UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE
FACULTY OF AGRICULTURE
DEPARTMENT OF ANIMAL SCIENCE

AN ASSESSMENT OF ANTIBIOTIC USAGE IN RUMINANT LIVESTOCK AND ANALYSIS OF ANTIBIOTIC RESIDUES IN BEEF AND CHEVON IN THE SUNYANI MUNICIPALITY, GHANA

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AUGUST, 2019

DECLARATION

I hereby declare that this research is my own handy work towards the award of MPhil in Animal Science, that to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any other degree in the University, except where due acknowledgment has been made in the text.

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Supervisors

I hereby declare that the preparation and presentation of this thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

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ABSTRACT

The objectives of this study were to evaluate the awareness, practices and attitudes of farmers on antibiotic usein ruminant livestock and to determine the prevalence of antibiotic residues in beef and chevon in the Sunyani Municipality of Ghana. Random sampling was used to select respondents (ruminant farmers and veterinary officers) and meat samples (beef and chevon) whiles meat selling points (Abattoir, Abism Market and Central Market) were purposevily selected. A total of 150 ruminant farmers and two veterinary officers were interviewed using structured questionnaires. Also, 36 samples comprising 18 beef and 18 chevon were analysed for antibiotic residues using Liquid-Chromatography Mass Spectrometer (LC-MS). The results showed that, 76.5% of the farmers were ignorant about antibiotic resistant strains, 38.7% had no knowledge that improper administration of antibiotics could worsen the animals' condition. Majority of the respondents (53.3%) did not observe withdrawal periods, while 34.7% did. The analysis of beef showed no significant differences ($P>0.05$) among all antibiotic residues in beef samples except ciprofloxacin.Similarly, no significant differences ($P>0.05$) were observed in residue levels of all the antibiotics detected in chevon except chlortetracycline and norfloxacin.Ractopanine was the only antibiotic residue found above its maximum residue limit in both beef and chevon. The misuse of antibiotics is capable of generating residues in foods obtained from animals.Residues of the antibiotics were detected at various levels.Farmers should ensure strict adherence to recommended withdrawal periods and drugs labeled instructions.Antibiotic monitoring and testing programs ought to become a component part of the quality control process in producing high quality meat products.

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DEDICATION

This work is dedicated to the late Mr. Akansale Akanda-am (The Night Watchman) who scratched everything he had to see me through my formative education.

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CHAPTER ONE

1.0 INTRODUCTION

Antibiotics are vital bioactive and chemotherapeutic antimicrobial substances that either exists naturally, semi-synthetically or synthetically manufactured and having the potential to anihiliate or hinder the survival of bacterial pathogens (Macarov *et al*., 2012; Barton, 2000). Antibiotics as well as other chemotherapeutics are administered at therapeutic quantities usually for the cure and prevention of specific infections of bacteria in animals (Tollefson and Miller, 2000). The efficacy and effectiveness of antimicrobial agents against diseases of bacterial origin makes them a frequently patronized veterinary drug in animal production (Fischer *et al.,* 2003). Antibiotics as a part of antimicrobials do play a vital part in the protection and enhancement of thehealth and well-being of humans and animals.

The aim of antimicrobiological treatment is to generate and ensure the maintenance of effective levels of the drug administered in order to boost the imunity of the body defense system at the parts of the body where infections are apparent assisting in the killing of the disease causing pathogens (Benet and Belleemain, 2005). Antimicrobials are administered either in sub-therapeutic and therapeutic quantities in animal production.

Antibiotic ingredients dispensed in subtherapeutic quantities in animal feed promote growth, development and feed conversion efficiency in productive animals (Swatantra *et al*., 2014). It is an established fact that, the observance of proper treatment protocols regarding the application of antibiotics in livestock production improves feed conversion index and average daily weight gain through the depletion of disease causing microorganisms in the gut, reducing the thickness of the mucous membrane lining which facilitates absorption and assimilation and brings about synthesis of proteins, inducing an increase in muscle mass via suppression of cytokine production by the animal. In a nutshell, antibiotics affect the intestinal flora and physiology, enhances immunity and improve growth of animals (Niewold, 2007). Also the use of these drugs in animal husbandry drastically minimizes morbidity and animal life mortality resulting from the activity of disease agents in the farm, prevention and control of transfer of diseases to humans through the consumption of infested animals (Baynes *et al*., 2016).

Antimicrobial use for growth enhancement has become a mainstream application in farm animals and therefore, the attention of regulatory authorities has been sought to consider taking steps to minimize the abuse and to bring antimicrobial usage to the barest minimum in livestock, poultry, and aquaculture. To achieve this, it is crucial to ensure strict adherence to antimicrobial treatment regimen to minimize resistance and to guarantee their continual effective activity and readily accessibility regarding these drugs and avert adverse effects on the health of humans (Plachouras *et al*., 2014).

When aiming at the therapy of clinical pathogenic infections of bacteria, antibiotics are dispensed in therapeutic quantities following directions specified on the label especially when targeting specific animal diseases (Tollefson and Miller, 2000). The frequently adopted practice is that large farm animals are often treated individually especially dairy cattle production but occurs in other species only when there is economic feasibility and logistic availability in other animals (McEwen and Fedorka-Cray, 2002). Drugs whose activity can persist for long periods are often used for dry-cow therapy (Botsoglou and Fletouris, 2001). For whatever purposes, the preventive and curative potential of any antimicrobial is dependant on the accurate diagnosis of the disease or need, the use of the correct antibiotic and the careful observance of all labeled instructions.

Antibiotic residues are minute raminants of drugs or active principles that remain and contaminate the edible tissues or products from treated animals. These residues defile the safety and wholesomeness of foodstuffs like muscle meat, milk and eggs derived from animals that have undergone treatment (Bergwerff and Schloesser, 2003) and poses deleterious effects on the health of the consumer (Sanders, 2007).

Mostly, residues of antibiotics make their way into the food marketing chain right from the farm. It is crucial therefore for producers to be educated on the factors that result in the accumulation of antibiotic contaminants in animal feedstuffs paying much attention on ways of averting these. Particularly there is the need for the institution of innovative antibiotic monitoring and survailance programs and sructures as a measure to address the knowledge deficits in animal production centered at the farmgate to ensure that fine and wholesome products are supplied into the food chain (Rushton, 2015).

The occurrence of these residues may result from the violation of the mandatory withdrawal times, illegal or missuse of the antibiotic during the therapy of animals, contact with animal feed contaminated with faecal matter of treated animals and the application of antibiotics that are unlicensed (Ivona and Mat, 2000). Other determinants of the presence of these contaminants include the biological make up of the animal, the chemical and physical properties of the drug including their pharmacodynamics and kinetics.

The numerous health hazards encountered by the human population may expressly be attributed to the consumption of foods obtained from animals in which antibiotic residues persist at higher amounts. There is considerable evidence that toxic effects, development and transfer of resistant strains of bacterial pathogens to humans (Martins da Costa *etal*., 2013), immune pathological adversities, carcinogenic effects (Al-Mustapha and Al-Ghamdi, 2002; Rakotoharinome *et al*., 2014), gene mutations, hepatotoxic effects including bone marrow poisons, nephropathy, disorder in reproduction, allergensity and hypersensitive effects in sensitive persons are among the reported debilitating health effects in humans due to high levels of antibiotic residues in animal-source foods (Sanders, 2007).

Given the fact that most antibiotic residues in beef and chevon persist at levels higher than the maximum residue limit (MRL) coud impact negatively on the wellbeing of the population when exposed to them (Donoghue, 2003). Meanwhile, much work has not been conducted in Ghana on antibiotic use and residue levels in ruminantlivestock production especially on samples of beef and chevon, hence this work.

1.1 Main objective

The main objective of this work was to evaluate the knowledge and practices of farmers on antibiotic administration in ruminant livestock and to analyse the concerntrations of selected antibiotic residues in beef and chevon in the Sunyani Metropolis of Ghana.

1.2 The specific objectives were to:

- Assess the knowledge and practices of ruminant livestock farmers in the Sunyani Municipality on antibiotic administration.
- Determine residues of chloramphenicol, amoxicillin, ciprofloxacin, norfloxacin, danofloxacin, Sulfadiazine, metronidazole, morantel tartate, ractopamin, oxytetracycline and chlortetracycline in beef and chevon using LC-MS.
- Compare the concentration of the drug residues with the MRL of the Joint FAO/WHO expert committee on food and additives (JECFA).
- Estimate the risk of consumption of beef and chevon.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1Classification of antibiotics

Veterinary drugs are classified either by their chemical structure, mechanism of action or by their range of activity. Veterinary drugs are categorized into five main groups based on their chemical structure. They are the beta (β)-lactams, tetracyclines, aminoglycocides, macrolides, and sulfonamides (Ruyck, 2003). According to their mechanism of action, they can be classified as having bactericidal (killing) or bacteriostatic (inhibiting) effect on bacteria and by their range of activity; they are grouped as either broad spectrum or narrow spectrum (Aminov, 2010).

2.1.1 β-lactam

β-lactam antibiotics are a broad spectrum antimicrobial agents that shares a common chemical specificity of a four-membered β-lactam ring nucleus entailing a three-carbon and a one nitogen cyclic amine as a constituent of their core molecular structure (Lara *et al*., 2012) used in controlling diseases with bacterial aetiology (Figure 1).

