at increasing distances from the sample. As diffusion progresses, the slope of the concentration gradient levels off, resulting in a broader gradient of decreasing concentrations within the agar medium (Barry, 1976). At first the diffusion proceeds radially from the source. When the bottom of the agar layer is reached, the direction turns to lateral. The thickness of the agar is inversely related to the zone size (Currie *et al*., 1998; Koenen-Dierick and De Beer, 1998). At some particular distance from the sample, the antimicrobial is diluted to a point that it no longer inhibits microbial growth, and an inhibition zone is formed. Under standardized conditions, the size of the inhibition zone shows a linear relationship with the log of the concentration of the drug (Schoevers *et al*., 1994).

The result of a diffusion-inhibition test is the result of a race between spreading of antimicrobial by diffusion and the grow-out of the test organisms.

When using spores the moment at which the germinating spores reach some critical stage – perhaps where the resulting vegetative organism first divides – is the "timing" mechanism for determining the position of the zone edge.

Within the zone the germinating organisms are prevented from passing the critical stage, whereas outside the zone the concentration of antimicrobial is too small to prevent the event (Davis and Stout, 1971).

The rate of diffusion through an agar gel depends e.g. upon the concentration of drug in the sample, the size and shape of the antimicrobial molecule, the viscosity of the agar gel and the temperature (Barry, 1976). Preincubation time, allowing the antimicrobial to diffuse in the agar before the test bacterium starts to grow, increases the sensitivity of an agar diffusion test, but the effect is not equally important for all substances (Koenen-Dierick and De Beer, 1998).

Inhibition zone widths can be measured with rulers or computerized image analysis systems (Schoevers *et al*., 1994). From the edge of the drug reservoir outward there may be a zone of complete inhibition of growth surrounded by a zone of partially inhibited microbial growth, enclosed in a zone of stimulated growth. The zone of partial inhibition probably represents the brief inhibition and eventual overgrowth that is often observed with subinhibitory concentrations of the antimicrobial agent. An inner zone of delayed growth can be seen with drugs that are primarily bacteriostatic (Barry, 1976). The phenomenon of double zones may also be due to the pH increase of the medium during incubation from a lower level to the optimum range of activity of e.g. aminoglycosides and macrolides (Korkeala and Pekkanen, 1977).

Antimicrobial agents with positively charged molecular structures may be electrostatically bound to acid or sulphate groups of the agar, and consequently the rate of diffusion through the agar gel falls off. E.g. aminoglycosides seem to have a tendency for electrostatic binding to active groups on the agar (Acar and Goldstein, 1996).

2.5.5 Growth Medium Composition

In agar diffusion tests the agar medium influences the zone size by its effect on the activity and the rate of diffusion of the antimicrobial, and its effect on the growth rate of the test organism. The general nutrient capacity of the medium will influence the length of the lag phase and the generation time of the test organism (Barry and Effinger, 1974); nutritionally deficient media produce larger inhibition zones because of a prolonged lag phase (Barry, 1976).

The pH of the medium affects the activity of certain antimicrobials. The activity of aminopenicillins and tetracyclines is increased in acidic pH, and the activity of macrolides, quinolones and aminoglycosides in alkaline pH (Yamada *et al*., 1981; De

Zutter *et al*., 1985). The mechanisms of the effect of pH on antimicrobial activity are incompletely understood and inconsistent from drug to drug (Amsterdam, 1996).

Trimethoprim is added in the growth media because of its synergistic action with sulphonamides (Gudding, 1976; Fabiansson and Rutegård, 1979; Bogaerts *et al*., 1981), and penicillin because of its synergistic action with streptomycin (Søgaard, 1979).

Phosphate is added to the media to inhibit the diffusion of aminoglycosides, which accumulate in kidney tissue (Van Schothorst and Peelen-Knol, 1970). It also inhibits the diffusion of natural inhibitory substances and buffers the medium pH against the possibly acidic or alkaline renal pelvis fluid/urine.

(Nouws *et al.,* 1988) Dextrose, especially in the presence of trimethoprim, increases the sensitivity to e.g. OTC (Koenen-Dierick and De Beer, 1998).

If the growth of the test organism produces a change in the medium pH in the absence of inhibition, the inhibition can be demonstrated with pH indicator dyes like bromcresol purple (Mac Neil and Ellis, 1995).

2.5.6 Currently Used Agar Diffusion Tests and their LODs

Very few commercially available test kits are designed for direct screening of animal tissues for veterinary drug residues in slaughterhouses (Boison, 2001).The LODs determined with similar antimicrobial concentrations, using same test bacteria and media vary because of the multitude of factors affecting the test result.

These factors include differences in test media, depth of the agar layers, inoculum levels, and differences in the application of the standard solutions.

Antimicrobial standards are commonly applied on the agar in filter paper discs or steel cylinders. The quantity of solution applied to a disc (Davis and Stout, 1971;

Bielecka *et al*., 1981) and charge volume of cylinders (Ragheb, 1988; Brady and Katz, 1990) influence the size of resulting zone diameters. The thinner the assay layer the less effect the cylinder charge volume has on the zone diameter (Brady and Katz, 1990).

There do not exists, a standardized procedure for the determination of LODs in agar diffusion tests. Therefore, various procedures have been applied in their determination (Nouws *et al*., 1988; Kondo *et al*., 1993; Koenen-Dierick *et al*., 1995; Currie *et al*., 1998; Okerman *et al*., 2001), complicating the comparison of results. The nature of the diluent also affects the LODs (Bielecka *et al*., 1981; Korsrud and Mac Neil, 1988; Braham *et al*., 2001).

Table 1. Agar Diffusion Tests Used for the Screening of Antimicrobial Residues in Meat.

Test method	Test bacterium	Test medium	Medium pH	Additional substances ($\mu g\ ml^{-1}$)	Test matrix	Reference/ producer
Four Plate Test (FPT)	*B.subtilis* BGA, *M. luteus* ATCC 9341	Test agar	6.0, 7.2, 8.0	Trimethoprim (pH 7.2)	Muscle, kidney, liver	Bogaerts and Wolf, 1980
Swab Test on Premises (STOP)	*B. subtilis*	Antibiotic medium No 5	7.9		Kidney tissue fluid, muscle, liver	USDA, 1979; Johnston et al., 1981
Two-plate test	*B. subtilis* BGA	Test agar	6.0, 8.0	Trimethoprim (pH 8.0)	Kidney, muscle	MAF, 2001a
The Live Animal Swab Test (LAST)	*B. subtilis* ATCC 6633	Antibiotic medium No 5	7.9		Urine from live animals	USDA, 1983
New Dutch Kidney Test (NDKT)	*B. subtilis* BGA	Standard nutrient agar	7.0	Dextrose, phosphate buffers, trimethoprim	Renal pelvis fluid	Nouws et al., 1988
Belgian Kidney Test (BKT)	*B. subtilis* BGA	Standard nutrient agar	7.0	Dextrose, trimethoprim	Kidney, muscle	Koenen-Dierick et al., 1995
Calf Antibiotic and Sulfa Test (CAST)	*B. megaterium*	Mueller-Hinton agar	7.4	Dextrose, bromcresol purple	Kidney tissue fluid, muscle, liver	USDA, 1984
The Fast Antibiotic Screen Test (FAST)	*B. megaterium*	Mueller-Hinton agar	7.4		Kidney tissue fluid	USDA, 1994
Premi^{R}Test	*B. stearothermophilus*		Muscle,		kidney, liver, urine	DSM Food Specialities

A limited number of studies have been performed to quantitatively correlate the results of microbiological and chemical analyses. Correlations between the mean areas of the inhibition zones and doxycycline and CTC concentrations have been determined (McEvoy *et al.*, 1994; De Wasch *et al*., 1998). The results of these studies indicate which portion of the total residue is released from the tissue in the microbiological analysis.

A poor correlation may result from the presence of fat tissue in the samples (De Wasch *et al*., 1998).

Table 2. Limits of detection (LODs) determined for various screening tests.

Test method[a] (Table 1)	Antimicrobial ($\mu g\ ml^{-1}$ or $\mu g\ g^{-1}$)						Reference
	Ampicillin	Pen G	OTC	Strepto-mycin/ DHS	Tylosin	Linco-mycin	
FPT[a]	0.13	0.06	1.0	1.6	1.0	0.3	De Zutter et al., 1985
FPT[a]	0.02	0.04	0.3	0.5	0.3	n. d.	Nouws et al., 1988
STOP[a]	0.4	0.03	1.6	1.0	1.6	25.0	Korsrud and Mac Neil, 1988
NDKT[a]	0.16	0.06	0.6	50.0	2.0	n. d.	Nouws et al., 1988
BKT[a]	0.13	0.06	1.4	1.9	0.4	2.2	Koenen-Dierick et al., 1995
CAST[a]	0.02	0.06	0.8	0.2	0.2	8.0	Korsrud and Mac Neil, 1988
BR Test[b]	0.001	0.0009	0.2	6.0	0.04	n. d.	Sundlof, 1990

n.d. not done
[a]Abbreviations in Table 1.
[b]Brilliant Black Reduction Test

Brilliant Black Reduction Test

Doxycycline residues in porcine kidney and muscle tissues far below MRL concentrations gave positive results in BKT (Koenen-Dierick *et al*., 1995) using *B. subtilis* as the test organism (Croubles *et al*., 1999).

Chromatographic methods (Neidert *et al.*, 1987; Oka *et al*., 1985) were more sensitive in the detection of CTC and OTC than the microbiological STOP test (Korsrud and Mac Neil, 1987). The stated LODs of four commercially available on-farm tests for pen G residues in bovine plasma were applicable also for incurred residues (Boison and Mac Neil, 1995).

Each of the current and potential screening tests has limitations in sensitivity, specificity, ruggedness, or other performance attributes (Korsrud *et al.,* 1998) making method refinement necessary. Although microbiological inhibition tests are suitable for large scale screening, it is obvious that there is no test which detects only samples with the residue concentrations of all antimicrobials above the MRL and does not miss any of them, and there will also be a number of positive test results for samples with concentrations below the MRL (Croubles *et al*., 1999). Most tests lack sensitivity to sulphonamides (Korsrud *et al*., 1998; Okerman *et al*., 1998), with only few execeptions (Braham *et al*., 2001; CFT, Charm Sciences Inc.).

Unspecific Inhibition Zones

Microbiological inhibition tests are unspecific in nature, i.e. any substance with antimicrobial activity may inhibit the growth of the test bacterium. Inhibition not caused by antimicrobial drugs is called unspecific, and the result of this inhibition is called a false positive reaction. False positive reactions have been shown to occur frequently.

Many antimicrobials were developed from naturally occurring fungal metabolites with high bacterial inhibition properties, but low animal toxicity.

Also naturally occurring fungal metabolites, mycotoxins possessing antibacterial characteristics - aflatoxin, ochratoxin A, patulin, citrinin, tenuazonic acid, rubratoxin, alternariol, altenuene and several tricothecenes - (Berdy, 1974), and unidentified antibacterial substances of mouldy grain (Williams *et al*., 1992) may cause inhibition of test bacteria.

Feed-unrelated substances in pig kidney tissue exposed to mechanical damage by freezing have been suspected to be the cause of unspecific inhibition. Nearly all kidneys of slaughtered animals contain a bacteriolytic enzyme lysozyme (Fleming, 1922; Heinert *et al.*, 1976), and inhibition zones resulting from the presence of lysozyme are rather often recognised in porcine kidneys. Especially frozen pig kidneys yield false-positive results (Korkeala *et al.*, 1983). Lysozyme is active against most gram-positive bacteria, particularly thermophilic spore formers (Beuchat and Golden, 1989).

Bacterial contamination may be an important cause of false positive results (Okerman *et al.*, 1998). Various microbial processes may cause these results: meat discs applied to test plates can contain natural inhibitors from earlier bacterial activity within the original sample, or contaminating organisms can produce inhibition in situ on assay plates during incubation (Smither, 1978; Smither *et al.*, 1980; Walton, 1983). Bacteriocins are substances produced by various species of bacteria, which in contrast to all other antimicrobials act mainly on strains of the same or closely related species (Reeves, 1965).

Cadmium in horse kidneys (Korkeala *et al*., 1976) and bile (Wilson *et al*., 1991) has also been attributed to unspecific inhibition. Lysine causes reversible inhibition of bacterial growth (Burger and Stahmann, 1952; Watson and Bloom, 1952).

Changes in pH may cause false positive reactions (Tritschler *et al*., 1987). Cattle urine at pH 7.5 inhibited the growth of *B. stearothermophilus* (Bielecka *et al*., 1981). High mean osmolarity and pH of urine samples correlated with the growth inhibition of *B. subtilis* (Terhune and Upson, 1989), but Erasmuson *et al*. (1998) found that although alkalinity in urine samples was associated with false positive reactions, the inhibition was actually caused by bicarbonate.

The occurrence of false positive reactions is associated with the test matrix used. False positive reactions have been detected in the CFT with muscle tissue (Korsrud *et al.*, 1995), but not with plasma as the test matrix (Boison *et al*., 1995). LAST with urine as the test matrix appeared to give false positive results (Tritschler *et al.*, 1987; Seymour *et al.*, 1988).

Dialysis membranes separate larger molecular weight proteins from the smaller molecular weight antimicrobial molecules. Their use in screening tests can reduce the incidence of false positive reactions (Forschner and Seidler, 1976), especially with frozen pig and horse kidney samples (Woodward and Shearer, 1995).

2.7 Identification of Residues with Agar Diffusion Tests

Although microbiological screening tests can reveal some information on the nature of the residue, a better post-screening characterization of the residue will significantly reduce efforts devoted to the identification and quantitation by chemical methods. The use of a microbiological method in the post-screening stage has many

advantages in terms of costs, practicability, and sample through put. Furthermore, simple or no extraction procedures are needed.

Microbiological identification tests use bacterial strains with different sensitivities to antimicrobials on media of varying pH values. The media may be supplemented with substances blocking or enhancing the action of certain antimicrobials or antimicrobial groups (Table 3).

Table 3. Certain Attributes of Microbiological Identification Tests

Test bacterium/ Growth medium attribute	Feature used in the identification
B. cereus K 250	Resistant to tetracyclines
M. luteus ATCC 9341a	Resistant to dihydrostreptomycin and streptomycin
M. luteus ATCC 15957	Resistant to erythromycin, lincomycin and spiramycin
S. epidermidis ATCC 12228	Resistant to tetracycline
Staphylococcus sp. ATCC 12715	Resistant to CTC and OTC
Penicillinase	Blocks the action of penicillinase sensitive penicillins
Decreasing pH (8→6)	Increasing activity of tetracyclines and cephalexin
Increasing pH (6→8)	Increasing activity of aminoglycosides, macrolides, fluoroquinolones, trimethoprim, sulphadoxine and sulphadiazine
$MgSO_4 \cdot 7H_2O$	Blocks the action of tetracyclines
Cystein	Blocks the action of streptomycins
PABA	Blocks the action sulphonamides
Trimethoprim	Synergistic action with sulphonamides

The integrity of a β-lactam ring is necessary for the mode of action of β - lactam antibiotics (Handal and Olsen, 2000). β -lactamases are bacterial enzymes capable of attacking either the penicillins (penicillinases) or the cephalosporins, or both. They catalyze the hydrolysis of the amino bond in the β -lactam ring (Citri, 1971), with production of the antibiotically inactive penicilloic or cephalosporoic acid (Hou and Poole, 1981). β -lactams can be further classified into three subgroups through selective hydrolysis of the β - lactam ring with PenaseTM or lactamase II (Medina *et al*., 1998; Moats *et al*., 1998).

Sulphonamides compete with PABA in the synthesis of folic acid compounds (Brown, 1962), and PABA is used to block the action of sulphonamides.

Cystein blocks the action of streptomycin (Greenstein and Winitz, 1961).

In addition to the use of various blocking agents, antimicrobials may be identified with selectively resistant bacteria (Table 3). The pH of the medium can also help in the identification of antimicrobials. A resistant test bacterium for the identification of fluoroquinolones has not been described, and the difficulty of distinguishing between e.g. aminoglycosides and quinolones has been encountered e.g. with the FSIS-USDA post-screening technique (Calderón *et al*., 1996).

Various test bacterium-medium combinations have been used in the identification of antimicrobial residues in meat (Fugate, 1974; Lund, 1986; Ferrini *et al*., 1997; Okerman *et al*., 2001). A specific, characteristic pattern or profile is obtained for each antimicrobial class.

A common feature of all identification tests is that when a complex mixture of antimicrobials is present in a sample, the pattern obtained does not allow the

identification of residues (Calderón *et al*., 1996). Synergistic and antagonistic effects of antimicrobials should also be considered.

Microbiological identification tests generally identify antimicrobials to group level. The identification is performed visually, by looking at the profiles obtained. Education and experience are needed to succesfully perform the identification.

2.7 Direct and Indirect Impediometry

When an electrolyte solution is exposed to an electric field, and the cations move toward the negative cathode and the anions towards the positive anode, current flows within the solution. Each ion carries a portion of the electrical current in proportion to its degree of mobility and concentration (Silley and Mortimer, 2003).

Impedance is defined as the resistance to flow of an alternating current through a conducting material. An impedance system measures the net changes in impedance in the culture medium at regular intervals (D zenclos *et al*., 1994; Silley and Mortimer, 2003).

When two metal electrodes are immersed in a conductive medium the test system behaves either as a resistor or capacitor in series or as a conductor and capacitor in parallel. Considering the case where the system is treated as a series combination, then the application of an alternating sinusoidal potential will produce a resultant current which is dependent on the impedance of the system. Impedance is a function of resistance, capacitance and applied frequency. Conductance is the reciprocal of resistance.

Resistance is a measure of the extent to which a substance opposes the movement of electrons among its atoms. Capacitance is an element that stores energy in an electric field (Firstenber-Eden and Eden, 1984; Kell and Davey, 1990).

Any increase in conductance or capacitance results in a decrease of impedance and an increase in current. The alternating current equivalent of conductance is admittance, defined as the reciprocal of the impedance, and the units of measurement are Siemens (S) (Silley, 1991).

In microbiological applications the incubation time required to reach certain curve features is called detection time (DT). DT is the point where the cellular growth has reached a critical mass, usually about 106 cells ml-1, and it is correlated to the number of bacteria by developing a calibration curve. It is a function of the culture medium, the temperature of incubation, the inoculums level and growth rate of the (test) bacterium. It is inversely proportional to the numbers of organisms present in the initial sample (Easter and Gibson, 1989; Bolton, 1990; Andrade *et al*., 1998; Wawerla *et al*., 1999).

In the direct impediometric technique the electrodes reach into the liquid culture medium that has been inoculated with the sample material. As a result of the metabolic activity of the microorganisms in the sample, large molecules are broken down into many smaller, electrically charged molecules. These changes in the molecular composition increase the conductivity of the liquid and the capacitance that arises mainly from the polarization of the electrodeliquid interface (Wawerla *et al.*, 1999).

In the indirect impediometric technique the metabolic activity of the bacterial cells determines the detection time. Most facultative microbes will respire aerobically if sufficient oxygen is present. In this situation carbohydrates such as glucose will be catabolized through the tricarboxylic acid cycle, to generate energy, carbon dioxide and water. This metabolism, which is common in the early stages of growth and generates large quantities of carbon dioxide, is later replaced by fermentation. Carbon dioxide

produced will dissolve in the aqueous growth medium until the solubility is exceeded and the volatile gas diffuses into the headspace. Using the indirect technique high impedance readings of media with high salt concentrations can be overcome (D zenclos *et al*., 1994; Silley and Forsythe, 1996; Silley and Mortimer, 2003).

In the rapid automated bacterial impedance technique (RABIT) (Don Whitley Scientific, Surrey, UK) potassium hydroxide is added to the impedance tube across the electrodes. The inoculated culture medium is in a separate chamber and not in contact with the electrodes or potassium hydroxide. The unit is tightly sealed such that any CO_2 produced as a result of normal metabolism is absorbed by the potassium hydroxide causing a resultant increase in impedance. The RABIT apparatus plots the conductance as a function of time (Bolton, 1990; Silley, 1991).

For KOH and NaOH solutions at pH values at or above 11 the carbon dioxide will be converted to carbonate ($CO_2 + 2OH^- \rightarrow CO_3^{2-} + H_2O$) with a reduced conductivity reading. The measured change in conductance of an absorbent solution depends on the amount of CO_2 produced by the culture, the volume and concentration of absorbent solution, and the cell constant of the electrode (Butler, 1982; Owens *et al*., 1989; Silley and Mortimer, 2003).

Impedance monitoring brings with it the associated benefits of an automated system, but the initial enumeration of bacterial cells has to be performed by an additional method and that value used as a baseline (MacKenzie *et al.*, 1999).

The conventional impedance-based method is not adequate for detecting bacterial concentrations lower than 100 CFU ml-1 in two hours or less. A microfabricated device for impedance-based detection of bacterial metabolism enabled the detection of a few

live bacterial cells. Rapid detection was possible since the cells were confined into a volume of the order of nanolitres (Gómez *et al*., 2002).

Impedance microbiology enables the monitoring of real-time observations on organism-drug interactions. The ability of a drug at a fixed concentration to inhibit or kill a bacterial population is measured over a fixed time period. This is in contrast with the single time point after overnight incubation used with most conventional methods (Silley and Forsythe, 1996).

Irrespective of the purpose of the impediometric method used to measure the interaction between bacteria and antimicrobials, the parameter(s) used to detect the growth inhibition have to be defined.

Direct impediometric technique has been used in antimicrobial residue testing of meat (Werber and Bergann, 1997) and milk (Okigbo and Richardson, 1985; Chen and Chang, 1994). Slope differences in conductivity readings (Okigbo and Richardson, 1985) and differences in detection times (Chen and Chang, 1994; Werber and Bergann, 1997) have been used to indicate the presence of antimicrobials. Indirect technique has not been used in antimicrobial residue testing.

Differences in detection times have also been used for efficacy testing of antimicrobials (Connolly *et al*., 1994; Zhou and King, 1995; Andrade *et al*., 1998). The effects have also been measured by determining the differences in time taken by antimicrobial-exposed and unexposed cultures to reach a certain cell density (Baquero *et al*., 1986; MacKenzie *et al.*, 1994 and 1999).

The difference in time to reach the maximum rate of change in conductance, and the difference in time for the growth of exposed and unexposed cultures to be first inferred have also been used to detect growth inhibition (Majcherczyk *et al*., 1994).

Certain problems of other techniques in assaying antibacterial effects of certain substances, like protamine, can be overcome by using impediometric techniques (Johansen *et al*., 1995). Methods based on colony counts, absorbance of bacterial suspensions or inhibition zones on agar plates may not be suited when assaying the antibacterial effect of protamine due to agglutination of the positively-charged protamine and the negatively-charged bacterial cells (Islam *et al*., 1984).

2.8 Automated Turbidometry

Turbidometry is an established method used to study bacterial growth since optical density (OD) measurements make it possible to follow bacterial population growth in real time (Begot *et al.*, 1995). An increase in turbidity is usually taken as an indication of bacterial multiplication. The slope of the growth curve describes the generation time and the sample turbidity correlates fairly well with the number of bacterial cells (Ali-Vehmas, 1998), although turbidity can also be influenced by other factors, such as changes in particle size due to swelling, capsule formation, aggregation and disaggregation.

With turbidimetric microplate technology and automated dispension, the analysis is simple, consumption of growth media is negligible and the technique requires a minimum amount of labour (Mattila, 1987), but the method requires solutions to be initially transparent (Ali-Vehmas, 1998).

As in impediometry, the growth curve parameters used for the measurement of growth inhibition have to be determined in order to detect and quantitate inhibitory effects of antimicrobials for any purpose.

In a microtitre plate assay (Buick *et al.,* 2000) for the detection of certain antimicrobials in porcine urine, inhibition of an enzyme activity of a *B. subtilis* strain

was measured. The OD data were expressed as a percentage of the maximum OD readings produced by the control samples.

The time taken for a microbial culture to achieve a specific OD or final absorbance, increase of the lag phase, slope of the logarithmic growth phase, and a certain level of growth reduction have all been used to study the efficacy of antimicrobial agents (Korkeala *et al*., 1992; Johansson *et al*., 1995; Lambert *et al*., 1998; Walsh *et al*., 2003).

A widely used approach is to relate the area under the OD/time curves to the degree of inhibition. This approach has been used to examine the kinetics of the disinfection process and inhibitory effects of food antimicrobials and preservatives, and it covers the entire growth period instead of referring to single points of time (Mattila and Sandhom, 1989; Skyttä and Mattila- Sandholm, 1991; Skyttä *et al*., 1993; Lambert and Pearson, 2000; Lambert, 2001).

Total inhibition of growth has been used to determine MICs (Hedin and Hambraeus, 1991; Lambert *et al*., 2001). The time course of the antibacterial activity (Korkeala and Männistö, 1988) has also been studied with automated turbidometry. Growth inhibition is detected more sensitively than with traditional procedures (Olsson-Liljequist and Hoffman, 1991; Van de Guchte *et al*., 2001).

2.9 Immunochemical Methods

Immunochemical methods are based on the ability of antibodies to bind specifically to different substances. The reversible association between antibodies and their corresponding antigens is called the immunological reaction. The binding forces involved are weak molecular interactions like Coulomb and Van der Waals forces as well as hydrogen bonding and hydrophobic binding (Märtlbauer *et al*., 1994). Veterinary

drugs, being low molecular weight compounds, generally do not prompt any immunogenic response in an animal. To produce such a response, the compounds have to be coupled to a large molecule such as bovine serum albumin (Dixon- Holland, 1992; Haagsma and Van de Water, 1992).

The assays are performed by bringing the antibodies into contact with the analyte and adding an amount of radio-, enzyme-, or fluorescent-labelled analyte, which competes with the non-labelled analyte for the available binding sites (Blake and Gould, 1984; Boison and Mac Neil, 1995).

In enzyme immunoassays, enzymes act as labels whether they are fluorogenic or not. When an enzyme substrate is used, enzyme activity proportional to the number of enzymes catalyzing the reaction is measured (Boison and Mac Neil, 1995).

Immunochemical methods can be used for the detection and identification of antimicrobial residues. However, for various reasons, immunological techniques have not commonly been used as post-screening methods: commercial EIA kits are expensive, have a limited shelf life and may demand laborious sample preparation (Okerman *et al*., 2003).

Enzyme-linked immunosorbent assay (ELISA) is the most popular method for testing chemical residues in milk, urine, blood and meat samples (Lee *et al*., 2001b). However, group-specific tests cannot be used to quantify a residue (De Wasch *et al*., 1998; Okerman *et al.,* 2003).

An electrochemical ELISA has been described for the detection of two macrolides in bovine muscle. After competition between free and coated analytes for the antibodies, the activity of the horseradish peroxidase-labelled antiglobulins was measured electrochemically (Draisci *et al.,* 2001).

Solid-phase fluorescence immunoassay has been succesfully used for the detection of certain tetracyclines and ß-lactams in bovine and porcine kidney tissue in a method described by Okerman *et al*. (2003).

Immunobiosensor is an alternative to enzyme immunoassay for the screening of residues. Rapid immunoassays using an optical biosensor have been described for the detection of sulphonamides in pork bile and muscle (Crooks *et al.*, 1998; Baxter *et al*., 1999; Bjurling *et al*., 2000), and streptomycins in meat, milk and honey (Ferguson *et al*., 2002).

Biochip assays are immunoassay-based tests with chemiluminescent detection. The biochip consists of substrate on which discrete test regions have been constructed, each test region representing a different analyte.

Digital imaging technology is used to measure light signals generated from individual test regions. The light signal generated is converted to provide the concentration of each chip parameter (McConnell *et al.*, 2000).

2.9.1 Other Techniques used in Residue Screening and Identification

Low tetracycline concentrations can induce ß-galactosidase synthesis in *E. coli* strains carrying *tetA-lacZ* gene fusions (Bertrand *et al.*, 1984). Chopra *et al*. (1990) developed a method for the detection of tetracyclines, in which an *E. coli* strain containing a cloned *tetA-lacZ* gene fusion was used. ßgalactosidase was readily detected with chromogenic substrates.

Korpela *et al.* (1998) developed a luminescence-based microbiological assay for the detection of tetracyclines. It uses *E. coli* carrying a sensor plasmid, in which a tetracycline-specific control unit regulates the expression of bacterial luciferase genes.

The bacteria emit visible blue light in the presence of tetracyclines. The sensor is self-luminescent (Chopra *et al*., 1990); i.e. light is emitted without additions of substrates or cofactors.

Tetracycline residues in bones can be detected by a specific fluorescence (Buyeske *et al.*, 1960). Haagsma and Mengelers (1989) described a fluorimetric screening method for the detection of tetracycline residues in pig meat and kidney tissues, and Kühne *et al*. (2000) for the detection of tetracyclines in bones.

The Charm II Test (Charm Sciences, Malden, MA, USA) uses two types of bacterial cells, which contain either the natural receptor sites for antimicrobials or an antibody coating, and a radiolabelled antimicrobial. A binding reaction occurs between drug functional groups and receptor sites on microbial cells. A radiolabelled analyte competes with incurred drug residue for receptor sites. A liquid scintillation counter is used to measure bound 14C or 3H from the labelled drug. Labelled drug not bound to receptor sites is removed from the substrate prior to counting, and therefore the greater the amount of incurred drug present in the sample, the lower the counts (Korsrud *et al*., 1994; Ferrini *et al*., 1997). Special equipment and precautionary measures are needed (Nouws *et al*., 1998). The test can be used both for the detection and identification of antimicrobial residues.

A method combining microbiological and chemical analyses was described for the detection of tetracyclines. High performance liquid chromatography (HPLC) was used for cleaning up and concentrating the analytes and the detection was facilitated by a microbiological assay (Hamscher *et al*., 2000; Sczesny, 2001). Another procedure combining a liquid chromatographic assay with a rapid screening test was developed for the determination and identification of ß-lactams in bovine urine (Musser *et al*., 2001).

The migration behaviour of most antimicrobials is governed by the effect of the net electrical charge on the molecule at a particular pH. In a method by Smither and Vaughan (1978) antimicrobials were electrophoretically separated and visualized with test bacteria. In a test system by Kondo *et al*.(1993) an agar block was taken from an inhibition zone caused by an antibacterial agent and identification carried out by reverse-phase HPLC.

2.9.2 Quantitative Confirmatory Methods

Commonly used procedures for the detection of veterinary drug residues include HPLC, gas chromatography (GC), thin layer chromatography (TLC) and mass spectrometry (MS) (Aerts *et al*., 1995; McCracken *et al.*, 2000).

Chemical methods usually proceed with a preliminary extraction in order to isolate the drugs of interest from the biological matrix. The main objectives of sample treatment are removal of macromolecules and other matrix constituents that may either adversely affect the chromatographic system or interfere with the detection, and enrichment of the analytes in order to achieve the required low limits of detection (Aerts *et al*., 1995). Compounds must be separated from another and the food matrix.

The low solubility of some antimicrobials in organic solvents has made it difficult to develop procedures to extract and concentrate their residues from biological matrices. Other antimicrobials are either insufficiently volatile or are too thermally unstable (or both) to permit their analysis using GC or GC-MS.

Liquid chromatography (LC) has emerged as the method of choice for determination of antimicrobials which are rather polar, non-volatile, and sometimes heat sensitive (Shaikh and Moats, 1993; Kennedy *et al*., 1998).

The development of coupled liquid chromatography-mass spectrometry, LC MS, has increased the range of antimicrobials for which assays, based on molecular spectrometry, can be developed (Niessen and Tinke, 1995).

Approaches to extraction include extraction with water or buffers, direct solidphase dispersion, extraction/deproteinization with tungstic acid, trichloroacetic acid, water-miscible organic solvents such as methanol, acetone or acetonitrile combined with buffers or acids, ultrafiltration, partitioning into water-immiscible organic solvents, heating to denature proteins, and extraction with supercritical carbon dioxide (Moats, 1997). With the automated sequential trace enrichment of dialysates sample pretreatment is restricted to homogenization and dilution of the samples; clean-up is by on-line dialysis and on-line solid-phase extraction (Zurhelle *et al*., 2000).