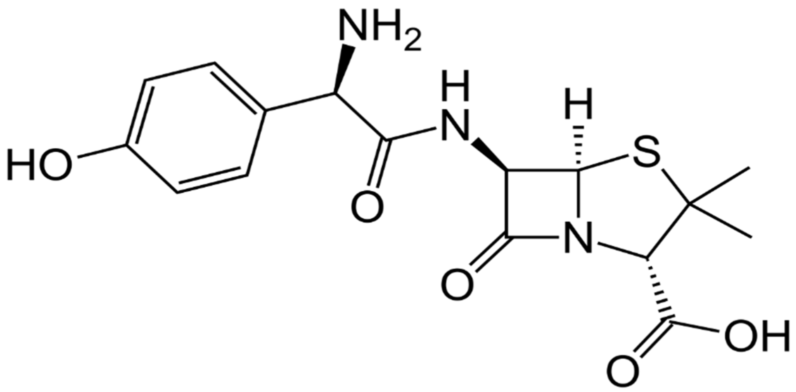


Figure 1: The Structure of amoxycilin

They are further classified into sub-groups according to their unique structural features namely the penicilins (e.g. ampicillin, amoxycilin), penems (e.g. imipenem, meropenem), cephalosporins (e.g. cephalexin, cefaclor), monobactams (e.g. aztreonam) and carbapenems (e.g. loracarbef) (Yao *et al*., 2007; Holten and Onusko, 2000).

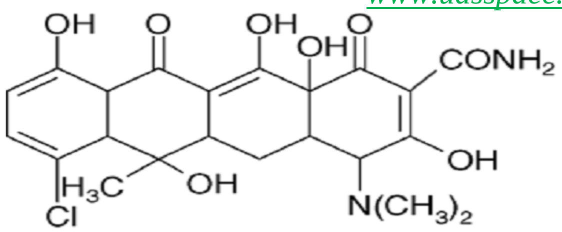
This distinctive ring of β-lactams is vulnerable to various nucleophiles, metal ions, acid-based reagents, oxidizing agents and solvents like water and alcohol in addition to enzymatic hydrolysis (Simoens*et al*., 2006). These circumstances make the ring unstable and subject to degradation through hydrolysis which compromises the structural integrity of the β-lactam ring and also depletes their antibacterial efficacy as well as the precipitation of potent allergensic characteristics because of the hydrolysed products. The magnitude and type of the products of hydrolysis of β-lactam antibiotic is often reliant on the strength of the chemical and enzymatic interferences (Cielecka-Piontek *et al.*, 2012).

Whenever a β-lactam antibiotic is administered to an infected animal or person, the drug diffuses through the cellulose cell wall to attach to the penicillin binding-proteins (PBPs) responsible for building up bacterial cell-wall (Boyce and Wanamaker, 2000). When these antibiotics bind to PBPs, the following bacteriostatic reactions proceeds, synthesis of bacterial peptidoglycan cell wall is severely disrupted due to cell wall lysis, cell shape is disfigured, and there is inhibition of cell division which eventually causes cell annihilation (Drlica*et al*., 2008).

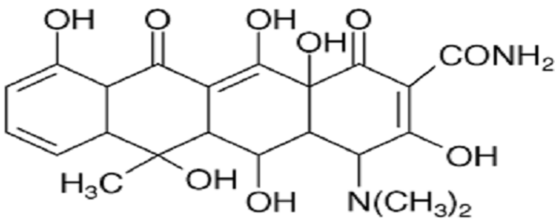
β-lactams retain lethal efficacy against gram positive bacteria genera like *streptococcus*, *staphylococus* and *gonococus* but are resisted by gram-negative bacterial may be due to an added lipopolysaccharide layer that inhibits antibiotic penetration, reduces the affinity of the PBP for the antibiotic or cause a change in the amount of PBP produced by the bacterium (Woodhead and Blasi, 2005). Penicillins comprising amoxicillin can poorly penetrate mammalian cells and also easily undrgo enzymatic hydrolysis by β-lactamases; they are therefore ineffective in the treatment of intracellular pathogens.β-lactamases are ubiquitous enzymes in bacterial cells with activity potential which inactivates the pharmacological effects of beta-lactam antibiotics (Moore *et al.*, 2008).

The prevalence of amoxicillin residues was studied in Egypt in laying chicken and commercial eggs (Khattab *et al.*, 2010). During this work the impact of cooking and storage on amoxicillin residues were also analysed. Varied concerntrations were found in both egg yoke and whites for a time of six consecutive days after the last administration of the antibiotic. It was revealed that the persistence of amoxicillin continued until the seventh day after the dispensation of the drug in eggs which were stored at room temperature at 4^{o}C. Boiling the eggs for ten minutes did not affect amoxicillin residue. The results of the work also found the withdrawal period for amoxicillin to be seven days.

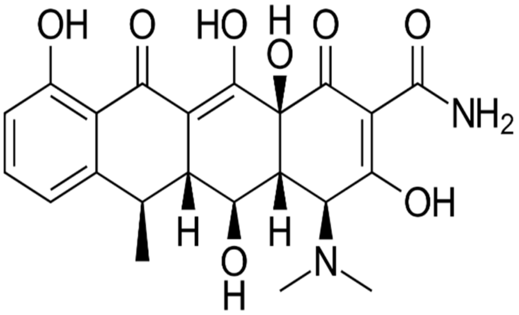
2.1.2 Tetracyclines



Chlortetracycline



Oxytetracycline



Doxycycline

Figure 2: Typical Structures of chlortetracycline, oxytetracycline and doxycycline

Tetracyclines are broad-spectum antimicrobial substances commonly used in veterinary and human medical practice with chemotherapeutic efficacy against a wide range of Gram-negative and Gram-positive bacteria including some anaerobes. Figure 2 shows typical structures of chlortetracycline, oxytetracycline and doxycycline. Tetracyclines also express strong activity against rickettsia, mycoplasmas, chlamydia and protozoan parasites (Ismail and Nelson, 2007). Theyare widely used in animal production for metaphylaxis, prophylactics, growth promotion and chemotherapeutics in food-producing animals to enhance effectiveness and productivity (Chopra and Roberts, 2001). The increasing application of tetracyclines for the purposes stated can potentially leave residues like toxins in edible tissues of animals which can affect human health.

The tetracyclines that enjoy frequent usage in veterinary practice include oxytetracycline, doxycycline, chlortetracycline and tetracycline (Fritz and Zuo, 2007). There are broadly classified first class tetracyclines and second class tetracyclines. First-class antibiotics can be first generation or second generation. First generation class is obtained by means of biosynthesis, for instance chlortetracycline, oxytetracycline, demeclocycline and tetracycline. The semi-synthesised tetracyclines like lymecycline, doxycycline, minocycline, methacycline and rolitetracycline are second generation. The third generation tetracyclines have recently been manufactured through total synthesis, example tigecycline for the therapy of infections hitherto resistant to other antimicrobials because it improved pharmacological and chemical properties with increased potency and efficacy (Olson *et al*., 2006).

Practical field therapeutic administration suggests the preferable use of the first-generation tetracyclines in food-producing animals while second-generation tetracyclines are offered to pets. Tetracyclines are used to treat therapeutic indications peritonitis, dermal and soft tissues diseases, infections of the respitory system and many enteric infections (Prescott *et al*., 2000).

For effective and less cumbersome administration of antimicrobials in farm animals, the drugs are given to the animals in groups orally as additives in drinking water or food for therapy or disease prophylactics (Giguère *et al.*, 2006). This notwithstanding oral administration of tetracycline preparations to ruminants should be avoided as they stand the risk of destruction of gut microbiota and creates a condition of indigestion. Horses also stand affected by the same effects. Intramuscular and intravenous infusion of tetracyclines are recommended for ruminants but if not done well can generate complications for the recipient animal. For instance, when tetrcyclines are administered intravenously in rapid maner, it can cause the dysfunction and collapse of the cardiovascular system of any animal species. Also intramuscular injections of tetracyclines are associated with swellings.

The chelation properties of tetracyclines are very strong (Aarestrup, 2003) and this readily determines the antibacterial and pharmacokinetics of antibiotics especially when tetracyclines undergo chelation with metal ions. Because of this fact, tetracycline medication shows contraindication when dispensed to young and pregnant animals, in such cases as indicated when tetracyclines reacts with calcium, chelation occurs especially on the surfaces of bones and teeth, and consequently consummates in stunted growth of the skeleton and the staining of the teeth as evidenced in children.

In the livestock industry, tetracyclines can be dispensed at low doses for the purpose of inducing growth in certain countries including fish farming.The long-term usage of antibiotics in this regard has been cited to be the cause of antibiotics, allergensity in both animals and humans. Also fluctuations in the population of environmental micro-flora and other detrimental outcomes are attributable to the excessive application of antibiotics (Chattopadhyay, 2014; Hao *et al*., 2014). Figure 3 shows the general structure of tetracycline.