In addition to sample preparation and analyte extraction, analytical methods are comprised of analyte clean-up and detection (Moats, 1997; McCracken *et al*., 2000). Sample clean-up procedures include column chromatography, TLC, liquid-liquid extraction, solid phase extraction and matrix solid phase dispersion.

The aim of chromatography in general is the resolution or separation of different molecular species (Klassen and Edberg, 1996). The mobile phase passes over the stationary phase at a constant rate; the two phases possess different chemical properties.

As the analytes in the mobile phase pass over the stationary phase, those with polarity closer that of the stationary phase are retained selectively for a time on the column. Conversely, the analyte molecules with polarity closer to that of the mobile phase tend to remain in the mobile phase, passing through the column faster. Passing through the instrument monitor sequentially, these “groups” of molecules give rise to peaks on the chromatogram (Klassen and Edberg, 1996).

HPLC methods utilise the same basic steps: extraction of the drug with a specific solvent, separation of the drug on the solid phase by HPLC, detection of the effluent from the solid phase by spectrometry and quantitation of the amount of antimicrobial present by peak height or peak area analysis. Of the detection methods used with LC analysis, UV absorbance is the simplest and most widely used. Derivatization, either pre- or postcolumn, is frequently used to enhance UV absorbance or to form fluorescent compounds. Compounds with little or no UV absorbance require other approaches (Moats, 1997).

Lanthanide sensitized luminescence is an alternative to UV detection and other luminescence techniques, i.e., fluorescence and phosphorescence, in separation science for the detection of drugs (Rietourd *et al*., 1997; Hernández-Arteseros *et al*., 2000).

Two limits are usually defined when validating an analytical technique, namely, the LOD, and the limit of quantification (LOQ). The LOD is a threshold concentration below which it is not possible to distinguish a signal because of the presence of the analyte, from the noise of the equipment. The LOQ is the limit from which a result is obtained with a given precision (Concordet and Toutain, 1997). The other critical parameters determined in method validation are day-to-day variation, relative standard deviation and the effect of the storage to analyte loss.

CHAPTER THREE

3.0 Materials and Methods

3.1 Study Area

Sokoto states located is the North Western part of Nigeria on longitudes 4^0 8^l E and 6^0 54^l E and latitudes 12^0 N and 13^0 58^l N. it boarders with Republic of Niger to the North; Kebbi State to the West and South West, and Zamfara State to the East. The state is divided into 23 local government areas (L.G.As). Farming is the main stay of the people's economy accounting for a significant proportion of the Gross Domestic Product (GDP) and responsible for about 70% of the total employment (NPC, 1991). Sokoto state ranks second in the nation livestock population with estimated 3 million cattle, 3 million sheep, 5 million goats, 4,600 camels and variable species of poultry including chickens, guinea fowls, ducks and turkeys (MOCIT, 2002). Consumption of meat, milk and their products (Yoghurt "Nono", "Kilishi", "Suya") form part of the food habits of the inhabitants of the state.

Sokoto state has a tropical continental type of climate dominated by two opposing air masses; the tropical marine from the south and tropical continental from the north, annual rainfall is about 550mm with the highest peak in August. Dry season sets in first with the cold harmattan from October to February, and hot period is from March to the end of May when temperature reaches 38^0C during the day, humidity less than 20% and the rain begins in June to September (Sokoto, 2000).

3.2 Sample Size

A pilot survey was conducted over a period of one week to determine the average slaughter per day at camel slaughtering section of Sokoto mmain abbatoir convenience samplinig technique was used to select representative samples.

3.3 Sample Collection.

From the initial pilot survey, an average number of 12 camels (both males and females) were slaughtered on daily basis. A total of 90 gluteal and 90 neck muscle samples were collected using tissue forcep and scapel blade from 90 convinently selected camels slaughtered over a period of 3 weeks was obtained.

The Samples were wrapped in polythene bags, properly labeled and then transported in an ice chest with ice packs to City Campus Central Laboratory, Usmanu Danfodiyo University, Sokoto and stored at 20^0C until the time of analysis.

3.4 Lateral-Flow Immunoassay (LFIA) Technique.

The obtained stored samples were analysed for the presence of oxytetracycline using Tissue Tetrasensor Kit ^{R} (KIT036).

3.5 Principle of the Procedure

Tetrasensor is a competitive test that exploits the activity of a receptor for the recognition of tetracycline molecules present in the sample. The test requires the use of two elements provided in the kit. The first element is a reagent containing a certain amount of labelled receptor and the second is a dipstick consisting of a set of membrane where two capture lines are printed in green. When there is a liquid format of the tissue sample, the sample supernatant is added together with the receptor and the dipstick. While starting to run vertically on the strip, the receptor binds tetracycline molecules present in the sample. When the liquid passes through the green capture lines, red colour appears. The first line captures the remaining active receptor and the second line takes a certain amount of the excess of reagent that has passed through the first line. This second upper line serves as a control line and becomes visible in all cases.

Composition OF Tissue Tetresensor Kit [R] (KIT036) manufactured by (UNISENSOR; BELGIUM): Each kit is for 96 assays:

- 12 pots each with 1 strip of 8 reagent microwells and 8 dipsticks;
- 1 bottle of 175ml of "Tissue Buffer 20X." to be diluted 20 times in pure water to reconstitute **Buffer 1;**
- 1 bottle containing 175ml of Buffer 2 **"**Urine Buffer". Ready for use;
- 1 Micropipette of 200 µl and disposable tips;
- 1 White tray for microwells
- 2 ml-microcentrifuge tubes (Eppendorf or Equivalent)
- Distilled water (deionised water)
- Balance, capable of accurately measuring 3-10 g
- Homogenize
- Timer

3.6 Procedures for Sample Preparation

For Raw Muscle Tissue

1. The Tetrasensor kit was taken out of the fridge and allowed to equilibrate at room temperature prior to use.
2. Tissue buffer 20x concentrate was diluted as follows; to make 1L of buffer 1 take 50 ml of the 20× concentrate and dilute to 1000ml using distilled water. 1L of extraction buffer 1 is sufficient for approximately 30 samples.
3. 10 g (± 0.3 g) of tissue was weighed and 30 ml of the buffer 1 (from step 2) to the tissue sample was added (equivalent to a 4 dilution of the tissue).

4. The tissue sample in the presence of the extraction buffer was macerate until homogenate slurry was achieved.
5. 1 ml of the slurry was removed using a disposable plastic Pasteur pipette and place in a clean centrifuge tube.
6. Homogenate was centrifuged for 1 minute at ambient temperature at 5000g using the microcentrifuge to separate supernatant from solid material.
7. 1 ml of the resulting supernatant was then removed for the Tetrasensor analysis (= 4x final dilution of original matrix sample).

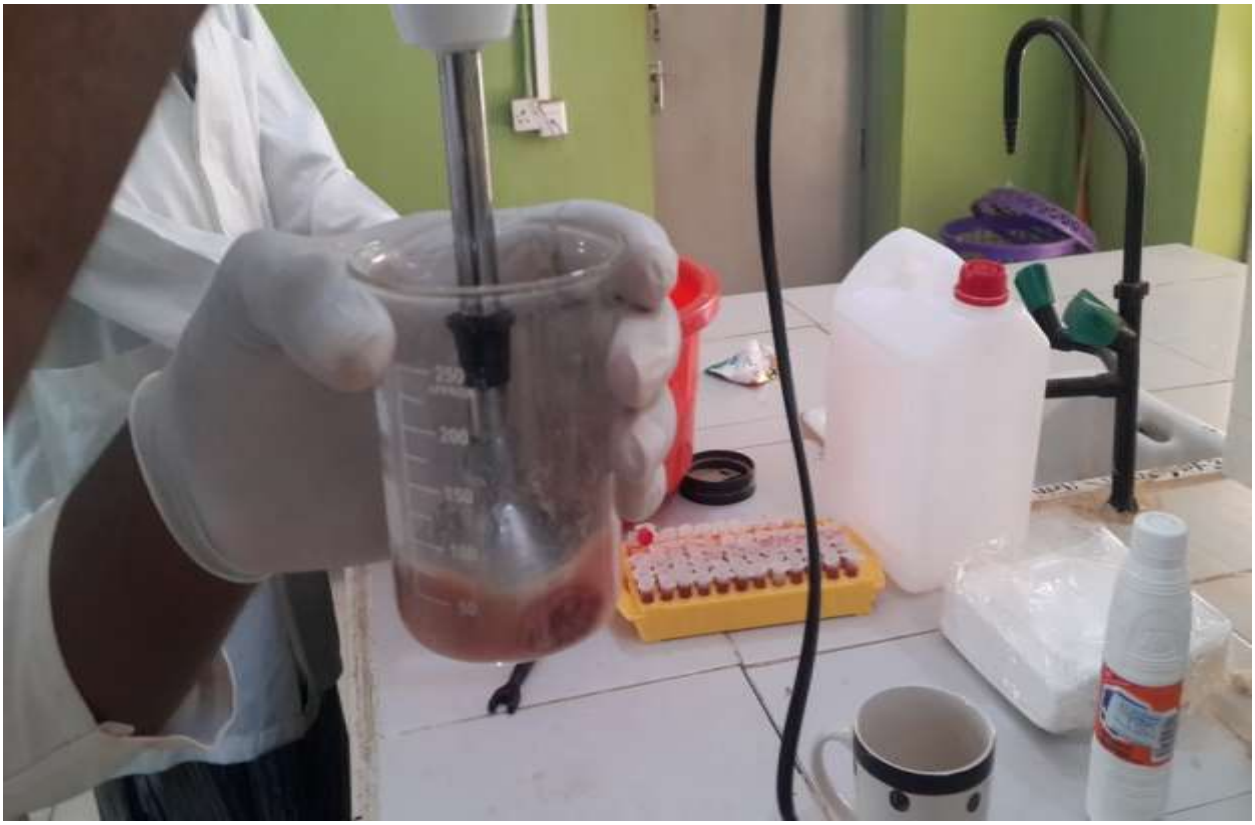

Plate 1: 10g of Tissue and 30 mls of Buffer (*) Being Homogenised in a 250mls Beaker.

3.7 Procedure for Dipstick Analysis:

1. Required number of individual reagent microwells was taken out and the rubber caps removed immediately prior to use.
2. The required number of dipstick was removed and label with the appropriate sample identification number.
3. Using a pipette, 200μl of the final liquid sample(s) was transferred to the reagent mircowell(s) containing dried reagent pellet.
4. Ensued the dried reagent pellet was thoroughly dissolved in the sample extract solution by manual, and over end mixing (crica 15 to 20 seconds).
5. Immediately after dissolving the pellet, one dipstick was dipped into the reagent microwell so that the bottom edge (indicated by the downward arrows) of the dipstick is fully submerged. The dipstick in the reagent microwell was Incubated for a 10minutes (± 3 minutes) period at room temperature.
6. Following the incubation period, the dipstick was removed from the microwell and its bottom sample pad removed. Immediately the presence/absence of the red coloured test and control lines was read.

3.8 Visual Readings:

1. First check whether the top control line is present. If not, regard the analysis as invalid and do not start (or continue) any interpretation.
2. When the top control line can be seen, interpret the test line as follows:
3. Examine one test line at a time and compare the intensity of the line colour of the test line with the intensity of the control line.

- If the test line is darker in colour than the control line, the result is NEGATIVE, which means that, given the sensitivity of our test, the sample contains no tetracyclines or tetracyclines at lower level than the minimum detection limit.
- If the test line is as distinct as or lighter in colour than the control line, the result is POSITIVE, which means that, the sensitivity of our test, the sample contains tetracyclines at or above the minimum detection limit.

4. If you hesitate, regard the sample as POSITIVE and confirm your interpretation by performing a second visual reading within the next 15 minutes;
5. Write down your assessment on each of the dipsticks;

Dipsticks can be archived as a permanent record if required by removing the sample pad and allowing it to dry before storage. N.B Line colour intensity will darken on drying.

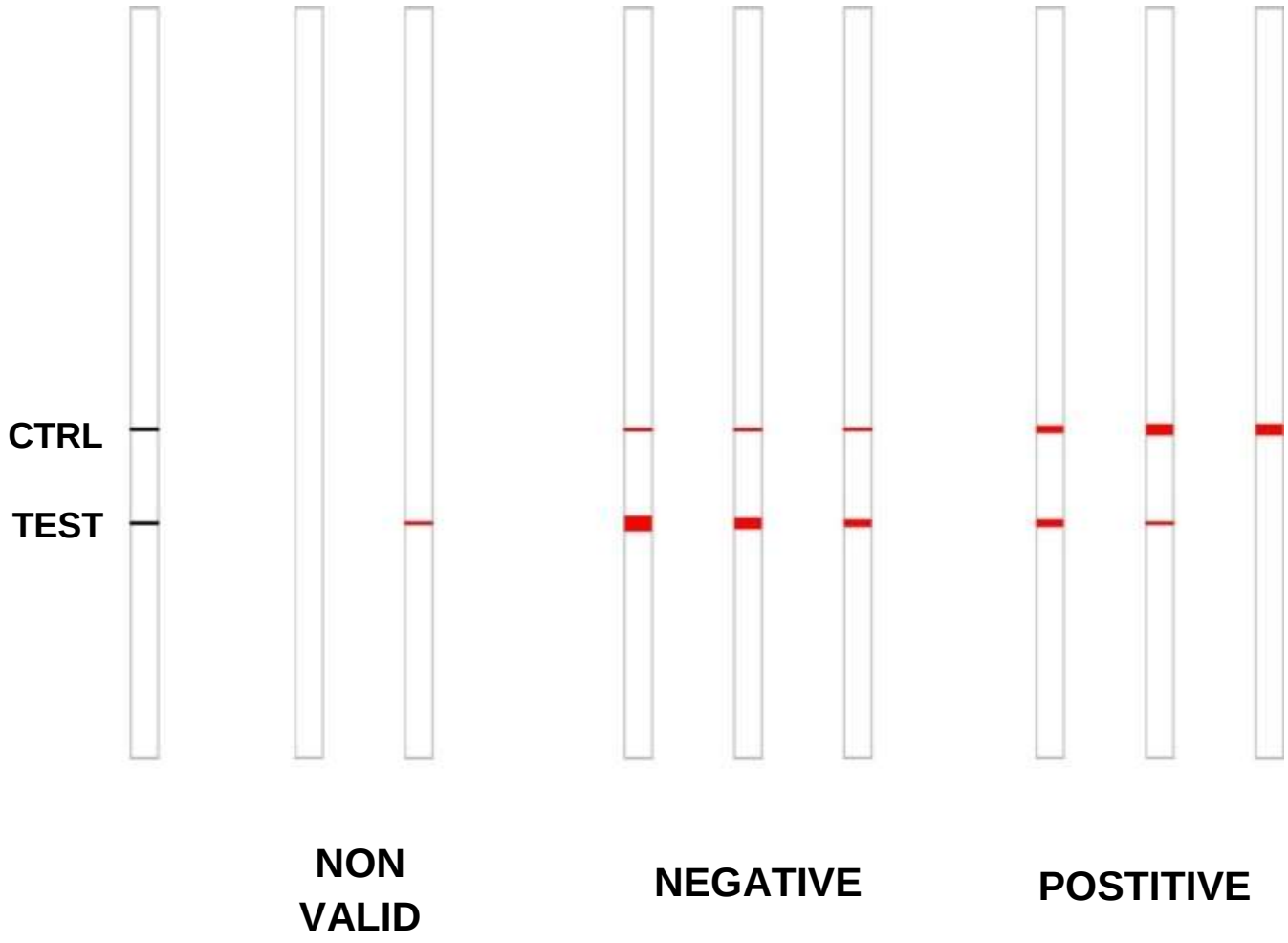

Plate 2: How the Strip is Interpreted

CHAPTER FOUR

4.0 Results

After 7 days of pilot survey, it was observed that an average number of 12 camels were slaughtered on daily basis at the Sokoto main abattoir.

Samples were collected from all the camels slaughtered on each day of visitation to the abattoir as shown in the table below.

Table 4: Number of Camel Samples Collected Per Day from Sokoto Main Abattoir

	Week 1			**Week 2**			**Week 3**		**Total**
Day	1	2	3	4	5	6	7	8	
No. of Camel samples	20	24	28	22	26	22	18	20	**180**

A total of 90 camels, comprising of 22 female and 68 male camels were sampled from the abattoir and analysed for presence of oxytetracycline residues.

Out of the 180 meat samples obtained from 90 camels during the study, 25 (13.89%) comprising of 16 (64%) glutted muscle sample

and 9 (36%) neck muscle sample had detectable levels of oxytetracycline residue at 10ppb or above.

Twenty two (22) of the overall total number of camles examined were female, out of which 5 (22.73%) had detectable level of oxytetracycline residue in their muscle, and 68 were male out of which 12 (17.65%) had detectable level of oxytetracycline residue in their muscle.

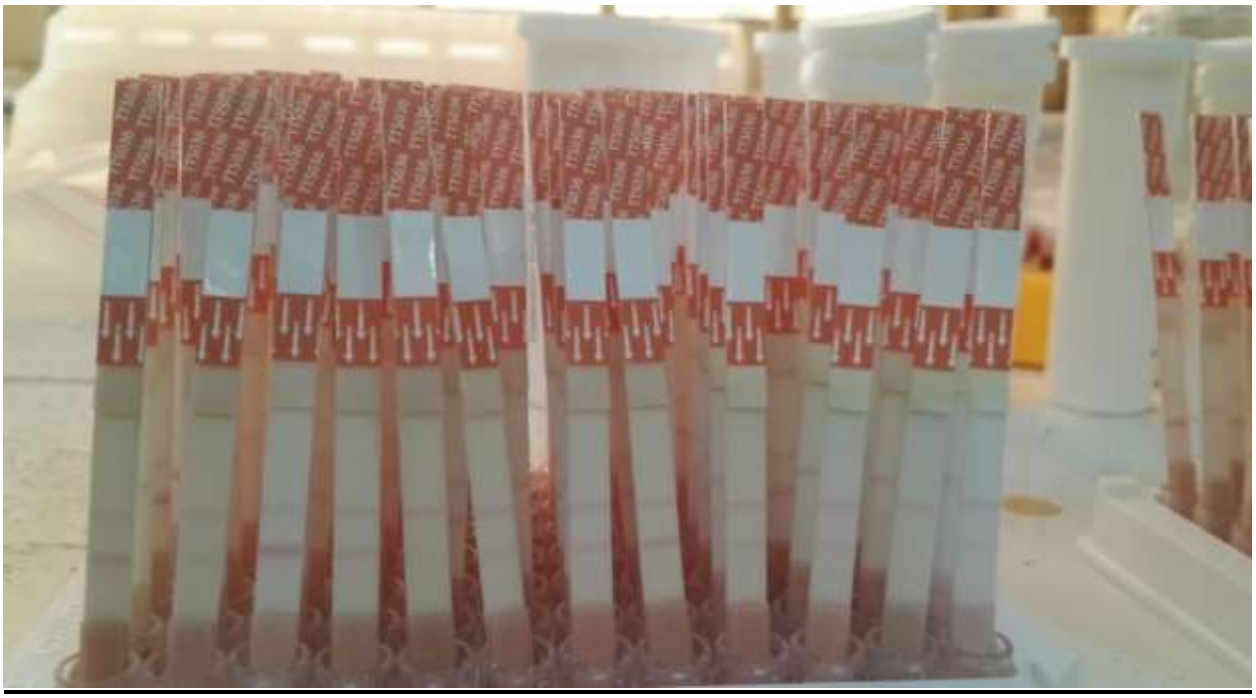

Plate 3: Dipsticks Inserted into Reagent Vials and Incubated at Room Temperature

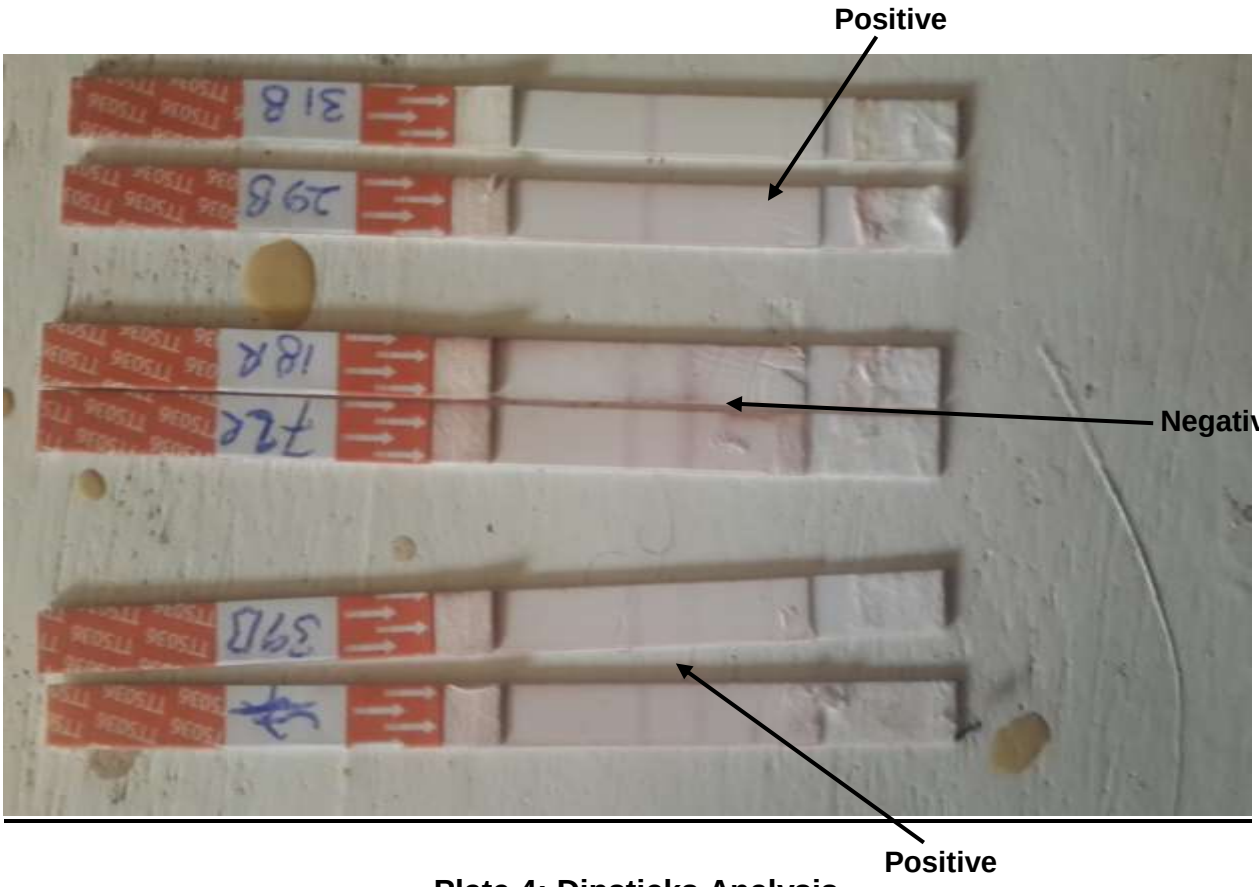

Plate 4: Dipsticks Analysis

CHAPTER FIVE

5.0 Discussion and Conclusion

5.1 Discussion

Result from this investigation revealed that out of overall of 180 camel meat examined, 25 (13.89%) had detectable levels of oxytetracyline residue; this could be an indication of indiscriminate use of veterinary drugs by nomadic herdsman as buttressed by Kabir *et al.,* (2002).

The proportions of oxytetracycline-positive samples found in this study is comparable with that of El Emam (2011) from Riyadh region, Kingdom of Saudi Arabia whose study revealed presence of oxytetracycline residues in slaughtered camle liver and kidney samples at a prevalence of 13.5% exceeding maximum residual limits however, the present study is in lower proportion than that reported by Jamela *et al.* (2015) which was 60% in muscle samples from camle carcasses slaughted at different slaughter hourse in Tripoli districts, Lybia using liquid chromatography-Mass spectroscopy (LC-MS) technique. This may be due to higher proportion of camel farmers employing traditional remedies in disease management over veterinary services (Chafe *et al.,* 2008) within the study area.

Other studies, such as that of Olatoye and Ehinmowo (2009) in catrtle, Dipeolu (2002) in goat, Dipeolu and Alonge (2002) in cattle and Kabir *et al.* (2004) in broilers in Nigeria, which were 54.44%,15.6%, 16.11% and 33.1% respectivlely: Mmbando (2004) in cattle from Morogoro and Dodoma municipalities in Tanzania, and Muriuki *et al.* (2001) in catle slaughtered in Kenya which were 41.2% and 45.6% respectively were also found to be higher than that obtained in this study.

This may be due to, the general rule, that antibiotics appear to have longer elimination half-lives in caemlids than in domestic ruminants, potentially prolonged their therapeutic effect but also increasing their risk of toxicity. This may be due to a lower rate of urine production in camelids (Lackey *et al.,* 1995), which may increase half-life of antibiotics excreted primarily through the kidneys (e.g., penicillins, aminoglycosides): and, another general rule, that the volume of distribution varies tremendously among individual camelids.

Of the 180 samples analyzed, 25 (13.89%) comprising of 16 (64%) gluteal muscle sample and 9 (36%) neck muscle sample had detectable levels of oxytetracycline residue, suggesting that the gluteal muscle may be a ore frequent used intramuscular injection site than the neck muscle in cael within this area.

Nisha (2008) reported that indiscriminate use of antibiotics to treat pyrexia, inflammation, wounds and viral diseases is associated with high levels of residues in edible tissues of food-producing animals being slaughtered before the end of the withdrawal period.

Administration of drugs to food-producing animals requires consideration not only of effects on the animals but also of effects in humans who consume food from these animals, because of the risk of sustained consumer exposure to drug residues.

Examples of such health effects include: induction of allergic reactions to some individuals (Rudzki and Rebandel, 1997); development of bacterials resistance (Rudzki and Rebandel, 1997), risk of teratogenicity when OTC is administered in the first trimester of pregnancy (Stauffer, 1967); and discoloration of prmary and permanent teeth (Stauffer, 1967).

5.2 Conclusion

The findings of this study may be indicative of the inappropriate use and management of veterinary drugs by livestock keepers. Therefore, further studies are necessary to evaluate other drug residues in edible camel tissues and stricter regulation of the use of veterinary drugs in the livestock industry as well as the inspection of livestock products prior to marketing is recommended.

Furthermore, livestock keepers need to be educated on the importance of adhering to the recommended drug withdrawal periods and possible human health effects associated with presence of veterinary drug residues in food of animal origin. Veterinarians and extension livestock officers should also promote alternative management options aimed at good animal husbandry and disease control measures.

REFERENCES

Abubakar, M.M. and Maigandi, S. (1994). Relative adaptability of cattle and camels to semi-arid zone of Nigeria. A paper presented at the 19th Annual Conference of Nigeria Society for Animal Production, University of Benin, March, 1994.

Acar, F.A. and Goldstein, F.W., (1996). Disk Susceptibility Test. In: Lorian, V. (ed.) Antibiotics in Laboratory Medicine. Williams & Wilkins, MA, USA **pp**. 1-51.

Aerts, M.M.L., Hogenboom, A.C. and Brinkman, U.A. (1995). Analytical strategies for the screening of veterinary drugs and their residues in edible products. *Journal of Chromatography* **667**, 1-20.

Agai, B.M., Magaji, A.A. and Sonfada, M.L. (1997). Slaughter of food Animals in Sokoto Metropolitan Abattoir and Meat Availability is Sokoto. *Nigerian Journal of Basic and Applied Sciences* **6**, 1-6

Ali-Vehmas, T. (1998). Microtitration tray technologies analyzing interactions of exogenous and indigenous antibacterials in milk. Thesis. Division of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland.

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 Additives and Contaminants* **27**, 291–301.

Amsterdam, A. (1996). Susceptibility Testing of Antibiotics in Liquid Media. *In: Lorian, V. (ed.) Antibiotics in Laboratory Medicine. Williams & Wilkins, MA, USA* **pp**. 52-111.

Anad´on, A., Martinez-Larranaga, M.R., Diaz, M.J., Bringas, P., Fernandez, M.C., Fernandez-Cruz, M.L., Iturbe, J. and Martinez, M.A. (1994). Pharmacokinetics of doxycycline in broiler chickens. *Avian Pathology* **23**, 79–90.

Anadón, A. (1985). Standard residue regulations for chloramphenicol in Spain. *Annals Veterinary Research* **16**, 149-153.

Anadón, A. and Reeve-Johnson, L. (1999). Macrolide antibiotics, drug interactions and microsomal enzymes: implications for veterinary medicine. *Research in Vetetrinary Science* **66**, 197-203.

Anadón, A. and Reeve-Johnson, L. (1999). Macrolide antibiotics, drug interactions and microsomal enzymes: implications for veterinary medicine. *Research in Vetetrinary Science* **66**, 197-203.

Anadon, A. and Reeve-Johnson, L. 1999 Macrolide antibiotics, drugs interactions and microsomal enzymes: implications for veterinary medicine. *Research of veterinary sciences* **66,** 197-203.

Andrade, N.J., Bridgeman, T.A. and Zottola, E.A. (1998). Bacteriocidal activity against *Enterococcus faecium* attached to stainless steel as determined by plate count and impedance methods. *Journal of Food Protection* **61**, 833-838.

Aureli, P., Ferrini, A.M. and Mannoni, V. (1996). Presumptive identification of sulphonamide and antibiotic residues in milk by microbial inhibitor tests. *Food Control* **7**, 165-168.

Baggott, J.D. (1980). Distribution of antimicrobial agents in normal and diseased animals. *Journal of the American Vetetrinary Medicine Association* **176**, 1085-1090.

Baquero, F., Culebras, E., Patrón, C., Pérez-Díaz, J.C., Medrano, J.C. and Vicente, M.F. (1986). Postantibiotic effect of imipenem on grampositive and gram-negative micro-organisms. *Journal of Antimicrobial Chemotherapy* **18**, Supplement E., 47-59.

Barry, A.L. (1976). Agar diffusion: general considerations. *In: Barry, A.L. (ed.) The antimicrobic susceptibility test: principles and practices. Lea & Febiger, Philadelphia, PA, USA.* **pp**. 163-179.

Barry, A.L. and Effinger, L.J. (1974). Performance of Mueller-Hinton agars prepared by three manufacturers. *Journal of American Clinical Pathol*ogy **62**, 113-117.

Bartels, H., Angersbach, H. and Klare, H.J. (1972). Nachweis Von Hemmstoffen bei Tieren aus Normalschlachtungen. *Fleischwirtsch* **4**, 479-482.

Barza, M., Giuliano, M., Jacobus, N.V. and Gorbach, S.L. (1987). Effects of broad-spectrum parenteral antibiotics on “Colonization resistance” of intestinal microflora of humans. *Antimicrobial Agents Chemotherapy* **31**, 723- 727.

Baxter, G.A., O'Connor, M.C., Haughey, S.A., Crooks, S.R. and Elliott, (1999). Evaluation of an immunobiosensor for the on-site testing of veterinary drug residues at an abattoir. Screening for sulfamethazine in pigs. *Analyst* **124,** 1315-1318.

Begot, C., Desnier, I., Daudin, J., Labadie, J. and Lebert, A. (1995). Recommendations for calculating growth parameters by optical density measurements. *Journal Microbiological Methodology* **25**, 225-232.

Berdy, J. (1974). Recent developments of antibiotic research and classification of antibiotics according to chemical structure. *Advanced. Applied Microbiol*ogy **18**, 309-406.

Berends, B.R., Van Den Bogaard, A.E.J.M., Van Knapen, F. and Snijders, J.M.A. (2001). Human health hazards associated with the administration of antimicrobials to slaughter animals. Part I. An assessment of the risks of residues of tetracyclines in pork. *The Veterinary Quarterly* **23**, 2-10.

Bertrand, K., Postle, K., Wray, L.V. and Reznikof, W.S. (1984). Construction of a single-copy promoter vector and its use in analysis of regulation of the transposon Tn10 tetracycline resistance determinant. *Journal of Bacteriology* **158**, 910-919.

Beuchat, L.R. and Golden, D.A. (1989). Antimicrobials ocurring naturally in foods. *Food Technology* **1989m**, 134-142.