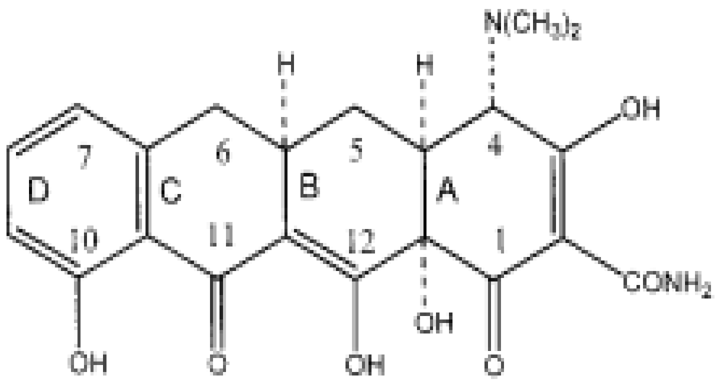


Figure 3: The general structure of tetracycline

A molecule of tetracycline is primarily composed of a hydronapthacene endowed with four tetracyclicnuclei (designated A, B,C and D as indicated in Figure 3) with varied functional groups attached conferring differentiability in the various tetracyclines (Chopra and Roberts,2001). Their MRLs set by FAO/WHO ranges from 100mg/kg, 300mg/kg, 600mg/kg and 200mg/kg for meat, liver, kidney and egg, respectively for animals like cattle, poultry, pigs, sheep (Codex Alimentarius Commission(CAC), 2012). A functional tetracycline that exhibits activity against bacteria should have a straight naphtacene ring containing an A-ring, diketo functional group on C1- C2 and an exocyclic carbonyl or amide group situated on C2. Evidence posits that tetracyclines that exact reversible inhibition of synthesis of proteins in bacterial cells require the attachment of an amino functional group on C4 and keta-enolic tautomers on C1 and C3 of the ring structure.The attachment of the amino group on C4 capacitates tetracyclines to carry out antimicrobial activities. Also a dimethylamino 4S isomer on C4 optimizes antibacterial activity buts it's decoupling from C4 decreases its activity against bacteria (Tariq, 1918; Fuoco, 2018).

Contemporary apprehension regarding the emerging bacterial resistance has prompted the withdrawal of these antibiotics from its capacity as growth promoters, particularly in Europe. Europe has since 1998 voluntarily stopped relying on all antibiotics as growth promoters in livestock production and eventuallythe swine industry did follow suit in 1999 (Aarestrup, 2003). In the EU, the law that bans the utilization of all kinds of antibiotics for growth inducement took effect from 2006 (Jensen and Hayes, 2014). The usage of tetracyclines as growth enhancers still finds place in the animal husbandry of certain countries (Chopra and Roberts, 2001; Van Boeckel, 2015). However, in the USA, beginning from 1st January, 2017, tetracyclines have been banned by legislation not to be used as growth promoters. Legislative authorityhas been exercised in this direction to ensure strict compliance with preventive application alone.

According to a research work by Abavelim (2014) to determine the levels of oxytetracycline in beef and mutton in three chosen markets in the Kumasi metropolis, Ghana. In all thirty (30) beef samples analysed in this work, the results showed that 15 (50%) samples composed of 5 (33.33%) from Central Market, 5 (33.33%) from Asafo market and 5 (33.33%) from Central Abattoir contained residues of oxytetracyclines at various concerntrations while in the rest 15 samples (50%), no residues of oxytetracyclines were found. Oxytetracycline has MRLs of 200µg/kg, 600µg/kg, and 1200µg/kg for beef, liver and kidney, respectively (Fagerquist and Lightfield, 2003). Out of the positive samples, 15 samples recorded levels of oxytetracycline residues beyond the MRLs in beef and 4 (26.67%) of these samples were from Central Abattoir. At Central Abattoir and Asafo Markets no samples recorded residue levels higher than the MRLs. The mean beefcontaminants of oxytetracycline were 86.18±17.20mg/kg, 87.17±13.07mg/kg, and 480.25±238.83mg/kg for Central, Asafo and Central Abattoir Markets, respectively. The ranges of oxytetracycline in the beef tissue are 68.70-110 mg/kg, 70.72-100mg/kg and 70.12-658.22mg/kg in Central, Asafo and Central Abattoir Markets, respectively.

For the 30 mutton samples analysed, 15 (50%) comprising of 5 (33.33%) each from Central Market, Asafo Market and Central Abattoir had detectable levels of residue of oxytetracycline but in the remaining 15 samples, no contaminants were identified. From the samples in which residues were detected, 5 (33.33%) had higher oxytetracycline residues above the recommended MRLs but 10 (66.67%) possessed concerntrations lower than the acceptable MRLs of oxytetracycline. The mean oxytetracycline residues in mutton were 181.13±53.33mg/kg, 239.70±141.65mg/kg and 105.08±57.68mg/kg for Central, Asafo and Central Abattoir Markets, respectively. The ranges of oxytetracycline in the mutton tissue were 110.35-240.99mg/kg, 87.41-400.45mg/kg and 75.88-190.88mg/kg for Central, Asafo and Central Abattoir Markets, respectively. Beef samples had higher concentration of residue than mutton samples at Central Abattoir Market for oxytetracycline. Significant differences ($P<0.05$) were recorded in the mean values of oxytetracycline residue in beef samples from the three markets. Mean oxytetracycline residue concerntration in mutton samples from the three markets were not significant ($P<0.05$) from each other. It was recommended to regulatory authorities to adopt strict monitoring systems to guarantee the safety and proper security of food.

2.1.3 Chloramphenicol

Chloramphenicol (CAP) is a wide spectrum antibiotic developed around the 1950's for the purpose of treatinginfections of bacterial origin in livestock (Bishop, 2005). It shows bacteriostatic potency against most Gram negative and Gram positive bacteria and also retards the growth and reproduction of rickettsia, chlamydiae and mycoplasmas (Bishop, 2005). Figure 4 shows the structure of chloramphenicol.

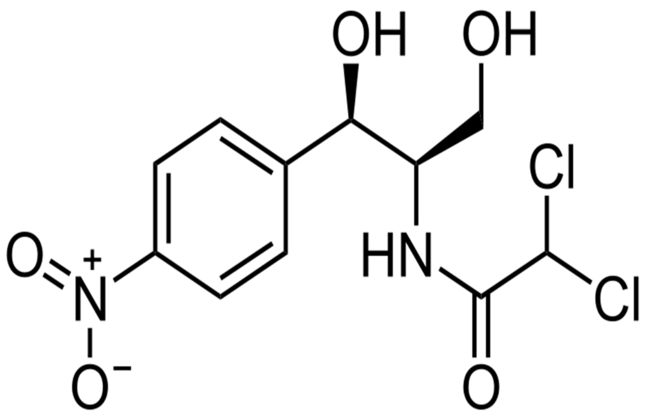


Figure 4: The structure of chloramphenicol

Chloramphenicol works by blocking of protein synthesis in bacterial cells and mammalian bone marrow cells. After the ingestion of food contaminated with chloramphenicol, it is often converted by means of biotransformation through glucoronidation in different biological organs such as the liver, kidney and muscle into metabolites which are transported in the whole body and excreted by kidneys. Plumb (2002) reported that, chloramphenicol undergoes biotransformation through glucoronidation which is a hepatic metabolism to become an inactive metabolite, chloramphenicol glucuronide.

Toxicologically, chloramphenicol is cytotoxic, genotoxic and hematotoxic and potentially causes dose related reversible myelo-supression affecting mostly precursors of red blood cells and, in the extreme cases, a rare but often lethal and irreversible aplastic anaemia that can lead to leukemia which has a high mortality rate determined as exceeding 50% in people. Also chloramphenicol contributes to the emergence of antibiotic resistant bacteria which in turn results in serious health problems (Ferguson *et al*., 2005). Chloramphenicol residues in human consumed food can cause precarious cardiovascular collapse in neonates, bone marrow toxicities and diseases, and a syndrome of cyanosis. The bioaccumulation of chloramphenicol in edible tissues of reared animals is raising concerns of its usage.

Following to reports of chloramphenicol residue toxicities in humans and the evidence of detecting residues of chloramphenicol in edible animal tissues, the drug has been banned in the US and the EU for administration in livestock production. Croatia has also banned the use of chlamhenicol in human consumed foods. In spite of the ban, traces of residues of chloramphenicol have been determined in foods of animal origin in the EU as reported by theRapid Alert System for Food Products (RASFF, 2002-2011) which raises questions of illegal use of the drug.

Mahmoudi *et al*. (2014) studied the prevalence of tetracycline, chloramphenicol and sulfonamide contaminants in cultured rainbow meat. In their investigation, they collected 100 samples of *Oncorhynchus mykiss* from different markets in northwest regions of Iran and prepared them for determination of residues of the antibiotics by ELISA method. It was found that 56% of the samples contained residues of the three antibiotics. Out of the 100 samples analysed, only seven contained chloramphenicol residues. This detection confirmed that inspite of the ban of the use of chloramphenicol in livestock production including aquaculture, there was continual illegal use. However, the low results obtained gave a positive indication in the matter of food safety and security.

The ban on the use of chloramphenicol has prompted the engagement of very sensitive detection methods to find chloramphenicol residues in edible matrixes. Frequently employed methods for determining chloramphenicol residues include the high performance liquid chromatography (HPLC), immunoenzymatic test (ELISA) and gas chromatography (Cerkvenik-Flajs, 2006).

In instances where samples are thought to contain higher amounts of chloramphenicol contaminants, methods such as gas and liquid chromatography-tandem mass spectrometry (GCMS/MS, LC-MS/MS) are used for effective quantification and confirmation of residue levels. These methods are preferred because of their high sensitivity, selectivity, accuracy and precision.

2.1.4 Sulphonamides (Sulfonamides)

Sulphonamides are one class of antimicrobials widely used in the livestock industry for therapy and prevention against a lot of diseases. They are largely employed and systematically appled in humans and animals for the purposes stated above (Schwarz and Chaslus-Dancla, 2001) and often also added to animal feed for the fact that prolonged intake of sulphonamides may have a growth-enhancing effect. Figure 5 shows the structure of sulfadiazine.

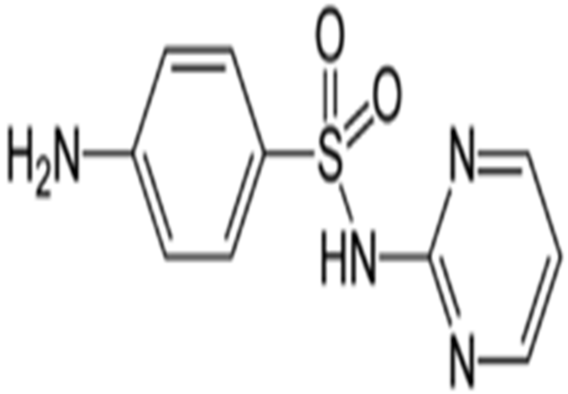


Figure 5: Typical Structure of sulfadiazine

The most common sulfonamide in frequent application are sulphadimidine, sulphamethizole, sulphamethoxazole, sulfadimethoxine, sulfadiazine, sulphadimidine and silver sulfadiazine which have the sulfonamide(RSO_2NH_2) as the base structure.They are all derivatives of para (p) aminobenzene sulphonamides and hence their chemical class contain a unique p-aminobenzoyl ring moiety containing an aromatic amino functional group positioned at N_4 and shows difference in substitution at the N_1 position (Chung *et al.*, 2009) as shown in Figure 6.

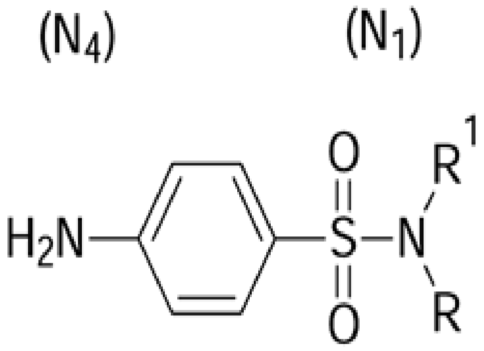


Figure 6: The basic structure of sulfonamide. If R_1=R_2=H=sulfanilamide.

From Figure 6, it can be seen that the nitrogen atom of $–SO_2NH_2$ is designated as 1 and that in $–NH_2$ is 2. Themechanism of action of sulphonamides involves a natural broad activity against gram-positive and gram-negative bacteria, nocardia, chlamydia, trachomatis and some protozoa and this antimicrobial activity is dependant on the substituents and their position of attachment on the benzene ring (Miller-Hjelle *et al*., 2004; Varagić, 2009). Sulfonamides have differential levels regarding their potency against bacteria but not in their spectrum of activity (Zessel *et al*., 2014). Studies confirm that sulphonamides that gives effective treatment results are derivatives of the compounds in which a single hydrogen atom of the $–SO_2NH_2$ is substituted by a heterocyclic ring structure (Lawrence *et al*., 2010). Microorganisms that are sensitive to sulfonamides need a p-amino benzoic acid (PABA) for the production of folic acid which is necessary for the synthesis of DNA and RNA (Lavanya, 2017). As a result of the similarity in structure of sulfonamides PABA, they competively stall PABA. This causes deficiency in folic acid which has bacteriostatic effect on the growth of bacteria and cell division (Lavanya, 2017). In effect there is a reversible blockage of the synthesis of folic acid, inducing an inhibition effect on bacterial growth (Varagić, 2009). More sulfanilamide derivatives with varying pharmacological properties have been synthesized from this main structure when R and R_1 are replaced by hydrogen, alkyl, aryl or hetero aryl (Lawrence *et al*., 2010).

Sulfonamides are among veterinary drugs that are inexpensive, readily available and commonly used in animal farms (Lee *et al*., 2001; Aboge *et al*., 2000). These medications can easily assimilate and be carried throughout the body system of chicken and ruminants, accumulate in different tissues and transported into their products (Kan and Petz, 2000; Weiss *et al*., 2007).

Although widely applied veterinary medical practice purposefully due to their effectiveness against infectious Coryza, Pollorum disease, Fowl Typhoid and Coccidiosis in poultry (Giguère *et al*., 2006). Sulphonamides such as sulfamethazine and other derivatives have been highly associated with conditions of ill-health including immune-pathological effects, hypersensitivity, resistance transfer and carcinogenicity (Wageh *et al*., 2013).

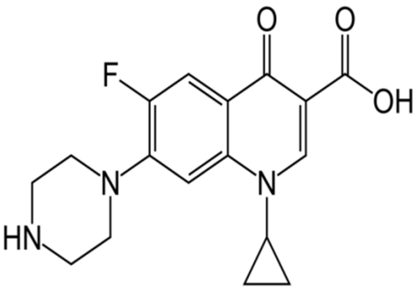
Mehtabuddin *et al*. (2012) reported thatmean levels of sulfonamide contaminants in poultry meat sampled from Rawalpindi and Islamabad confirm concerns of high levels of residues.In this study the total samples collected from Rawalpindi were 19. Out of this 8(42.10%) contained detectable levels of sulfonamides with 5 samples being higher in concrntration than the recommended MRLs. The range of concentration of sulfonamide residues was between 0.02µg/kg to 0.8µg/kg. The samples from Islamabad totaled 11 poultry meats. Out of the samples 5(45%) showed detectable concentration of sulfonamides of which 2 exceeded the MRL.The mean residue concentration ranged from 0.02-0.6 µg/kg.

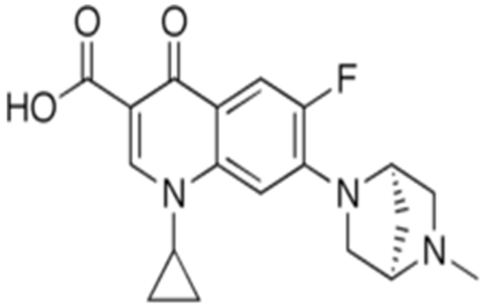
In total there were 30 samples in this study to determine sulfonamide residues in meat samples of poultry, of which 45% samples contained levels of residues which ranged from (0.02-2.0µg/kg). Out of the 13 samples that were positive for sulfonamide residues, 7(23.3%) were higher than the recommended MRL (0.12-0.8 µg/kg). The results recorded in this study were in conformity with Salem (2004) and Shaikh *et al*. (2000) who detected sulfonamide contaminants above the recommended MRL level in chicken meat samples.

One measure to reduce dangers to human health is aimed at minimizing sulphonamide contaminants in edible animal parts to tolerable levels by requiring that these substances are administered only in accordance with labeled instructions and their respective withdrawal periods observed (Kozarova *et al*., 2004). The MRL of sulfonamides should not exceed 100 µg/kg (EUX-Lex, 2010) in edible organs.MRLs are used in regulating the maximum permissible level of drug residues in food for each antibiotic which is considered safe and secured.

2.1.5 Quinolones

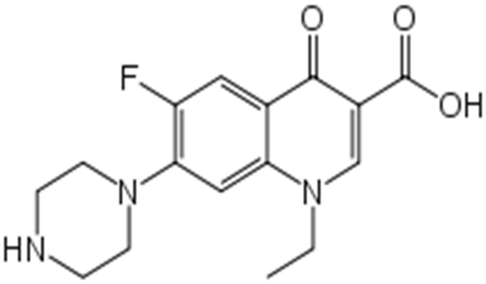
Quinolones and fluoroquinolones are vital synthetic antibiotics that have been admitted into human and veterinary medical practice and prescribed for use due to their clinical relevance (Velissariou, 2006; Cháfer-Pericás *et al*., 2010). Their broad activity against bacterial makes them a preffered drug in the treatment and prevention of animal dieases.Figure 7 shows typical structures of fluoroquinolones.





Ciprofloxacin

Danofloxacin



Norfloxacin

Figure 7: Typical structures of fluoroquinolones (ciprofloxacin, danofloxacin and norfloxacin)

The advents of fluoroquinolones which are second generation derivatives of quinolones improve the treatment integrity of the compound in clinical practice. Fluoroquinolones are known to possess improved activity, greater Gram-negative penetration and enhanced pharmacodynamics and pharmacokinetic properties (Aldred *et al*., 2014; Fabrega *et al*., 2009; Bisacchi, 2015).

The quinolone structure was modified by introducing fluorine at the sixth position and a ring substitute at position seven. Quinolones are deravatives of of nalidixic acid and oxolinic acid. A look at the structure of the compound (Figure 7) reveals that, there is a nitrogen atom on position 1 in the aromatic bicyclical ring structure with an alkyl group (cyclopropyl or ethyl) often joined to it. The carboxylic acid at position 4 confers antibacterial strength in the same manner like the kito group in position 4. There is a florene atom situated at position 6 on the quinolone carboxylic acid nucleus which improves the effectiveness of these drugs against gram-negative and gram-positive pathogens. The fundamental nitrogen containing moiety induces tissue penetration and reduces the central nevous system toxicities. Many other modifications on the structure are thought to improve the antibacterial properties of quinolones. When fluoroquinolones are administered, they penetrate the targeted phagocytic cells, reach the site of bacterial replication where theyremain microbiologically active within the cells and exert their antibacterial effect against pathogenic bacteria such as *Legionella pnewmophya* (Bermudez, 2001).