Bevill, R.F. (1984). Factors influencing the occurrence of drug residues in animal tissues after the use of antimicrobial agents in animal feeds. *Journal of American Veterinary Medicine Association* **185**, 1124-1126.

Bielecka, M., Baldock, J.D. and Kotula, A.W. (1981). Determination of antibiotics in meat using Bacillus stearothermophilus spores. *Journal Food Protection* **44**, 194-200.

Bjurling, P., Baxter, A., Caselunghe, M., Jonson, C., O'Connor, M., Persson, B. and Elliott, C.T. (2000). Biosensor assay of sulfadiazine and sulfamethazine residues in pork. *Analyst* **125**, 1771-1774.

Black, W.D. and Gentry, R.D. (1984). The distribution of oxytetracycline in the tissues of swine following a single oral dose. *Canaddian Veterinary Journal* **25**, 158- 161.

Blake, C. and Gould, B.J. (1984). Use of enzymes in immunoassay techniques. A review. *Analyst* **109**, 533-545.

Bogaerts, R. and Wolf, F. (1980). A standardized method for the detection of residues of anti-bacterial substances in fresh meat. *Fleischwirtsch* **60**, 672-674.

Bogaerts, R., De Groodt, J.M. and De Vos, D. (1981). An ultra-sensitive microbiological method for the semi-quantitative detection of low-level sulfonamides. *Journal Food Science* **46**, 158-160.

Boison, J.O. (2001). Committee on drugs and related topics. Drug residues in foods, diagnostics and test kits. *Journal of Official Analytical Chemists International* **84**, 190-191.

Boison, J.O. and Mac Neil, J.D. (1995). New test kit technology. In: Oka, H., Nakazawa, H., Harada, K. and Mac Neil, J.D. (eds.) *Chemical analysis for antibiotics used in agriculture, Association of Official Agricultural Chemists International, Arlington, VA, USA*. **pp**. 77-119.

Boison, J.O., Korsrud, G.O., Mac Neil, J.D., Yates, W.D.G. and Papich, M.G. (1992). Effect of cold-temperature storage on stability of benzylpenicillin residues in plasma and tissues of food-producing animals. *Journal of Association of Official Analytical Chemist* **75,** 974-978

Boison, J.O., Nachilobe, P., Cassidy, R., Keng, L., Thacker, P.A., Peacock, A., Fesser, A.C., Lee, S., Korsrud, G. and Bulmer, W.S. (1996). Determination of trimethoprim and sulphadoxine residues in porcine tissues and plasma. *Canadian Journal Veterinary Research* **60**, 281-287.

Boisseau, J. (1993). Basis for the evaluation of the microbiological risks due to veterinary drug residues in food. *Veterinary Microbiology* **35**, 187-192.

Bolton, F.J. (1990). An investigation of indirect conductimetry for detection of some food-borne bacteria. *Journal Applied Bacteriology* **69,** 655-661.

Bowen, J.M. (1979). The aminoglycosides. In: Steele, J.H. (ed.) *Antibiotics, sulfonamides and public health, Vol 1. CRC Press, LLC, Boca Raton, FL, USA.* **pp**. 289-304.

Brady, M.S. and Katz, S.E. (1990). Factors influencing optimization of diffusion assays for antibiotics. *Journal of the Association Official Analytical Chemist* **73**, 202-205.

Braham, R., Black, W.D., Claxton, J. and Yee, A.J. (2001). A rapid assay for detecting sulfonamides in tissues of slaughtered animals. *Journal Food Protection* **64**, 1565-1573.

Brown, G.M. (1962). The biosynthesis of folic acid. II Inhibition by sulfonamides. *Journal Biological Chemist* **237**, 536-540.

Bugyei, K., Black, W., McEwen, S. and Meek, A.H. (1994). Detecting oxytetracycline residues in chicken tissues using the Delvotest P system. *Journal Food Protection* **57**, 141-145.

Buick, R.K., Greer, N.M. and Elliott, T. (2000). A microtitre plate assay for the detection of antibiotics in porcine urine. *Analyst* **125**, 395-396.

Burger, W.C. and Stahmann, M.A. (1952). The agglutination and growth inhibition of bacteria by lysine polypeptides. *Archives Biochemistry and Biophysics* **39**, 27-36.

Butler, J.N. (1982). Carbon Dioxide Equilibria and their Applications. Addison-Wesley, Raeding, Mass., USA.

Buyeske, D.A., Eisner, H.J. and Kelly, R.G. (1960). Concentration and persistence of tetracycline and chlortetracycline in bone. *Journal of Pharmacology Experiment Therapeutics* **130**, 150-156.

Calderón, V., Gonzáles, J., Díez, P. and Berenguer, J. A. (1996). Evaluation of a multiple bioassay technique for determination of antibiotic residues in meat with standard solutions of antimicrobials. *Food Additves and Contaminants* **13**, 13-19.

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. *Antimicrobial Resistant Infectection Control* **1**: 11.

Carman, R.J., Van Tassell, R.L. and Wilkins, T.D. (1993). The normal intestinal microflora: ecology, variability and stability. *Veterinary and Human Toxicology* **35**, 11-14.

Chafe, U.M., Musa and Dogara, B. (2008). Status of some health aspects of traditional camel management in North-West Nigeria. Livestock Research for Rural Development, Volume 20, Artivle No. 31, Retrieved November 23, 2008 http://www.cipav.org.co/irrd20/2/chaf2003i.htm.

Chen, H.C. and Chang, T.C. (1994). Detection of penicillin G in milk using a conductimetric method. *Journal Dairy Science* **77**, 1515-1520.

Cherlet M., De Backer, P. and Croubels S. (2006) Control of the ketoenol tautomerism of chlortetracycline for its straightforward quantitation in pig tissues by liquid chromatographyelectrospray ionization tandem mass spectrometry, *Journal Chromatography A*; **1133**, 135–141.

Choi, J., Yee, A., Thompson, D., Samoluk, J. and Mitchell, M. (1999). Determination of fluoroquinolone residues in animal tissues using Escherichia coli as indicator organism. *Journal of the Association of Official Analytical Chemists International* **82**, 1407-1412.

Cholas, G. (1976). Withdrawal times and limitations for use of animal drugs and certifiable antibiotics used in food-producing animals. *Southwest Veterinary* **29**, 144-158.

Chopra, I., Hacker, K., Misulovin, Z. and Rothestein, D.M. (1990). Sensitive biological detection method for tetracyclines using a tetA-lacZ fusion system. *Antimicrobial Agents Chemother*apy **34**, 111-116.

Citri, N. (1971). Penicillinases and other β-lactamases. In: Boyer, B. D. (ed.) *The Enzymes*. Vol. IV. Academic Press, New York, NY, USA, **pp**. 23-46.

Clark, C.H. (1977). Toxicity of aminoglycoside antibiotics. *Modern Veterinary Practice* **58**, 594-598.

Comparison of four commercially available rapid test kits with liquid chromatography for detecting penicillin G residues in bovine plasma. *Journal of the Association of Official Analytical Chemists International* **78**, 1144-1152.

Concordet, D. and Toutain, P.L. (1997). The withdrawal time estimation of veterinary drugs: a non-parametric approach. *Journal of Veterinary Pharmacology Therapeutics* **20**, 374-379.

Connolly, P., Bloomfield, S.F. and Denyer, S.P. (1994). The use of impedance for preservative efficacy testing of pharmaceuticals and cosmetic products. *Journal Applied Bacteriology* **76**, 68-74.

Cooper, K.E. (1972). The theory of antibiotic diffusion zones. Kavanagh, F. W. (ed.) *Analytical Microbiology,*Vol. II. Academic Press, New York, NY, USA, **pp**. 13-30.

Corpet, D.E. (1987). Antibiotic residues and drug resistance in human intestinal flora. *Antimicrobial Agents Chemotherapy* **31**, 587-593.

Corpet, D.E. (1993). An evaluation of methods to assess the effect of antimicrobial residues on the human gut flora. *Veterinary Microbiology* **35**, 199- 212.

Craigmill, A.L., Miller, G.R., Gehring, R., Pierce, A.N., and Riviere, J.E. (2004). Meta-analysis of pharmacokinetic data of veterinary drugs using the Food Animal Residue Avoidance Databank: Oxytetracycline and procaine penicillin G. *Journal Veteriary Pharmacology Therapeutics* **27**, 343–353.

Crooks, S., Baxter, A., O'Conner, M. and Elliott, C. (1998). Immunobiosensor- an alternative to enzyme immunoassay screening for two sulfonamides in pigs. *Analyst* **123**, 2755-2757.

Croubles, S., Baert, K., Okerman, L., Robbrecht, V., Aerden, L. and De Backer, P. (1999). The detection of residues of doxycycline in porcine kidney and muscle tissue: correlation between results of microbiological screening and confirmation tests. *Vlaams Diergeneesk. Tijdschr* **68**, 22- 26.

Currie, D., Lynas, L., Kennedy, D.G. and Mc Caughey, J. (1998). Evaluation of a modified EC four plate method to detect antimicrobial drugs. *Food Additives Contaminants* **15**, 651-660.

CVMP. (1995). Committee for Veterinary Medicinal Products. Note for Guidance: Approach towards Harmonisation of Withdrawal Periods. EMEA/CVMP, 036/95. EMEA website

CVMP. (2001a). Revised guidance on pre-authorisation studies to assess the potential impact of veterinary medicinal products to antimicrobial resistance. EMEA/CVMP, 244/01 EMEAwebsite (http://www.emea.eu.int/index/indexv1.htm) **3,** 4.

Czeizel, A.E., Rockenbauer, M. and Olsen, J. (1998): Use of antibiotics during pregnancy. *European Journal of Obstetrics, Gynecology and Reproductive Biology* **81**, 1-8.

D zenclos, T., Ascon-Carbera, M., Ascon, D., Lebeault, J.M. and Pauss, A. (1994). Optimisation of the indirect impedancemetry technique; a handy technique for microbial growth measurement. *Applied Microbiology and Biotechnology* **42**, 232-238.

Davis, W.W. and Stout, T.R. (1971). Disc plate method of microbiological antibiotic assay, I. Factors influencing variability and error. *Applied Microbiology* **22**, 659-665.

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

De Wasch, K., Okerman, L., Croubles, S., De Brabander, H., Van Hoof, J. and De Backer, P. (1998). Detection of residues of tetracycline antibiotics in pork and chicken meat: correlation between results of screening and confirmatory tests. *Analyst* **123**, 2737-2741.

De Zutter, L., Koenen-Dierick, K. and Van Hoof, J. (1985). Detection of antimicrobial residues in slaughtered animals. II. Sensitivity of some detection methods to different antibiotics. *Vlaams Diergeneesk. Tijdschr* **54**, 445-454.

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 and Chemical Toxicology* **29**, 477-483.

Dina, O.A. and Arowolo, R.O.A. (1991): Some considerations on veterinary drug use and supply in Nigeria. *Revue d'Elevage de Medicine Veteriary des Pays Tropicaux* **44**, 29-31.

Dipeolu, M.A. (2002). Residues of tetracycline antibiotic in marketed goats and pigs in Lagos and Ogun States Nigeria. *Tropical Journal of Animal Science* **5**:47-51.

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. *Archiva Zootechnica* **51**, 477-480.

Dixon-Holland, D.E. (1992). ELISA and its applications for residue analysis of antibiotics and drugs in products of animal origin. In: Agarwal, V.K. (ed.) Analysis of Antibiotic Drug Residues in Food Products of Animal Origin. *Plenum Press, New York, USA*, **pp**. 57-74.

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.

Easter, M.C. and Gibson, D.M. (1989). Detection of microorganisms by electrical measurements. Rapid Methods in Food Microbiology. In: Adams, M.R. and Hope, C.F.A. (eds.) *Progress in Industrial Microbiology*. *Elsevier, New York, USA*, **pp**. 57-100.

EC. (2001). European Community. Notice to applicants and note for guidance. *Establishment of maximum residue limits* (MRLs) *for residues of veterinary medicinal products in foodstuffs of animal origin*. **Volume** 8.

EEC. (1970). European Economic Community. Council Directive 70/524/EEC of 23 November concerning additives in feedingstuffs. *Official Journal European Communities* L 270, **pp**. 1.

EEC. (1990). European Economic Community. Council Regulation 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Official Journal European Communities*. L 224, **pp**. 1-8.

Ehsani, A., Fazlara, A., Maktabi, S. and Najafzadeh, H., (2010). Measurement of tetracycline residue in consumed broiler meats in Ahvaz city by HPLC. *Journal of Veterinary Medical Scienes* **2**,119–129.

El Emam, O.M. (2011). Residues of Antibiotics in Camel Products and Tissues In: Sudan University of Science and Technology.

Ellerbroek, L. (1991). Zum mikrobiologischen Nachweis der Chinoloncarbonsäurederivate Enrofloxacin, Ciprofloxacin und Flumequin, *Fleischwirtsch* **71**, 187-189.

Elliott, C., McCaughey, W.J., Crooks, S.R.H. and McEvoy, J. (1994). Effects of short term exposure of unmedicated pigs to sulphadimidine contaminated housing. *Veterinary Record* **134**, 450-451.

Engel, H.W.B., Van Leusden, F.M. and Nouws, J.F.M. (1983). Evaluation of the European Communities (EC) four-plate method for the detection of residues of antimicrobial drugs in slaughtered animals. Antimicrobials and Agriculture. Proceedings of 4th International Symposium on antibiotics in agriculture: benefits and malefits. Butterworths, London, UK, **pp**. 491-499.

e-publication (www.nam.fi/uploads/laakeinfo/ellaakkeet2002). 3.4.2004

Erasmuson, A.F., Cairns, E.R. and O'Kane, B.O. (1998). Bicarbonate as an interference in B. stearothermophilus assays. *Analyst* **123**, 2497- 2499.

Fabiansson, S. and Rutegård, Å. (1979). A modified method for the detection of antibiotic residues in slaughter animals. *Acta Veterinary Scandinavica* **20**, 477-491.

FEDESA (2004). e-publication www.fedesa.be 3.4.2004.

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

Ferguson, J.P., Baxter, G.A., McEvoy, J.D.G., Stead, S., Rawlings, E. and Sharman, M. (2002). Detection of streptomycin and dihydrostreptomycin residues in milk, honey and meat samples using an optical biosensor. *Analyst* **127**, 951-956.

Ferrini, A.M., Mannoni, V. and Aureli, P. (1997). The combined plates microbial assay (CPMA) for the detection of β-lactam, sulfonamide, streptomycin and tetracycline residues in meat. *Archiv fur Lebensmittelhygien* **48**, 133-135.

Fink-Gremmels, J. and Van Miert, A.S. (1994). Veterinary drugs: disposition, biotransformation and risk evaluation. *Analyst* **119**, 2521- 2528.

Firstenberg-Eden, R. and Eden, G. (1984). What is impedance microbiology. In: Impedance Microbiology. Research Studies Press Ltd., Letchworth, Hertfordshire, UK, **pp**. 7-20.

Fisch, R.D. (2000). Withdrawal time estimation of veterinary drugs: extending the range of statistical methods. *Journal of Veterinary Pharmacology and Therapeutics* **23**, 159-162.

Fitzpatrick, S., Brynes, S. and Guest, G. (1995). Dietary intake estimates as a means to the harmonization of maximum residue levels for veterinary drugs. I. Concept. *Journal Veteriary Pharmacology Therapeutics* **18**, 325-327.

Fleming, A. (1922). On a remarkable bacteriolytic element found in tissues and excretions. *Proceedings of the Royal Soc*iety of London **93**, 306-310.

Fodey, T.L., Crooks, S., Elliott, C.T. and McCaughey, W. (1997). Comparison of porcine urine and bile as matrices to screen for the residues of two sulfonamides using a semi-automated enzyme immunoassay. *Analyst* **122**, 165-168.

Forschner, E. and Seidler, M. (1976). Alternativvorschläge zum Hemmstofftest, Rationalisierung und Absicherung. *Fleischwirtsch* **56**, 1008-1013.

Fugate, H.G. (1974). Determination of antibiotic residues in animal tissues. In: *Microbiology Laboratory Guidebook*. FSIS/USDA, Washington DC, USA.