Like many other antibiotics, quinolones may directly induce toxic effects or result in the emergence of resistance of bacteria to drugs and can causehealth hazards to man. The quinolone resistance is acquired through these ways: mutation of the bacterial chromosomes resulting in the alteration of the target enzymes and their drug-binding capacity; reduced drug accumulation by reducing uptake or increasing efflux plamid-acquired resistant genes and producing either target proteins, drug modifying enzymes and drug efflux pumps (Redgrave, 2014; Hooper and Jacoby, 2016). Antibiotic residues are generally known to produce deleterious health effects such as allergy and hypersensitivity activity or toxicity in human physiology (Juan-Garcia *et al*., 2006).

Quinolones have been approved for use in several jurisdictions in animal production. It is used for the therapy of cattle, poultry, and fish in several countries of the world. Antibiotics are used for curtailing the incidence of diseases and for other purposes as prophylaxis. Non-adherence regarding the use of the antibiotic in animal-origin foods potentially leaves quinolone residues in those products. Doctor *et al*. (2011) gave a report that, as a result of the presence ofresidues of quinolones in human food and the consequent emergence of resistance strains of the antibiotic, there has been evidence of the ineffectiveness of the use of quinolones in human therapy. In Ghana, the approved and certified quinolones designated for use in animal farming include the following danofloxacin, norfloxacin andciproflaxin.

According to JECFA, the maximum residue limit for danofloxacin should not exceed200µg/kgin beef and chicken meat, enrofloxacin and ciprofloxacin in chicken muscle and beef should not also be above 100 µg/kg and difloxacin should not exceed 300 µg/kg in chicken and 400 µg/kg in beef.

2.1.6 β-adrenoceptor agonist

B_2-agonists are employed for the cure of lung diseases such as tocolytics, heart tonics and bronchitis (Courtheryn *et al*., 2002). Their usage as feed additives has been given approval in certain jurisdictions especially, Canada, Mexico and Japan for growth enhancement of fattening pigs, turkey and cattle but currently prohibited in fattening farm animals in 80 countries including European Union (EFSA, 2009; Allemanno *et al*., 2012). Ractopamine hydrochloride is classified under this group of antibiotics. Despite their legal use, the drug is often times illegally misapplied for purposes of improving weight gain and carcass deposition by decreasing buildup of fat mass while improving muscle leanness. The biochemical basis of ractopamine activity implies incrasing nitrogen retention, improving protein synthesis and and lipolysis, and suppression of lipogenesis in biological systems (Mitchell, 2009; Apple *et al*., 2007; Amstrong *et al*., 2004; Carrs *et al*., 2005). Figure 8 shows the structure of ractopamine.

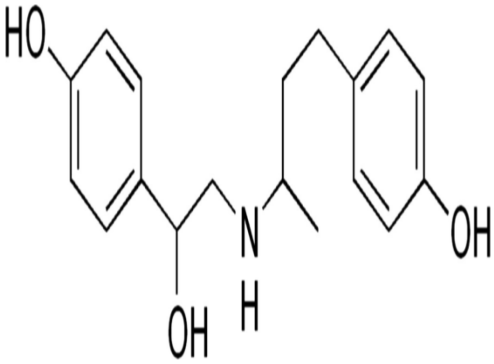


Figure 8: The structure of ractopamine

B-adrenergic receptors situated at the bronchi, heart, skeletal muscle, central nervous system, blood vessels, uterus and other organs are stimulants given their physiological effect onthese structures. Long-term exposure to beta-agonist can lead to these health hazards vasodilation,tachycardia,muscle tremors, nervousness, impaired metabolism and reduction in sensitivity to beta-adrenergic receptors. These adversities are dose related and pharcologically predictable. There are also non-pharmacological effects including airway reactivity. In livestock, ractopamine are associated effects like “downer” syndrome and acute cardiovascular stress andalso has been linked to heart disorders and poisoning in humans. Ractopamine is a lameness-facilitating agent that endangers food safety and security due to overdose and illegal application of the drug in animals (Huang *et al*., 2016).

The permissible limit of ractopamine residues for muscle, liver, and kidneys were 10.0µg/kg, 40.0µg/kg and 90.0µg/kg, respectively (Codex, 2012)**.**

2.2 Uses of antibiotics in livestock production

Antibiotic drugs are dispensed for purposes of treatment and prevension of infections in animal production (Saini *et al.*, 2012; Aarestrup, 2008; Tollefson and Miller, 2000).

2.2.1 Therapeutic uses

Antibiotics are administered to food animals for purposes of preventionor control of the development and establishment of infectious diseases (Ungemach, 2000). Prudent dispensation of antibiotics to animals that shows disease indications is usually carried out to minimize morbidity and mortality caused by pathogenic organisms in the farm in addition to the control of zoonosis in food animals that could infest humans via the food supply chain (Baynes *et al*., 2016). The infected animal is normally put on treatment dosage for a period of time, following the prescription and advice of a practicing veterinarian or an experienced farmer. The administration of antibiotics aimed at treatment or control should follow strictly the drugs labeled directions in order not to trigger more pressure resulting in selection pressure in the emergence of antibiotic resistance (Dale, 2009).

It is critical to ensure that the antibiotic used is safe and does not impose toxicity to the treated animal, giving way for their application as chemotherapeutic agents for the treatment of infections of bacterial origin. In order to reduce toxicity of the antibiotic to the sick animal, and minimize the development of antimicrobial resistance, proper identification and diagnoses of the diseases' causal agent is required to allow for definitive therapy (Leekha *et al.*, 2011).

In livestock therapy, the prime focus is to restrict the progression of diseases in the population, as illness imposes limitation on the performance of productive animals. Treatment of individual animals is easily carried out in the case of large ruminants like cattle but in flocks or broiler chickens, it is not practical to capture, handle and treat individual animals. In situations of this sort, the entire flock is treated, including clinically sick animals, those that maybe incubating the disease and those not infected (McEwen and Fedorka-Cray, 2002). The treatment of flock or herd is often initiated when clinical signs are first noticed in small fractions of the animals.For instance, one of the signs for medication in animals is physical stress incurred during transportation (EFSA, 2011). Mass treatment regimes can facilitate animal performance and the general welfare of animals, however, it does result in increased application of antimicrobials. The limitation regarding mass treatment programmes generally has to do with giving antibiotics to animals that do not need them whiles the selective therapy of animals with recognized clinical cases errs on the side of withholding treatment from some individuals that would have benefited. During the therapy of infections, the aim is to eradicate bacterial pathogens as quickly as possible with reduced adverse effects on the recipient animal (Capitano and Nightingale, 2001).

All medications legally applied in animals must be approved by the institution of government responsible for veterinary medicine and its accompanying instructions must dully be observed, to avoid unintended consequences. These institutions are tasked with the duty of setting rules for antibiotic use such as permissible routes of drug administration, forms of dose, maximum residue levels, withdrawal periods and limitations. Drug manufacturers, farmers, regulatory agencies, veterinary officers and farm employees who constitute the stakeholders involved in antibiotic drug administration have a collective responsibility to ensure that the right treatment protocols are adhered to so as to control public health impacts of antibiotic usage.

2.2.2 Non therapeutic uses

The non-therapeutic dispensation of antibiotics involves their application for prophylaxis, metaphylaxis and growth promotion purposes. Prophylaxis and metaphylaxis are the preventive uses of antibiotics. Prophylaxis is the administration of antimicrobials to healthy animals that are exposed and said to be at risk, but before the onset of the expected disease for which no etiological agent has yet been cultured (NCCLS, 2002). Prophylaxis is a preventive measure conducted either individually or in groups of healthy animal to avert the onset of diseases (Jaime *et al*., 2012). In young animals, prophylaxis helps to minimize enteric diseases whose development is induced by the incompete development of the immume system during the first week of life while in adult animals, it prevents the onset of feed-related pathology (acidosis in steers). Metaphylaxis is a mass medication procedure which focuses on the treatment of sick animals while medicating other healthy ones to prevent disease (Jaime *et al*., 2012). Group-level prophylactic treatments of livestock deemed to be at risk of infection is very common and may be characterized by the administration of antibiotics at therapeutic or sub-therapy levels for a much longertime of treatment than for therapy (Chee-Sanford *et al*., 2001; Heuer *et al*., 2009). Very often prophylactic antibiotics are not only dispensed through water and feed but occasionally also by means of injection (Heuer *et al*., 2009).

Several circumstances may prompt veterinarians or ruminant farmers to administer medications to animals that are not currently ill with a particular disease but stands the risk of contracting infections. For example, animals can receive treatment with antimicrobials after undergoing surgery or injurious trauma (Chee-Sanford *et al*., 2001). Also livestockmay be dispensed with antimicrobials if there is a higher probability of outbreak of contagious disease as a result of exposure to pathogenic bacteria or unfavourable environmental conditions (metaphylaxis). In livestock production, the mass dispensation of antibiotic medications is undertaken during transport or movent of young animals, during dry-cow therapy in dairy cows and in averting disorders in the respiratory system and intestines during stressful situations (WHO, 2015).

Under intensive animal husbandary systems, livestock are housed under stressful management with higher stocking densities, poor biosecurity practices but with expectationsof higher performance (higher production of offspring, meat, milk or eggs). These adverse production conditions can affect their health and also impair their immume competences making the animals more susceptible to these unfavourable conditions leading to the development and spread of infectious diseases (European Medicines Agency (EMA), 2017; WHO, 2015). Curtailing these requires the regular administration of antimicrobials to whole flocks or herds kept under intensively managed farm animals through water or feed.

In preventive or curative use of antibiotics, the knowledge regarding the pathogen involved and the anbiotic's pharmacology taking into account the species of the animal is vital tool in the control and prevention of diseases. However, this use of antibiotics should never be a replacement for proper biosecurity measures in the farm, taking into account the adverse impacts on the health of humans because of misuse of antibiotics (Mitchell, 2000).

Several calls to stop the regular use of antibiotics for prophylatics and metaphylactics continue to echo. For instance, the European Commissions's 2015 guidelines for the prudent use of antimicrobials in veterianary medicine state that, "routine prophylaxis must be avoided" (EC, 2015). A joint scientific opinion pubished in 2017 by the European Medicines Agency (EMA) and the European Food Safety Authority (EFSA) states, "there should be an aim to phase out the preventive use of antimicrobials except in exceptional circumstances" (EMA and EFSA, 2017).

The Federation of Veterinarians of Europe states: "Routine use of antibiotics as prophylaxis should be phased out and, in a longer time perspective, completely come to an end. Disease prevention must be based on proper husbandry practices and we should move away from the use of antibiotics against expected bacterial infections at certain points in time of the life of food animals" (FVE, 2016). The WHO recommends "complete restriction of use of all classes of medically important antimicrobials in food-producing animals for prevention of infectious diseases that have not yet been clinically diagnosed" (WHO, 2015).

Growth promotion involves the administration of antimicrobials in sub-therapeutic doses as supplements in livestock feed to enhance growth. This practice has been in existence since the 1940s and it is estimated to make up more than half of the total antimicrobial worldwide. The measure was discovered accidentally when fowls fed with the by-products of tetracycline fermentation were found to grow faster than those that were not fed those by-products. Many other antibiotics have since been discovered to have average daily weight gain efficacy and feed coversion efficiency in livestock in a variety of applications (Gaskins *et al*., 2002). Several types of antibiotics namely polypeptides (bacitracin), glycolipids (bambermycin), ionophores (salinomycin) and beta-lactams (penicillin) are applied for growth enhancement and prevention of diseases (Singer and Hofacre, 2006; Butaye *et al*., 2003).

The precise physiological basis by which antimicrobials work as growth enhancers is vague, but knowledge on the matter has improved (Gaskins *et al*., 2002). Hypothesis confirms that the efficiency of growth promoters is mediated by their antibacterial effects through alterations of the composition and activities of intestinal microbiota (Collier *et al*., 2003) resulting in more efficient digestion of feed and metabolism of nutrients. Further actions of antibiotics are seen in the selective inhibition of pathogenic bacterial species, disease expression and immune system release which are other antibacterial effects that results in growth enhancement. For instance, mention can be made of increased rate of post-weaning scours because of the prohibition of the use of antibiotics as growth promotors in Sweden (Wierup, 2001). The Danish discovered after banning the use of growth promoters, low doses of antibiotics aid in warding off the pathogenic effects of *Lawsonia intracellularis* and minimize the occurrence and severity of ileitis and diarrhea (Bager and Emborg, 2001). The stated mechanisms potentially reduce the normal populations of gut microflora and inhibit the progression of susceptible harmful bacteria inhabited in the intestines.

The employment of antibiotics as growth enhancers in livestock keeping contributes to the emergence of antibiotic resistance. The selection for resistance may not be towards only that singular antibiotic but other antibiotics that belong to the same class and even unrelated antimicrobials when resistant genes to both drugs exist within the bacteria (O'Brien, 2002). Works done in the United States and Europe has revealed that the risk of death and hospitalization due to resistance is higher than sensitive *Salmonella* infection (Varma*et al*., 2005; Helms *et al*., 2004).

Collignon (2003) found that, the application of the antibiotic ovapocin as a growth enhancer in food animals in Europe brought about the development and spread of vancomycin-resistant enterococci (VRE) and subsequent colonization of a significant percentage of human population through the food chain. A prohibition in the use of ovapocin in livestock in the EU resulted in a marked reduction in the percentage of the general population carrying VRE in the bowel.

In view of the potential health risk several legislations have received ratification from regulatory authorities and governments to ban the administration of antimicrobials as growth enhancers in farm animals. The International Coalition for Farm Animals Welfare (ICFAW) affirms that the dispensation of antimicrobials for growth enhancers should be banned worldwide (ICFAW, 2018).

2.3 Causes for occurrence of antimicrobial residues

The following have been identified as responsible largely for residues in food animal products, inadequate guidance on withdrawal regimen, extra-label application of antibiotics, inability to identify drug's withholding times, accessibility to antibiotics to laymen, poor therapeutic records, difficulty in identifying treated livestock, poor management, long period of use and over-dose of antimicrobials, lack of enforcement of restrictive legislation regarding antibiotic usage, lack of consumer awareness about consumption of food animal containing residues (CAC, 2001).

2.4 Antibiotics resistance

Antibiotic resistance is a kind of drug resistance that arises when bacteria aquire the ability to survive exposure to an antibiotic medicated to kill or hinder its growth. When bacteria are exposed to subclinical amount of antibiotics, this can induce resistance to develop, be selected for, multiply and spread by passing genetic material to other unrelated bacteria, making them resistant as well, that is bacteria in animals behave in the same way as in humans (Silbergeld *et al*., 2008).

2.4.1 Developement of drug resistance

The health of man can either be affected through contaminants of antimicrobials in foods of animal origin, which may cause direct detrimental effects (Beyene and Tesega, 2014) or indirectly through the selection of antibiotic resistance determinants either gradually or at a much faster pace (Marshall and Levy, 2011; Zhang *et al*., 2003) that may spread human pathogens. Resistant microorganisms can infect humans, either through direct contact (Chang *et al*., 2014) or indirectly via meat, milk and other meat products. Bacteria that is inhabiting the human body have capability to colonise human indegenious flora and superimpose an added loaded burden the reservoir of resistant genes already present in man. Beyene (2016) concluded in a research work, that the potential for transfer of resistance from animals to man exist. Evidence confirms that, the application of antibiotics in animal production has been associated with the emergence of antibiotic resistance in humans (Chang*et al*., 2014; Landers *et al*., 2012). Research confirms that humans contract antibiotic resistance from bacteria such as *Salmonella*, *Campylobacter* and *Staphylococcus* from animals (Chang *et al*., 2014). For example, fluoroquinolones and ovaopocin have been identified as drugs to which bacteria have developed resistance to.

The most vital index that influences resistance selection and prevalence is noncompliance to antibiotic usage protocols in people and in food animals (Barza, 2002). Resistance to antibiotics proceeds gradually. Any time an animal or human receives a dose of an antimicrobial; there is the propensity to develop resistance to the said drug. The rising use of antibiotics for sub therapeutic results takes center stage because drugs are administered at low doses, effects sub-lethal injury and selective advantage to resistant mutants for longer time periods. Also higher stocking densities, poor housing, insanitary conditions and poor animal welfare under intensive farming are other factors that foster the development and spread of bacterial resistant strains (WHO, 2002/2003) due to the constant use of antibiotics to counter the growing interference of microbes on the productivity of animals.

2.4.2 Mechanisms of bacteria resistance and transference

Bacteria have developed several resistant mechanisms (Simon *et al*., 2009). Many species of bacteria have acquired features that are able to counter the effect and adapt to environmental variations after undergoing genetic mutations to their response in the new environment through natural selection and drift. In addition, bacteria cease the opportunity to take advantage of mobile genetic elements, such as plasmids (circles of DNA that can self-replicate) and transposable ('jumping genes') elements. Bacteria utilize these large reservoirs of itinerant genes that move from one bacteria cell to another and can spread via bacterial populations. Some of these genes are able to confer resistance of bacteria to antibiotic effects. Antibiotic resistance is either natural or acquired. Natural or intrinsic resistance occurs when a bacterial species has an innate functional ability to resist the activity of particular antimicrobials. This may be attributed to the development of certain defensive meachanisms by the bacterial cell as in blocking the antibacterial agent from reaching the target site, or a lack of affinity between the antibacterial and the target site or the absence of the target site in the cell. Evidence confirms the inherent resistance of certain species of bacteria to whole class of antibiotics. Under such circumstances, all analogues of that bacterial species are resistant to all members of the antibacterial classes.

Acquired resistance is a major cause for concern due to its natural mechanism of transmissibility of resistance. In this case, resistance to antibiotics is a function of chromosomal gene mutations and horizontal gene transfer executed byacquired mobile genetic elements, such as plasmids and transposons, which carry the antibiotic resistant genes (Bogaard*et al*., 2002). The transfer of resistant genes among bacteria occurs through:

transformation, where bacteria gain genes from the uptake of DNA from the environment; transductions, in which bacteria aquire genes through infection with contagious DNA; and conjugation, where bacteria gain genetic material by cell to cell mating. During this process, a plasmid is transported from one organism to another through the pilus (Aminov, 2011). This is possible between bacteria belonging to the same or different species. Plasmids carrying resistant genetic materials to one or more antibiotics have been identified in the *Salmonella* and *E.coli* isolated from humans in Asia, Europe, US and Africa and in farm animals (Villa *et al*., 2000). Bogaard *et al*. (2002) again confirmed that, the transmission of gene coding for resistance to antibiotics is facilitated by mobile genetic elements known as transposons, which has the capability of moving from plasmids to the bacterial chromosome and the reverse direction. Figure 9 is a diagram showing the transfer of antibiotic resistant plasmids between bacteria.