Glazko, A.J., Dill, W.A. and Rebstock, M.C. (1950). Biochemical studies on chloramphenicol (chloromycetin). III. Isolation and identification of metabolic products in urine. *Journal Biological Chemistry* **183**, 679-691.

Glazko, A.J., Wolf, L.M., Dill, W.A. and Bratton Jr., A.C. (1949). Biochemical studies on chloramphenicol (chloromycetin). II. Tissue distribution and excretion studies. *Journal of Pharmacology Experimental Therapeutics* **96**, 445-459.

Gómez, R., Ladisch, M., Bhunia, A.K. and Bashir, R. (2002). Microfabricated device for impedance-based detection of bacterial metabolism. *Material Research Society Symposium Proceedins* **729**. U4.6.1-4.6.6.

Gorbach, S.L. (1993). Perturbation of intestinal microflora. *Veterinary Human Toxicology* **35**, 15-23.

Greenstein, J.P. and Winitz, M. (1961). Cystine and cysteine. In: J. Wiley & Sons (eds.) *The Chemistry of The Amino Acids. J., Wiley, New York, USA*, **pp**. 1879-1928.

Greko, C. (2003). Tissue cages in calves for studies on pharmacokinetic/pharmacodynamic relationships of antimicrobials. Thesis. Swedish University of Agricultural Sciences, Uppsala, Sweden.

Guardabassi, L. and Dalsgaard, D. (2004). Occurrence and faith of antibiotic resistant bacteria in sewage. *Environmental Project No.* **722.** The royal Veterinary and Agricultural University, Department of Veterinary Microbiology. Copenhagen, Denmark. E-publication (www.mst.dk) **3,** 4.

Gudding, R. (1976). An improved bacteriological method for the detection of sulfonamide residues in food. *Acta Veterinary Scandinavica* **17**, 458-464.

Guest, G.B. and Paige, J.C. (1991). The magnitude of the tissue residue problem with regard to consumer needs. *Journal of the American Veteriary Medical Associ*ation **198**, 805-808.

Guillot, P., Sanders, P. and Mourot, D. (1989). Chloramphenicol and oxytetracycline residues in milk and tissues from cows and bullocks treated with an injectable formulation. *Food Additives Contaminants* **6**, 467-473.

Gustafson, R. (1993). Historical perspectives on regulatory issues of antimicrobial resistance. *Veteriary Human Toxicology* **35**, 2-5.

Haagsma, N. and Mengelers, M.J. (1989). A rapid fluorimetric screening method for chlortetracycline, oxytetracycline and tetracycline in pig meat and kidney tissues. Z. Lebensm. Unters. *Forsch* **188**, 227-231.

Haagsma, N. and Van de Water, C. (1992). Immunochemical methods in the analysis of veterinary drug residues. In: Agarwal, V. K. (ed.) Analysis of antibiotic drug residues in food products of animal origin. Plenum Press, New York, USA, **pp**. 81- 97

Haasnoot, W., Stouten, P., Cazemier, G., Lommen, A., Nouws, F.M. and Keukens, H.J. (1999). Immunochemical detection of aminoglycosides in milk and kidney. *Analyst* **124**, 301-305.

Hamscher, G., Sczesny, S., Abu-Qare, A., Höper, H. and Nau, H. (2000). Stoffe mit pharmakologischer Wirkung einschließlich hormonell aktiver Substanzen in der Umwelt. Nachweis von Tetracyclinen in güllgedüngten Böden. *Deutsche Tierärztliche Wochenschrift* **107**, 332-334.

Handal, T. and Olsen, I. (2000). Antimicrobial resistance with focus on oral beta-lactamases. *European Journal of Oral Sci*ence **108**, 163-174.

Hedin, G. and Hambraeus, A. (1991). Screening tests for the detection of methicillin resistance in Staphylococcus epidermidis. *Journal of Antimicrobial Chemotherapy*.

Heinert, H., Van Der Wall, G. and Brehmer, H. (1976). Lysozym-Ursache von unspezifischen Reaktionen im mikrobiolologischen Hemmstofftest. *Archiv fur Lebensmittelhygiene* **27**, 55-60.

Heitzman, R.J., (ed.) (1994). Veterinary drug residues. Residues in food producing animals and their products: reference materials and methods. 2nd ed. *Commission of the European Communities*. Blackwell Scientific Publications, Oxford, UK.

Hernández-Arteseros, J.A., Compano, R., Ferrer, R. and Prat, M.D. (2000). Application of principal component regression to luminescence data for the screening of ciprofloxacin and enrofloxacin in animal tissues. *Analyst* **125**, 1155-1158.

Higgins, H.C., McEvoy, J.D.G., Lynas, L. and Fagan, N.P. (1999). Evaluation of a single plate microbiological growth inhibition assay as a screening test for the presence of antimicrobial agents in compound animal feedingstuffs at therapeutic and contaminating concentrations. *Food Additive and Contaminant* 543-554.

Hornish, R.E. and Kotarski, S.F. (2002). Cephalosporins in veterinary medicine-ceftiofur use in food animals. *Current Topics in Medicinal Chemistry* **2**, 717- 731.

Hou, J.P. and Poole, J.W. (1981). β-lactam antibiotics: their physicochemical properties and biological activities in relation to structure. *Journal Pharmaceutical Sciences* **60**, 503-532.

(http://www.emea.eu.int/indexv1.htm) 3.4.2004.

Huber, W.G., Carslon, M.B. and Lepper, M.H. (1969). Penicillin and antimicrobial residues in domestic animals at slaughter. *Journal of American Veterinary Medical Association* **154**, 1590-1595.

IPCS. (1987). International Programme on Chemical Safety in cooperation with the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Environmental Health Criteria 70, Principles for the safety assessment of food additives and contaminants in food. *World Health Organization, Geneva, Switzerland.*

Islam, N., M.D., Itakura, T. and Motohiro, T. (1984). Antibacterial spectra and minimum inhibition concentration of clupeine and salmine. Bull. *Japanese Society for the Science Fish* **50**, 1705-1708.

Isoherranen, N. and Soback, S. (1999). Chromatographic methods for analysis of aminoglycoside antibiotics. *Journal Association of Official Analytical Chemist Internation* **82,** 1017-1045.

Jackson, G.S. (1980). Safety assessment of drug residues. *Journal American Veterinary Medical Association* **176**, 1141-1144.

Jamela, E., Amer, E., Ibrahim, E. and El-Mahmoudy, A. (2015): Residual evaluation of oxytetracycline in camel edible tissues in Tripoli region, Libya. Current Research in Microbiology and Biotechnology **3**, 718-724.

Johansen, C., Gill, T. and Gram, L. (1995). Antibacterial effect of protamine assayed by impedimetry. *Journal Applied Bacteriology* **78**, 297-303.

Johansson, A., Sunzel, B., Holm, S., Söderberg, T. and Gref, R. (1995). Antimicrobial screening of zinc in the absence or presence of oleoresins and various resin acids. *All Purpose Medical Information System* **103**, 419-427.

Johnston, A. (1998). Use of antimicrobial drugs in veterinary practice. *British Medical Journal* **317,** 665-667.

Johnston, R.W., Reamer, R.H., Harris, E.W. and Fugate, H.G. (1981). A new screening method for the detection of antibiotic residues in meat and poultry tissues. *Journal of Food Protection* **44**, 828-831.

Kaartinen, L., Pyörälä, S., Moilanen, M. and Räisänen, S. (1997). Pharmacokinetics of enrofloxacin in newborn and one-week-old calves. *Journal Veterinary Pharmacology Therapeutics* **20**, 479-482.

Kaartinen, L., Salonen, M., Älli, L. and Pyörälä, S. (1995). Pharmacokinetics of enrofloxacin after single intravenous, intramuscular and subcutaneous injections in lactating cows. *Journal Veterinary Pharmacology and Therapeutics* **18**, 357-362.

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.

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

Kabir, J., Umoh, J.U., Umoh, V.J. (2002). Characterization and Screenig for Antimicrobial substances of Slaughtered cattle in Zaria, Nigeria. *Meat Science* **61,** 435-439.

Kaneene, J.B. and Miller, R. (1997). Problems associated with drug residues in beef from feeds and therapy. *Revenu Scientifique et Technique Office International des Epiz*ooties **16**, 694-708.

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

Kell, D.B. and Davey, C.L. (1990). Conductimetric and impedimetric devices. In: Cass, A.E.G. (ed.) Biosensors, a practical approach. Oxford University Press, Oxford, UK, pp. 124-154.

Kennedy, D.G., Cannavan, A. and McCracken, R.J. (2000). Regulatory problems caused by contamination, a frequently overlooked cause of veterinary drug residues. *Journal Chromatography* **882**, 37-52.

Kennedy, D.G., McCracken, R.J., Cannavan, A. and Hewitt, S.A. (1998). Use of liquid chromatography - mass spectrometry in the analysis of residues of antibiotics in meat and milk. *Journal of Chromatography* **812**, 77-98.

Kietzmann, M., Markus, W., Chavez, J. and Bollwahn, W. (1995). Arzneimittelrückstände bei unbehandelten Schweinen. *Deutche Tierärztliche Woochenschrift* **102**, 441-442.

Klassen, M. and Edberg, S.C. (1996). Measurement of antibiotics in human body fluids: techniques and significance. In: Lorian, V. (ed.) Antibiotics in Laboratory Medicine. Williams & Wilkins, MA, USA, **pp**. 231-295.

Koenen-Dierick, K. and De Beer, J.O. (1998). Optimization of an antibiotic residue screening test, based on inhibition of Bacillus subtilis BGA, with experimental design. *Food Additve Contaminant* **15**, 528-534.

Koenen-Dierick, K., Okerman, L., De Zutter, L., Degroodt, J.M., Van Hoof, J. and Srebrink, S. (1995). A one plate microbiological screening test for antibiotic residue testing in kidney tissue and meat: an alternative to the EEC four-plate method? *Food Additive Contaminant* **12**, 77-82.

Kondo, F., Tsai, C.E., Hamada, E., Lin, S.Y. and Saitanu, K. (1993). A continous, simple and rapid method for the detection, extraction and identification of residual antibacterial agents in meat. *Microbios* **73**, 237- 247.

Korkeala, H. and Mäki-Petäys, O. (1984). Detection of chloramphenicol residues in pigs with different agar diffusion methods. *Acta Veterinary Scanddinavica* **25**, 352-364.

Korkeala, H. and Männistö, P. (1988). Time course of the antimicrobial activity of erythromycin stearate and erythromycin acistrate against two Staphylococcus aureus strains in vitro. *Journal Antimicrobial Chemotherapy* **22**, 127-133.

Korkeala, H. and Pekkanen, T. (1977). The testing of the antibiotic sensitivity of bacteria on an agar medium: the problem of a double zone of inhibition. *Acta Pathology Microbiologica Scandinvica* **85**, 174-176.

Korkeala, H., Alanko, T. and Tiusanen, T. (1992). Effect of sodium nitrite and sodium chloride ion growth of lactic acid bacteria. *Acta Veterinaria Scandinva* **33**, 27-32.

Korkeala, H., Alanko, T. and Tiusanen, T. (1992). Effect of sodium nitrite and sodium chloride ion growth of lactic acid bacteria. *Acta* Vet.Scand. **33**, 27-32.

Korkeala, H., Sorvettula, O., Mäki-Petäys, O. and Hirn, J. (1983). Comparison of different agar diffusion methods for the detection of residues in the kidneys of pigs treated with antimicrobial drugs. *Meat Science* **9**, 294-304.

Korkeala, H., Stabel-Taucher, R. and Pekkanen, T.J. (1976). The problem of testing horse kidneys for the presence of antibiotics at meat inspection: how to avoid a false positive reaction. *Nordisk Veterinaermedicin* **28**, 377-380.

Korpela, M.T., Kurittu, J.S., Karvinen, J.T. and Karp, M.T. (1998). A recombinant Escherichia coli sensor strain for the detection of tetracyclines. *Analytical Chemistry* **70**, 4457-4462.

Korsrud, G. and Mac Neil, J.D. (1987). A comparison of three bioassay techniques and high performance liquid chromatography for the detection of chlortetracycline residues in swine tissues. *Food Additives and Contaminant* **5**, 149-153.

Korsrud, G. and Mac Neil, J.D. (1988). Evaluation of the Swab Test on Premises, the Calf Antibiotic and Sulfa test, and a microbial inhibitor test with standard solutions of 22 antibiotics. *Journal of Food Protection* **51**, 43-46.

Korsrud, G., Salisbury, C.D.C, Fesser, A. and Mac Neil, J. (1995). Laboratory evaluation of the Charm Farm Test for antimicrobial residues in meat. *Journal of Food Protection* **58**, 1129-1132.

Korsrud, G.O., Salisbury, C.D.C., Fesser, A.C.E. and Mac Neil, J.D. (1994). Investigation of Charm Test II receptor assays for the detection of antimicrobial residues in suspect meat samples. *Analyst* **119**, 2737-2741.

Korsrud, G.O., Salisbury, C.D.C., Rhodes, C.S., Papich, M.G., Yates, W.D.G., Bulmer, W.S., Mac Neil, J.D., Landry, D.A., Lambert, G., Yong, M.S. and Ritters, L. (1998). Depletion of penicillin G residues in tissues, plasma, and injection sites of market pigs injected intramuscularly with procaine penicillin G. *Food Additive and Contam*inant **15**, 421-426.

Kühne, M., Wegmann, S., Kobe, A. and Fries, R. (2000). Tetracycline residues in bones of slaughtered animals. *Food Control* **11**, 175-180.

Kundrat, W. (1968). Methoden Zur Bestimmung Von Antibiotika- Rckständen in tierischen Produkten. Zeitschr. *Analytical Chem*istry **243**, 624- 630.

Küng, K., Riond, J.L., Wolffram, S. and Wanner, M. (1992). Comparison of an HPLC and bioassay method to determine antimicrobial concentrations after intravenous and oral administration of enrofloxacin in four dogs. *Research in Veterinary Sci*ence **54**, 247-248.

Lackey, M., Belknap, E. and Salman, M., et al. (1995). Urinary indices in llamas fed different diets. *American Journal of Veterinary Research* **56**, 859-865.

Lambert, R.J.W. (2001). Advances in disinfecion testing and modeling. *Journal of Applied Microbilogy* **91,** 351-363.

Lambert, R.J.W. and Pearson, J. (2000). Susceptibility testing: accurate and reproducible minimum inhibitory concentration (MIC) and noninhibitory concentration (NIC) values. *Journal of Applied Microbiol*ogy **88**, 784-790.

Lambert, R.J.W., Johnston, M.D. and Simons, E.A. (1998). Disinfectant testing: use of the Bioscreen microbiological growth analyser for laboratory biocide screening. *Lettersin. Applied Microbiol*ogy **26**, 288-292.

Lambert, R.J.W., Joynson, J. and Forbes, B. (2001). The relationships and susceptibilities of some industrial, laboratory and clinical isolates of Pseudomonas aeruginosa to some antibiotics and biocides. *Journal of Applied Microbiol*ogy **91**, 972-984.

Larkin, C., Poppe, C., Mcnab, B., Mcewen, B., Madhi, A. and Odumeru, J. (2004): Antibiotic resistance of Salmonella isolated from hog, beef, and chicken carcass samples from provincially inspected abattoirs in Ontario. *Journal of Food Protocol* **67**, 448-455.

Lathers, C.M. (2001). Role of veterinary medicine in public health: antibiotic use in food animals and humans and the effect on evolution of antibacterial resistance. *Journal of Clinical Pharmacology* **41**, 595-599.

Lee, H.J., Lee, M.H. and Han, In K. (2001b). Application of ELISA for the detection of sulfamethazine residues in live cattle. Asian-Australian *Journal of Animal Sci*ence **14**, 378-381.

Lee, H.J., Lee, M.H. and Ruy, P.D. (2001a). Public health risks: chemical and antibiotic residues. *Asian-Australian Journal of Animal Sci*ence **14**, 402- 413.

Lee, H.J., Lee, M.H., Ryu, P.D., Less, H. and Cho, M.H. (2001c). Enzyme-linked immunosorbet assay for screening the plasma residues of tetracycline antibiotics in pigs. *Journal of Veterinary Medical Science* **63,** 553-556.