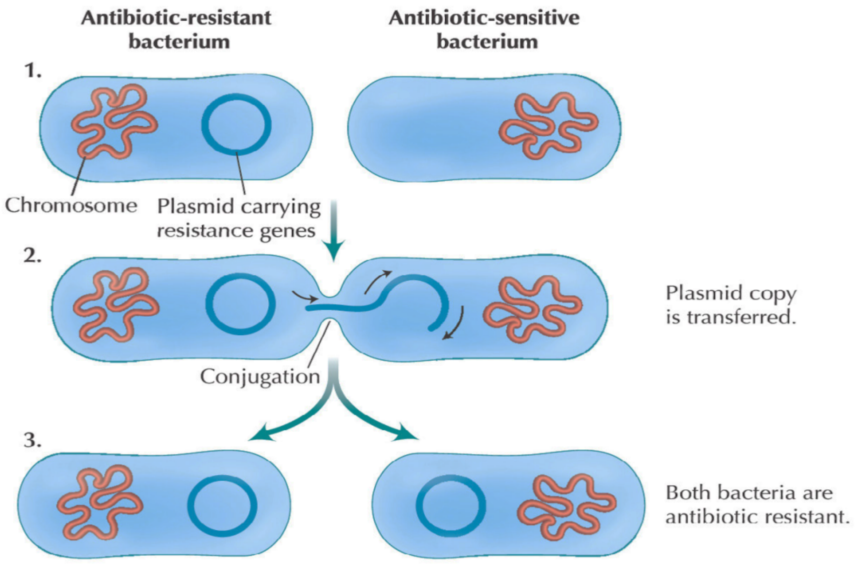


Figure 9: The Transfer of antibiotic resistant plasmids between bacteria (Barton *et al*., 2007).

2.5 Antibiotic residues and public health

Human health has direct dependence on the surrounding environment, most especially the nature and wholesomeness of food. Veterinary antimicrobials are regarded as challenge to public health when they gain access into animal-origin foods in the form of contaminants especially from treated animals (Landers *et al*., 2012; Butaye *et al.*, 2001). However, this subject is often omitted and not frequently or not entirely understood when public health or food and security issues are being handled.

The non-restrictive exploitation of antimicrobials in animal husbandry may violate public health standards due to the contamination of food with antibiotic residues and edible products of animal origin.Human health stands the risk of being dislodged via contaminants of antimicrobials animal-origin foods, which may produce direct or indirect adverse impacts via the selection of antimicrobial resistance determinants that may be transmitted to humans (Hughes and Heritage, 2001). The problems encounted by humans may result from the ingestion of sub-chronic exposure of levels of antibiotic residuescontained in foods including hypersensitivity, toxicities and carcinogenisity. Beta-lactam antibiotics such as cephalosporins and penicilins can cause allergies if high concentrations of residues persist in animal-source foods consumed by penicillin allergic persons.Tetracycline residues also have the potential to stain teeth of young children (Phillips *et al*., 2000). The Food Safety Authority of Ireland (FSAI), (2007) confirmed that, the judicious use of antibiotics in the manner of averting feed contamination is crucial.

2.5.1 Drug adverse reactions, hypersensitivity and allergy

Adverse drug reactions include all adverse issues relative to drug dispensation, regardless of the mechanism of action of pathogenic bacteria against the drug and disease aetiology. Adverse drug reactions as defined by the World Health Organisation encompases all unintended and noxious response to a drug occurring at a dose normally applied in man. A pharmacological categorization puts adverse antimicrobial reactions into two major sub-groups. Type (A) reactions, which are dose-related with predictable reactions and can occur in anyone depending on the known pharmacological actions of the antimicrobial (example overdose, side effects, drug interactions);Type B) reactions are not dose-dependant and reactions are unpredictable occurring in susceptible individuals having no relations to the pharmacological properties of the antimicrobial (Khan and Solensky, 2010; Sylvia,2010).

Drug hypersensitivity is matter of response to an arbitration between a drug agent and the human immume system especially in a sensitize patient. Allergy to drugs refers to a hypersensitivity reaction where a definite immunological mechanism, either IgE or T-cell mediated, is demonstrated, while nonallergic hypersensitivity refers to reaction, such as those to acetylsalicylic, in which other pathogenic mechanisms play a role (Johansson *et al.*, 2004). The IgE-mediated response occurs shortly after exposure to drugs and these include urticaria, angioedema, life threatening anaphylaxis and bronchospasm. Non IgE-mediated responses include thrombocytes, hemolytic anemia, acute intestinal nephritis, serum sickness, erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis (Granowitz and Brown, 2008). Several concerns expressed are that antibiotic drug residues in meat and their products might be the cause of similar hypersensitive in a number of individuals when they are consumed.

Drugs are foreign chemical moieties too small in weight to cause immunogenic effects, as a result, they act as haptens, forming conjugal associations with the proteins of antimicrobial sensitive persons to be immunogenic and trigger formation of antibodies (Riedl and Cassilas, 2003). In certain instances, allergy or hypersensitivity appears immediately during the first few hours after drug administration and are very heterogeneous in etiology and presentation (nausea, vomiting, skin reactions, respiratory symptoms, and hypotension). Although generally, non-toxic in nature β-lactams are frequently implicated in reported cases of allergy to antimicrobials. Other antibiotics that may cause allergy include sulphonamides, tetracyclines and aminoglycosides. Drug allergy may include serum sickness, anaphylaxis and cutaneous reaction. These adverse effects are passed on to humans after consuming foods containing drug residues with potential allergic effects of antibiotics used as food additives. It is reported that about 50% is said to be hypersensitive to the antibiotic penicillins, but quickly added that the extent of hypersensitivity to drugs in animals is not certain. Some macrolides in rare cases are the cause of liver injuries, induced by a particular allergic reaction to macrolid modified hepatic cells (Addisalem and Bayleyegn, 2012). Streptomy residues are found to have possible anaphylactic reactions on sensitive individuals. Tightness in the chest and angioneurotic edema may be caused by penicillin residues in meat, also penicillin residues in milk provokes allergy in sensitized persons. It must also be mentioned that in human chemotherapy, penicillin and streptomycin appear to cause hypersensitivity and allergensity than others in contemporary use.

2.5.2 Carcinogenicity

According to the American Cancer Society (ACS), (2014), a carcinogenic reaction is an expression generated by an antimicrobial possessing a cancerous producing effect. The carcinogenic veterinary medical products commonly applied in several farms are nitrofurance, quinoxaline and nitromidazoles. The residues of these drugs are obtained via foods obtained from animals. Theevidential truth about these drugs nitrofurance, quinoxalinedinoxides and nitromidazoles regarding their carcinogenicity and mutagenicity cannot be overemphasized and require concerted restrictions to overcome their carnage.

The potential dangers of carcinogenic drugs have to do with their reaction as regards their mediation or covalent bonding to varied intracellular components such as deoxyribonucleic acid (DNA), phospholipids, glycogen, glutathione, ribonucleic acid (RNA) and proteins.This leads to the alteration of cellular components such as DNA (Beyene, 2016; Aiello *et al*., 2005). These inherent hazards of genotoxicity imposed by these drugs can be overcome by prudent utilization and adherence to proper withholding periods.

2.5.3 Disruption of normal intestinal micro- bioactivities

Disturbance of normal human intestinal microflora delecterous affect antimicrobial contaminants consumed in human foods. The pathogenic bacteria that inhabit the intestinal environment serve as a barricade blocking incoming pathogens from getting rooted and causing diseases. Antimicrobials may cause a reduction or in severe cases deplete the total load of these benign bacteria or selectively kill some beneficial species that engage in gut microbioactivities (Myllyniemi *et al*., 2000). The use of broad-spectrum antimicrobials may bring into effect several adversities to a wide range of intestinal microflora and eventually result in gastrointestinal malfunction (Cotter *et al*., 2012). For example, the application of some antibiotics such as streptomycin, flunixin and tyosin in livestock and the medical administration of nitroimidazole, vancomycin and metronidazole in humans (Cotter *et al*., 2012) are noted for these disruptional effects (Beyene, 2016).

A work conducted to establish the effect of antimicrobial residues on the bacterial flora of the human intestinal tract concluded that, residues exert selective pressure on the dominant intestinal microbiota,promotes directly or indirectly, the development of acquired resistance in pathogenic enteric bacteria, enhances the growth of micro-organisms with natural or acquired resistance, impairs colonization resistance, or altering metabolic enzyme activity of the intestinal microflora (Hernandez, 2005). Eventhough, there is scarcity ofscientific information available in this area, few studiesaffirmed that subtherapeutic exposureof antibiotics yields negative effects on the human intestinal microflora (European Medicines Evaluation Agency (EMEA), 2001).