Lees, P., Concordet, D., Shojaee Aliabadi, F. and Toutain, P.L. (2006). Drug selection and optimisation of dosage schedules to minimize antimicrobial resistance, in Antimicrobial Resistance in Bacteria of Animal Origin, Aarestrup FM, ed., ASM Press, Washington, DC. **pp**. 49–71.

Lindsay, D.G. (1983). Monitoring and testing for residues of therapeutics in meat. *Veterinary Record* **112**, 469-471.

Lloyd, D. and Van Der Merwe, D. (1987). The use of a diffusion test for the detection of antibiotics in the tissues of slaughter stock. *Journal of South African Veterinary Assoc*iation **58**, 183-186.

Lott, A.F., Smither, R. and Vaughan, D.R. (1985). Antibiotic identification by high voltage electrophoresis bioautography. *Journal Association of Official Analytical Chemist Internation* **68**, 1018-1020.

Lund, C. (1986). Identification of antibiotic residues in animal tissues under practical conditions. In: Proceedings of the 2nd World Congress, Foodborne Infections and Intoxications, Volume II, Institute of Veterinary Medicine - Robert Von Ostertag Institute, Berlin (West), FRG, **pp**. 819- 824.

Lynas, L., Currie, D., McCaughey, W.J., McEvoy, J.D.G. and Kennedy, D.G. (1998). Contamination of animal feedingstuffs with undeclared antimicrobial additives. *Food Additive Contam*inant **15**, 162-170.

Mac Neil, J.D. and Ellis, R.L. (1995). Regulatory overview of antibiotic use in food-producing animals in North America and current methods of detection and analysis. In: Oka, H., Nakazawa, H., Harada, K. and Mac Neil, J.D. (eds.) Chemical analysis for antibiotics used in agriculture. *Journal of Official Analytical Chemist Internation* Arlington, VA 22201-3301, USA, **pp**. 1-29.

MacKenzie, F., Gould, I.M., Chapman, D. and Jason, D. (1994). Postantibiotic effect of meropenem on members of the family Enterobacteriacae determined by five methods. *Antimicrobial Agents Chemother*apy **38**, 2583-2589.

MacKenzie, F., Milne, K.E., and Gould, I.M. (1999). The post-exposure response of Enterobacteriacae to ceftibufen. *Journal of Antimicrobial Chemother*apy **43**, 71-77.

MAF. (2001a). Degree on microbiological detection of antimicrobial drugs in meat insection. 21/EEO/2001.

MAF. (2003). Recommendations for the use of antimicrobial agents in the treatment of the most significant infectious diseases in animals. The ministry of Agriculture and Forestry Memorandum 2003:9a Helsinki, Finland.

Majcherczyk, P.A, Kunz, S., Hattenberg, M., Vaxelaire, J., Zaki, O. and Reilly, T.O. (1994). Isolation and In-Vitro and In-vivo characterisation of a mutant of Pseudomonas aeruginosa PAO1 that exhibited a reduced postantibiotic effect in response to imipenem. *Journal of Antimicrobial Chemotherapy* **34,** 485-505.

Mamber, S.W. and Katz, S.E. (1985). Effects of antimicrobial agents fed to chickens on some gram-negative enteric bacilli. *Applied Environmental Microbiology* **50,** 638-648.

Martlbauer, E., Usleber, E., Schneider, E. and Dietrich, R. (1994) Immunochemical detection of antibiotics and sulfonamides. *Analyst* **119,** 2543-2548.

Mattila, T. (1987). Automated turbidometry - A method for enumeration of bacteria in food samples. *Journal Food Protection* **50**, 640-642.

Mattila, T. and Sandholm, M. (1989). Antibacterial effect of the glucose oxidase-glucose system on food-poisoning organisms. *Intertional Journal of Food Microbiol*ogy **8**, 165-174.

Mawhinney, H., Oakenfull, S.M. and Nicholls, T.J. (1996). Residues from long-acting antimicrobial preparations in injection sites in cattle. *AustralianVeterinary. Journal* **74**, 140-142.

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. *Veterinary Record* **126**, 351-354.

McConnell, R.I., Lamont, J.V., Fitzgerald, S.P., Farry, L.T. and Mills, J.A. (2000). In: Proc. EURORESIDUE IV, Conference on residues of veterinary drugs in foods. Van Ginkel, L.A. and Ruiter, A. (eds.), Veldhoven. The Netherlands, May 8-10, **pp**. 746-752.

McCracken, A., O'Brien, J.J. and Campbell, N. (1976). Antibiotic residues and their recovery from animal tissues. *Journal of Applid Bacteriology* **41**, 129-135.

McCracken, R.J., Spence, D.E. and Kennedy, D.G. (2000). Comparison of extraction techniques for the recovery of veterinary drug residues from animal tissues. *Food Additive Contaminant* **17**, 907-914.

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

McEvoy, J.D.G., Crook, S.R.H., Elliot, C.T., McCaughey, W.J. and Kennedy, D.G. (1994). Origin of chlortetracycline in pig tissue. Analyst **119,** 2603-2606. Medicine *Nepal Agriculture Research Journal* **45,** 66-70.

Medina, M.B., Poole, D.J. and Anderson, M.R. (1998). A screening method for ß-lactams in tissues hydrolyzed with penicillinase.

Meijer, L.A., Ceyssens, K.G.F., De Jong, W.T. and De Grève, B.I. (1993). Correlation between tissue and plasma concentrations of oxytetracycline in veal calves. *Journal of Toxicology and Environmental Health* **40**, 35-45.

Mesgari, A.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 Journal of Veterinary Animal Sci*ence **33**, 345–349.

Mestorino, N., Hernandez, E.M., Marchettl, L., Errecalde, J.O. (2007). Pharmacokinetics and tissue residues of an oxytetracycline/diclofenac combination in cattle. *Revue Scientifique technique Office International Epiz*ooties **26**(3), 679-690.

Mevius, D.J., Vellenga, L., Breukink, H.J., Nouws, J.F.M., Vree, T.B. and Driessens, F. (1986). Pharmacokinetics and renal clearance of oxytetracycline in piglets following intravenous and oraladministration, *Veterinary Quartly* **8**, 274-284.

Mitchell, J.M., Griffiths, M.W., McEven, S.A., McNab, W.B. and Yee, J. (1998). Antimicrobial drug residues in milk and meat: causes, concerns, prevalence, regulations, tests, and test performance. *Journal of Food Protection* **61**, 742-756.

Mmbando L.M.G. (2004). Invetigtion of Oxytetacycline use and abuse. Determination of its residue in meat consumed in Dodoma and Morogoro. A thesis submitted for the Award of a Master of Veterianry Medicine Degree at Sokoine University of Agriculture, Morogoro, Tanzania, **pp:** 240

Moats, W. (1997). Advances in Determination of Antibiotic Residues. *Journal Association of Official Analytical* **80**, 1-4.

Moats, W.A., Romanowski, R.A. and Medina, M. (1998). Identification of ß-lactam antibiotics in tissue samples containing unknown microbial inhibitors. *Journal Association of Official Analytical Chemist Internation* **81**, 1135-1140.

MOCIT, (2002). Guide to Sokoto State's Economic Potentials. Commerce Department, Ministry of Commerce, Industry and Tourism, Sokoto State, **pp:** 4-18.

Mohammed, I. (2000). Study of Integration of the dromedary in the smallholder crop-livestock production systems in North Western Nigeria. Goattingam: *cuvillier Verlag* 228pp.

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. *Journal of Veterinary Sci*ence **2**, 97–101.

Musser, J.M.B., Anderson, K.L. and Moats, W.A. (2001). Screening method for identification of ß-lactams in bovine urine by use of liquid chromatography and a microbial inhibition test. *American Journal Veterinary Research* **62**, 326- 330.

Mustapha, L.G. and Olusiyi, A.J., (1993). The Status of Camel (Camelus Dromedarius) as a food Animal in the Semi-Arid Zone of Nigeria. A paper presented at the 18th Annual Conference of Nigerian Society for Animal Production. Federal University of Technology, Owerri, March 2013.

NAM. (2004). National Agency for Medicines

Neidert, E., Saschenbrecker, P.W. and Tittiger, F. (1987). Thin layer chromatographic/bioautographic method for identification of antibiotic residues in animal tissues. *Journal Association of Official Analytical Chemist* **70**, 197-200.

Nhiem, D.V., Paulsen, P., Suriyasathaporn, W., Smulders, F.J., Kyule, M.N., Baumann, M.P., Zessin, K.H. and Ngan, P.H. (2000) Preliminary analysis of tetracycline residues in marketed pork in Hanoi, Vietnam. *Annals of the New York Academy of Sci*ences **1081**,534–542. [PubMed].

Niessen, W.M.A. and Tinke, A.P. (1995). Liquid chromatography - mass spectrometry. General principles and instrumentation. *Journal of Chromatography Analyst* **703**, 37- 57.

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

Nord, C.E. and Edlund, C. (1990). Impact of antimicrobial agents on human intestinal microflora. *Journal of Chemotherapy* **2**, 218-237.

Nouws, J.F.M. (1979). Techniques de dosage des residus d'antibiotiques et methodes de fixation des delais d'attente. *Revue Médicine Véterinaire* **130**, 1193-1206.

Nouws, J.F.M. (1981). Tolerances and detection of antimicrobial residues in slaughtered animals. *Archiv fur Lebensmittelhygiene* **32**, 103-110.

Nouws, J.F.M. (1984). Irritation, bioavailability, and residue aspects of 10 oxytetracycline formulations administered intramuscularly to pigs, *Veterinary Quartly* **6**, 80–84.

Nouws, J.F.M. and Ziv, G. (1977). Tissue distribution and residues of beta-lactam antibiotics in normal dairy cows. *Tijdschrift. Diergeneeskunde* **102**, 1173-1186.

Nouws, J.F.M. and Ziv, G. (1978). Pre-slaughter withdrawal times for drugs in dairy cows. *Journal Veterinary Pharmacology and Therapeutics* **1**, 47-56.

Nouws, J.F.M., Broex, N.J.G., Den Hartog, J.M.P. and Driessens, F. (1988). The New Dutch Kidney Test. *Archiv fur Lebensmittelhygiene* **39**, 133-156.

Nouws, J.F.M., Smulders, A. and Rappalini, M. (1990). A comparative study on irritation and residue aspects of five oxytetracycline formulations administered intramuscularly to calves, pigs and sheep. *Veterinary Quartly* **12**, 129-138.

Nouws, J.F.M., Van Egmond, H., Loeffen, G., Schouten, J., Keukens, H., Smulders, I. and Stegman, H. (1998). Suitability of the Charm HVS and a microbiological multiplate system for detection of residues in raw milk at EU Maximum Residue Levels. *Veterinary Quartly* **21**, 21-27.

NPC, (1991). Sokoto State Censors Figures of 1991, National Populatio Commission, Nigeria.

Oka, H., Matsumoto, H., Uno, K., Harada, K., Kadowaki, S. and Suzuki, M. (1985). Improvement of chemical analysis of antibiotics. VIII. Application of prepacked C18 cartridge for the analysis of tetracycline residues in animal liver. *Journal of Chromatography* **325**, 265-274.

Okerman, L., Croubels, S., Cherlet, M., De Wasch, K., De Backer, P. and Van Hoof, J. (2004). Evaluation and establishing the performance of different screening tests for tetracycline residues in animal tissues. *Food Additive Contaminant* **21**, 145-153.

Okerman, L., Croubles, S., De Baere, S., Van Hoof, J., De Backer, P. and De Brabander, H. (2001). Inhibition tests for detection and presumptive identification of tetracyclines, beta-lactam antibiotics and quinolones in poultry meat. *Food Additive Contaminant* **18**, 385-393.

Okerman, L., De Wasch, K., Van Hoof, J. and Smedts, W. (2003). Simultaneous determination of different antibiotic residues in bovine and in porcine kidneys by solid-phase fluorescence immunoassay. *Journal Association of Offiicial Analytical Chemist Internation* **86**, 236-240.

Okerman, L., Van Hoof, J. and Debeuckelaere, W. (1998). Evaluation of the European Four-plate test as a tool for screening antibiotic residues in meat samples from retail outlets. *Journal Association of Offociai Analytical Chemist Internation* **81**, 51-56.

Okigbo, O.N. and Richardson, G.H. (1985). Detection of penicillin and streptomycin in milk by impedance microbiology. *Journal of Food Protection* **48**, 979-981.

Olatoye, I.O. and Ehinmowo, A.A. (2009) Oxytetracycline residues in edible tissues of cattle slaughtered in Akure, Nigeria. *Journal of Food Safety*, **Vol.** 11, **p**. 62-66

Olsson-Liljequist, B. and Hoffman, B.M. (1991). In-vitro activity of clarithromycin combined with its 14-hydroxy metabolite A-62671 against Haemophilus influenzae. *Journal of Antimicrobial and Chemotherapy Supplement* **27**, 11-17.

Owens, J.D., Thomas, D.S., Thompson, P.S. and Timmerman, J.W. (1989). Indirect conductimetry: a novel approach to the conductimetric enumeration of microbial populations. *Letter Applied Microbiology* **9**, 245-249.

Paige, J.C. (1994). Analysis of tissue residues. *Food and Drug Administration Centre for Veterinary Medicine* **9**, 4-6.

Paige, J.C. and Kent, R. (1987). Tissue residue briefs. *Food and Drug Administration Centre for Veterinary Medicine* **11**, 10- 11.

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

Pakkala, P., Stabel-Taucher, R. and Pekkanen, T.J. (1976). Oxytetracycline residues in meat and kidney tissue after intramuscular or intramammary treatment as determined by a chemical-physical method and compared to a microbiological method. *Nordisk Veterinaer Medicin* **28**, 610-614.

Papich, M.G., Korsrud, G.O., Boison, J.O., Yates, W.D., MacNeil, J.D., Janzen, E.D., Cohen, R.D. and Landry, D.A. (2009). A study of the disposition of procaine penicillin G in feedlot steers following intramuscular and subcutaneous injection. *Journal of Veterinary Pharmacology and Therapeutics* **16**, 317-327.

Perrin-Guyomard, A., Cottin, S., Corpet, D.E., Boisseau, J. and Poul, J.M. (2001). Evaluation of residual and therapeutic doses of tetracycline in the human-flora-associated (HFA) mice model. *Regul. Toxicology and Pharmacology* **34**, 125-136.

Pijpers, A., Schoevers, E.J., Van Gogh, H., Van Leengoed, L.A., Visser, I.J., Van Miert, A.S. and Verheijden, J.H. (1991). The influence of disease on feed and water consumption and on pharmacokinetics of orally administered oxytetracycline in pigs, *Journal of Animal Science* **69**, 2947–2954.

Prats, C., E.l., Korchi, G., Giralt, M., Cristofol, C., Pena, J., Zorrilla, I., Saborit, J., Perez, B., P.K. (2005). PK/PD of doxycycline in drinking water after therapeutic use in pigs, *Journal of Veterinary Pharmacolpgy and Therapeutics* **28**, 525-530.

Prescott, J.F and Walker, R.D. (2000). Principles of antimicrobial drug selection and use. In: Prescott, J.F., Baggot, J.D. and Walker, R.D. (eds) *Antiicrobial Therapy in Veterinary Medicine,* 3rd edition. Ames, lowa, USA, **pp.** 88-104.

Prescott, J.F. (2000a). Antimicrobial drug action and interaction: an introduction in: Prescott, J.F., Baggot, J.D. and Walker, R.D. (eds) *Antimicrobial Therapy in Vererinary Medicine,* 3rd edition. Ames, lowa, USA, **pp.** 3-11.

Prescott, J.F. (2000b). Beta-lactam antibiotics: penam penicillins. In: Prescott, J.F., Baggot, J.D. and Walker, R.D. (eds.) *Antimicrobial Therapy in Veterinary* Medicine, 3rd edition. Ames, Iowa, USA, **pp**. 105-126.

Prescott, J.F. (2000c). Beta-lactam antibiotics: cephalosporins and cephamycins. In: Prescott, J.F., Baggot, J.D. and Walker, R.D. (eds.) *Antimicrobial Therapy in Veterinary Medicine,* 3rd edition. Ames, Iowa, USA, **pp**. 134-159.

Prescott, J.F. (2000d). Tetracyclines In: Prescott, J.F., Baggot, J.D. and Walker, R.D. (eds.) *Antimicrobial Therapy in Veterinary Medicine,* 3rd ed. Ames, Iowa, USA, **pp**. 275-289.

Prescott, J.F. (2000e). Aminoglycosides and aminocyclitols. In: Prescott, J.F., Baggot, J.D. and Walker, R.D. (eds.) *Antimicrobial Therapy in Veterinary Medicine,* 3rd edition Ames, Iowa, USA, **pp**. 191-228.

Prescott, J.F., Baggot, J.D. (1993). *Antimicrobial Therapy in Veterinary Medicine* 2nd edition. Iwoa State University Press: Iowa, 215-228.

Ragheb, H.S. (1988). Effect of volume of solution per cylinder on estimation of antibiotic potency in diffusion assay. *Journal Association of Official Analytical Chemist Internation* **71**, 1071-1074.

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

Randecker, V.W., Reagan, J.A., Engel, R.E., Soderberg, D.L. and Mc Neal, J.E. (1987). Serum and urine as predictors of sulfamethazine levels in swine muscle, liver and kidney. *Journal of Food Protection* **50**, 115-122.

Ranheim, B., Ween, H., Egeli, A.K., Hormazabal, V., Yndestad, M. and Soli, N.E. (2002). Benzathine penicillin G and procaine penicillin G in piglets: comparison of intramuscular and subcutaneous injection. *Veterinary Research Community* **26**, 459-465.

Reeves, P. (1965). The bacteriocins. *Bacteriology Review* **29**, 24-45.

Renard, L., Moulin, G. and Sanders, P. (1992). Using experimental design to optimize a microbial diffusion assay. *Journal Association of Official Analytical Chemist Internation* **75**, 1045-1048.

Riedl, M.A. and Casillas, A.M. (2003). Adverse drug reactions: types and treatment options. *American Family of Physician*. **68**, 1781-1790.

Rietourd, A., Prognon, P., Brion, F. and Mahuzier, G. (1997). Liquid chromatographic determination using lanthanides as time-resolved luminescence probes for drugs and xenobiotics: advantages and limitations. *Analyst* **122**, 59R-66R.

Riviere, J.E. and Spoo, J.W. (1995). Tetracycline antibiotics. In: Adams, R.H. (ed.) Veterinary Pharmacology and Therapeutic., 7th edition. Iowa State University Press, Ames, Iowa, USA, **pp**.784-796.

Rollins, L.D., Gaines, D.W., Pocurull, D.W. and Mercer, H.D. (1975). Animal model for determining the no-effect level of an antimicrobial drug on drug resistance in the lactose-fermenting enteric flora. *Antimicrobial Agents and Chemotherapy* **7**, 661-665.

Rudzki, E., and Rebandel, P. (1997). Sensitivity to Oxytetracycline contact Dermatitis, **37,** 136-139.

Sande, M.A. and Mandell, G.L. (1980). Antimicrobial agents: the aminoglycosides. In: Goodman, L.S. and Gilman, A. (eds.) The pharmacological basis of therapeutics. Macmillan, New York, USA, **pp**. 1162-1180.

Schmid, A. (1983). Chloramphenicolrückstände in Lebensmittel tierischer Herkunft als potentielle Ursache der aplastischen Anämie des Menschen. *Deutsche Tierärztliche Wochenschrift* **90**, 201-248.

Schoevers, E.J., Terlou, M., Pijpers, A. and Verheijden, J.H.M. (1994). An image analysis system: an objective and accurate alternative for reading the agar diffusion test. *Journal Veterinary Pharmacology Therapeutics* **17**, 38-42.

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

Sczesny, S. (2001). Analysis of tetracyclines in environmental and food samples using a selective extraction procedure and a sensitive detection system by coupling HPLC with a microbiological assay and tandem mass spectrometry. Dissertation. Tierärztliche Hochschule, Hannover, Germany.

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

Settepani, J.A. (1984). The hazard of using chloramphenicol in food animals. *Journal of American Veterinary Medical Association* **184**, 930-931.

Seymour, E.H., Jones, G.M. and Mc Gilliard, M. L. (1988). Comparison of on-farm screening test for detection of antibiotic residues. *Journal of Dairy Science* **71**, 539-544.

Shaikh, B. and Allen, E.H. (1985). Overview of physical-chemical methods for determining aminoglycoside antibiotics in tissues and fluids of food-producing animals. *Journal Association of Official Analytical Chemist Interation* **68**, 1007-1013.

Shaikh, B. and Moats, W.A. (1993). Liquid chromatographic analysis of antibacterial drug residues in food products of animal origin. *Journal of Chromatography* **643**, 369-378.

Silley, P. (1991). Rapid automated bacterial impedance technique (RABIT). *Society for General Microbiology Quartly* **18**, 48-52.

Silley, P. and Forsythe, S. (1996). Impedance microbiology - a rapid change for microbiologists. *Journal of Applied Bacteriology* **80**, 233-243.

Silley, P. and Mortimer, F. (2003). Impedance In: Rapid microbiological methods in the pharmaceutical industry. Easter, M.C. (ed.) Interpharm/CRC, New York, USA, **pp**. 99-123.

Skyttä, E. and Mattila-Sandholm, T. (1991). A quantitative method for assessing bacteriocins and other food antimicrobials by automated turbidometry. *Journal of Microbiological Methodology* **14**, 77-88.

Skyttä, E., Haikara, A. and Mattila-Sandholm, T. (1993). Production and characterization of antibacterial compounds produced by Pediococcus damnosus and Pediococcus pentosaceus. *Journal of Applied Bacteriology* **74**, 134- 142.

Smither, R. (1978). Bacterial inhibitors formed during the adventitious growth of micro-organisms in chicken liver and pig kidney. *Journal of Applied Bacteriology* **45**, 267-277.

Smither, R. and Vaughan, D.R. (1978). An improved electrophoretic method for identifying antibiotics with special reference to animal tissue and animal feeding stuffs, *Journal of Applied Bacteriology* **44,** 421-429.

Smither, R., Lott, A.F., Dalziel, R.W. and Ostler, D.C. (1980). Antibiotic residues in meat in the United Kingdom; an assessment of specific tests to detect and identify antibiotic residues. *Journal Hygiene Cambridge* **85**, 359-369.

Søgaard, H. (1979). An improved microbiological procedure for detection of streptomycin residues in milk and animal tissues. *Acta Veterinaria Scandinavica* **20**, 38-44.

Sokoto State, (2000). Guide to Sokoto State's Economic Potentials. Commerce Department, Ministry of Commerce, Industry and Tourism, Sokoto-Nigeria **pp:** 7-10

Stauffer, U.G. (1967). Tooth changes caused by tetra. In the fetus, infant and child. Scheizerische Mediziniscge Wochenschrift **97,** 291-293.

Sternesjö, Å. and Johnsson, G.J. (1998). A novel rapid enzyme immunoassay (Fluorophos BetaScreen) for detection of beta-lactam residues in ex-farm raw milk. *Journal of Food Protection* **61**, 808-811.

Sullivan, Å., Edlund, C. and Nord, C.E. (2001). Effect of antimicrobial agents on the ecological balance of human microflora. *Lancet Infectious Diseases* **1**, 101-114.

Sundlof, S.F. (1989). Drug and chemical residues in livestock. *Clinical Toxicology* **5**, 411- 449.

Sundlof, S.F. (1990). Residue detection: a survey of available technology. *The Bovine Practitioner* **25,** 15-17.

Sundlof, S.F. (1994). Human risks associated with drug residues in animal derived foods. *Journal of Agromedicine* **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.

Tancrede, C. and Barakat, R. (1989). Ecological impact of low doses of oxytetracycline on human intestinal microflora. *Journal of Veterinary Med*icine **42**, Supplement, 35-39.

Terhune, T.N. and Upson, D.W. (1989). Factors affecting the accuracy of the live animal swab test for detecting urine oxytetracycline and predicting oxytetracycline residues in calves. *Journal of American Veterinary Medical Association* **194**, 918-921.

Teske, R.H., Rollins, L.D. and Carter, G.G. (1972). Penicillin and dihydrostreptomycin serum concentrations after administration in single and repeated doses to feeder steers. *Journal of American Veterinary Medical Association* **160**, 873- 878.

Thamdrup Rosdahl, V., Christensen, S.G. and Jacobsen, M. (1979). A rapid agar-diffusion test for the detection of antibiotic residues in kidneys from slaughter-animals. *Acta Veterinaria Scandinavica* **20**, 466-468.

Timoney, J.F. (1979). The tetracyclines In: Steele, J. H. (ed.) Antibiotics, sulfonamides and public health, vol 1. CRC Press, LLC, Boca Raton, FL, USA, **pp**. 267-279.

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

Tollefson, L. and Miller, M.A. (2000). Antibiotic use in food animals: controlling the human health impact. *Journal Association of Official Analytical Chemist Internation* **83**, 245-256.

Toutain, P.L. and Raynaud, J.P. (1983). Pharmacokinetics of oxytetracycline in young cattle-comparison of conventional vs. longacting formulations, *American Journal of Veterinary Research* **44**,1203–1209.

Tritschler, J.P., Duby, R.T., Oliver, S.P. and Prange, R.W. (1987). Microbiological screening tests to detect antibiotic residues in cull dairy cows. *Journal of Food Protection* **50**, 97-102.

Tsai, C.E. and Kondo, F. (2001). Improved agar diffusion method for detecting residual antimicrobial agents. *Journal of Food Protection* **64**, 361- 366.

Tscheuschner, I. (1972). Anaphylaktische Reaktion auf Penicillin nach Genuβ Von Schweinfleisch. *Z. Haut-Geschl. Kr*. **47**, 591-592.

USDA. (1979). United States Department of Agriculture. A self Instructional Guide: Performing the Swab Test (On Premises) for Antibiotic Residues. *Food Safety and Quality Service.* Washington, D.C., USA.

USDA. (1983). United States Department of Agriculture. How to perform the Live Animal Swab Test for antibiotic residues. USDA, Handbook No. 601, Washington, D.C., USA.

USDA. (1984). United States Department of Agriculture. Performing the Calf Antibiotic and Sulfa Test. A Self-Instructional Guide, Food Safetyand Inspection Service, Washington, D.C., USA.

USDA. (1994). United States Department of Agriculture. Fast Antimicrobial Screen Test, For detection and antibiotic and sulfonamide residues in livestock kidney tissue. A self-insructional guide. *Food Safety and Inspection Service,* Washington, D.C., USA.

Vaden, S.L. and Riviera J.E. (1995). Penicillins and related ß-lactam antimicrobials. In: Adams, H.R. (ed.) Veterinary Pharmacology and Therapeutics. Iowa State University Press, Ames, USA, **pp**. 774-783.

Van de Guchte, M., Ehrlich, S.D. and Maguin, E. (2001). Production of growth-inhibiting factors by Lactobacillus delbrueckii. *Journal of Applied Microbiology* **91**, 147-153.

Van den Bogaard, A.E. and Stobberingh, E.E. (1999). Antibiotic usage in animals: Impact on bacterial resistance and public health. Drugs **58**, 589- 607.

Van der Waaij, D., Berghuis-de Vries, J.M. and Lekkerkerk-Van Der Wees, J.E.C.M. (1971). Colonization resistance of the digestive tract in conventional and antibiotic treated mice. *Journal of Hygiene* **69,** 405-411.

Van Dresser, W.R. and Wilke, J.R. (1989). Durg residues in food animals *Journals of American Veterinary Medical Association* **194,** 1700-1710.

Van Schothorst, M and Van Leusden, F. (1972). Evaluation of the kidney test for the detection of antibiotic residues in slaughter animals. Tijdschr. Diergeneesk **97,** 1043-1047.

Van Schothorst, M. and Peelen-Knol, G. (1970). Detection and identification of some abiotics in slaughter animals. *Netherland Journal of Veterinary Science* **3,** 85-93

Vancutsem, P.M., Bapish, J.G. and Schwark, W.S. (1990). The fluoroquinolone antimicrobials: structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. *Cornell Veterinary* **80,** 173-186.

Vazquez-Moreno, L., Bermudez, M., Langure, A., Higuera-Ciapara, I., Diaz de Aguayo, M. and Flores, E. (1990). Antibiotic residues and drug resistant bacteria in beef and chicken tissues. *Journal of Food Science* **55,** 632-634, 657.

Vollaard, E.J. and Claesner, H.A.L (1994).Colonization resistance. *Antimicrobial Agents Chemotherapy* **38,** 409-414

Wageh S.D. Elsaid, A., Eldaly, T.E.Y. and Ikenaka, S. (2013). Scenario. *Japanese Journal of Veterinary Research* **61**(Supplement): S13-S22.

Walker, R.D. (2000). Fluoroquinolones In: Prescott, J.F., Baggot, J.D. and Walter, R.D. (eds) *Antimicrobial Therapy iin Veterinary Medicine,* 3rd ed. Ames, lowa, USA, **pp.** 315-338.

Walsh, S.E., Maillard, J.Y., Russel, A.D., Catrenich, C.E., Charbonneau, D.L. and Bartolo, R.G. (2003). Activity and mechanisms of action of selected biocidal agents on Gram-positive and –negative bacteria. *Journal of Applied Microbiology* **94,** 240-247.

Waltner-Toews, D. and McEwen, S. (1994). Residues of antibacterial and antiparasitic drugs in food of animal origin: a risk assessment. *Previous Veterinary Medicine.* **20,** 219-234.

Walton, J.R. (1983). Antibiotics, animals, meat and milk *Zimbabwe Veterinary Mecidine.* A.

Watson, W. and Bloom, W.L. (1952). Antimicrobial activity of a natural and synthetic polypeptide. *Proceedings of the Socoety for Experimental Medicine* **81,** 29-33.

Wawerla, M., Stolle A, Schalch, B. and Eisgruber, H. (1999). Impedance microbiology: applications in food hygiene. *Journal of Food Protection* **62,** 1488-1496.

Werber, D. and Bergann, T. (1997). Zum impedimetrischen Nachweis Von Antiinfektiva in Fleisch. Fleischwirtsch **77,** 556-559.

WHO. (1988). Joint FAO/WHO Expert Committee on Food Additives (JECFA) Taxicological evaluation of certain veterinary drug residues in food: Monograph prepared by the thirty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 763. WHO Geneva.

WHO. (1991). Joint FAO/WHO Expert Committee on Food Additives (JECFA). Taxicological evaluation of certain veterinary drug residues in food: Monograph prepared by the thirty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 815. WHO Geneva.

Williams, K.C., Blaney, B.J. and Mahwinney, H. (1992) Bacterial inhibitory residues in pig fed mould grain. *Australian Veterinary Journal* **69,** 38-39.

Wilson, D.J, Franti, C.E. and Norman, B.B. (1991). Antibiotic and sulfonamide agents in bob veal calf muscle, liver, and kidney. *American Journal of Veterinary Research* **52,** 1383-1387.

Woodward, K.N. (1991) Hypersensitivity in humans and exposure to veterinary drugs *veterinary Toxicology* **33,** 168-172.

Woodward, K.N. and Shearer, G. (1995). Antibiotic use in animal production in the European Union - regulation and current methods for residue detection In: Oka, J., Nakazawa, H., Harade, K. and Mac Neil, J.D. (eds) Chemical analysis for antibiotics used in agriculture. *Association of Official Agricultural Chemists International*, Arlington, VA, USA, **pp,** 47-76.

www.ultimateungulate.com/artiodactyla/camelus_dromedarius.html.

Yamada, Y., Sasaki, J, Matsuzaki, T. and Shiki, K. (1981). Influence of medium and diluents pH and diffusion time on antibiotic bioassay. *Tokai Journal of Experimental and Clinical Medicine* **6,** 23-33.

Zhou X. and King, V.M. (1995). A rapid bactometer method for screening of biocides against sulfate-reducing bacteria. *Applied Microbiology and Biotehnology* **43,** 336-340.

Zurhelle, H., Miller-Seitz, E. and Petz, M. (2000). Automated residue analysis of tetracyclines and their metabolites in whole egg, egg white, egg yolk and hens's plasma utilizing a modified ASTED system. *Journal of Chromatography* **B 739,** 191-203.