2.5.4 Mutagenicity

The term mutagen describes any physical or chemical agent that can inflict harm to the genetic material of a cell or organism. In all living organisms excluding some virus, the DNA is genetic material. Many chemicals including analougous bases and alkalizing agents have been proved to be directly involved in inducing mutagenic activity (Brown, 2002). There has been increasing concern that antimicrobial drugs in addition to chemicals in the environment that could trigger potential health effects to the human population through mutagenesis or chromosome breakagethat may adversely affect human fertility (Beyene, 2016; Foster and Beecroft, 2014). Understandably during mutagenesis, either the somatic or the general cell may be affected; meanwhile any aberration or damage inflicted to any group of these cells results in dare consequences on the health of the bio-population. Fron the standpoint of, genetic mutation in the general cells is more immediately significant as a result of its dangers to succeeding generations.

2.5.5 Antibiotic toxicities

The toxicity of streptomycin has been widely reported and three types: local sensitization with dermatitis is only of significance to veterinary surgeons who frequently handle powder or solution who become candidates to sensitization or allergensity. Systemic toxicity is vital in both veterinary and human medical administration and mainly responsible for the limited utilization of streptomycin in the veterinary field. Acute or chronic toxicity is characterized by anaphylactoid response with nausea, vomiting and rapid collapse with loss of consciousness (Johannes *et al*., 2007). Antibiotics caused acute toxicity to the host when administrated in high dosage. For example, aminoglycosides could cause acute tubular necrosis when given in a dose more than 35 micrograms per milliliter. Also aminoglycoside toxicity occurs either in the cochlea or vestibulum causing hearing loss or disequilibrium respectively (Palomar *et al*., 2001). Sulphonamides could produce crystalline aggregates in kidneys, ureters and bladder when given in a dose more than 125 micrograms per milliter. The products of animals whose products contain drug residues could cause effects in man when consumed. Moreover, sulfonamides are associated with the devastating condition of the dermonecrolytic Stevens-Johnson syndrome (See and Mumford, 2001).

More specifically the activity of certain drugs or pharmacological agents produces inherent toxicity that affects embryonic or fetal development at critical periods of gestation. Eventually, a congenital malformation that dislodges the functional and physiostructural integrity of the animal is produced. The well known documented issue of thalidomide involving several children in Europe was a factual evidence of lethal effects that could occur when such agents are dispensed during pregnancyand confirms this fact of teratogenicity. The antihelminthic, benzimidazole is tetarogenic and embryo toxic when administered at early periods of pregnancy as a result of the antihelmintic activity of the antimicrobial (Aiello *et al*., 2005; El-Makawy, 2006).Additionally, oxfendazole which is a benzimidazole has been implicated in mutagenic effects (El-Makawy *et al.,* 2006).

2.6 Incidence of antibiotic residues in various animal products in Ghana

Dapaah (2014) collected samples of liver, kidney and muscle tissues of chicken from Offinso-South Municipality, Kwabre District and Kentinkrono in the Ashanti region of Ghana. These products were analyzed for veterinary drug residues (including chloramphenicol, sulphathiazine, sulphamethoxazole, tiamulin, oxy-tetracycline and other veterinary drugs) using HPLC equipped with photodiode array (PDA) detector. Offinso-South Municipality recorded the highest mean concentration of 1542.0±62.73 µg/kg drug residues and was followed by residues in liver samples from Kentinkrono (1424.0±49.82 µg/kg). Samples from Kwabre District showed the least level of accumulation (1328.0±16.68 µg/kg) residues in the liver and this was very close to the kidney residue accumulation in the same District. Kwabre District however recorded the highest concentrations of residues (1247.0±42.39 µg/kg) in the kidney followed by Offinso municipality. Kentinkrono had the least accumulation of veterinary drug residue in the kidney. The concentrations of residue in the muscles from all the five farms visited were insignificantly different ($p < 0.05$). Offinso recorded the highest accumulation of 390.0±40.97 µg/kg closely followed by Kwabre, 374.8±47.11 µg/kg. Kentinkrono registered the lowest concentration of 343.7±35.85 µg/kg. Residues of all veterinary drugs were higher in liver and/or kidney tissue as compared to muscle tissue. Liver parts registered the highest mean concentration (1455.0±85.7 µg/kg) whiles kidney parts registered 887.5±45.8 µg/kg mean concentration. Muscle parts had the lowest concentration (371.2±33.6) in all the farms.

Addo *et al*. (2015) analysed 200 liver and 200 kidney samples for antimicrobial residues using the Premi® test in the Greater Accra region and found that 36 (18%) were positive for antibiotic residues. The highest number of positives was recorded from 24 (12%) out of the 200 kidney samples analyzed. The liver samples recorded the least with 12 (6%) positives out of the 200 liver samples examined for antibiotic residues. At the abattoirs the highest number of positives, 13(6.5%) was recorded at Amasaman followed by Madina and Ghana Industrial Holding Corporation (GIHOC) with 9 (4.5%) and 8(4%) positives respectively. Whilst no antibiotic residue was detected in beef from University of Ghana farms (UG, 6 (3%) of the carcasses from the Accra abattoir were positive for antibiotic residue.

In a study to analyze milk, cheese and yoghurt being sold at different points in Kumasi metropolis detected levels of the residues of chloramphenicol, sulfathiazole, sulfamethoxazole and oxytetracycline- four commonly used veterinary drugs in the country. The concentrations found were generally very low and close to the method detection limit of 0.1µg/kg. Concentration of chloramphenicol in yoghurt (0.8µg/L) was the highest among all the residues studied (Darko *et al.*, 2017).

Another report showed that 35% of the raw milk marketed in two major cities, Accra and Kumasi, were contaminated with antibiotics (Aning *et al.*, 2007). Additionally, 3.1% of the raw milk samples contained antibiotic levels above the European Union maximum residue limit. The antimicrobials detected included β-lactams, sulphonamides, aminoglycosides, tetracyclines and macrolides (Addo *et al.*, 2011)

2.7 Antibotics drug residues in parts of Africa

In several parts of Africa, majority of livestock farmers are implicated concerning the indiscriminate and frequent exploitation of antibiotics as drugs meant to progress the physiological performance as in improved feed conversion efficiency, rate of weight gain and therapy of diseases in food producing animals via direct administration or its incorporation as additives in livestock feed.

The threat of antibiotic adulteration is a critical concern not only confronted by Africans but also the whole global family (Cars *et al.*, 2008). Such residues are rapidly spreading, irrespective of geographical, legal and economic variability between countries (Harbath and Samore, 2005). Moreover, a study reported by EU in 2004 established that, a higher proportion of residues detected in animal products are antibacterial agents (EC, 2010).

That notwithstanding, limited information regarding the magnitude of veterinary drug residue worldwide is still apparent. This calls for an extensive work to determine the extent of prevalence to avert the occurrence of veterinary antibacterial residues and to orientate animal health professionals about the requisite knowledge on the pharmacodynamics, pharmacokinetics and toxicological adversities of veterinary drugs in order to reduce the potential public consequences posed by these drug residues.

Now in Africa, studies regarding the prevalence of antibiotic contaminants in animal-source foods are gaining the attention of researchers though at a marginal scale. For instance, Abiola *et al*. (2005) reported in a study conducted in Dakar (Senegal) of the detection of antimicrobial residues in chicken meat at prohibited concentrations of chloramphenicol and nitrofuran residues in different matrices. The prevalence of antimicrobial residues was found to be 30.8% for beef, 28.6% for pork, 29.3% for for chevon, 24% for mutton and 6.8% for eggs (Donkor*et al*., 2011). In addition, figures reported in Nigeria puts prevalence rates of antibiotic contaminants at 0.1%-1% for eggs, 4.8 for% for local chicken, 23.6% for laying hens, 21.8% for chicken faeces and 23.6% in laying hens (Fagbamila*et al*., 2010; Kabir *et al*.,2004).Higher prevalence values of 52% and 81% were obtained in gizzards and chicken livers respectively in Senegal (Abiola *et al*., 2005), as well as in Kenya(Kang'ethe *et al.*, 2005) and Tanzania (Kurwijila *et al*., 2006).

In advanced jurisdiction like US and the EU, there are systems put in place that allow for the conduct of regular and systematic checks through periodic screening for antimicrobial residues for the moninitoring, regulation and control of the usage of veterinary drug residues (EC, 2010). In the case of Africa, there has been unencouraging efforts in the conduct of studies to evaluate antibiotic residues in edible tissues of animals and raw milk residues with the exception of some counties in North Africa, due to the fact that milk is a well patronized delicacy in these countries (Donkor *et al*., 2011). Detectable concentrations of inhibitory compounds have been determined in raw milk, yoghurt, pasturized milk and the milk curd popularly known as *raïbi* in the sorroundings of Kenitra and Rabat. Inthis work, it was found that, the contamination of *raïbi*, raw milk and pasteurized milk stood at prevalence levels of 3.33%, 42.87% and 6.65% respectively. A report from Algeria by Tarzaali (2008) indicated that 89.09% of milk sampled from Blida, Tipaza, Wilayas and Medea tested positive for tetracycline residues while 65.46% tested positive for beta-lactamines. In the regions of Algiers, 9.87% of raw milk samples tested positive for antibiotic residues. Out of this 97.33% of the samples contained tratracyclines and/or penicillins contaminants, while 2.67% tested positive for macrolids and/aminogllycosides(Ben-Mahdi and Ouslimani, 2009). Kouame-Sina *et al*. (2010) reported a prevalence of 24.7% in Cote d'Ivoire compared with Mali whereantimicrobial concentrations of 6%-16% were found in raw cow's milk (Bonfoh *et al*., 2009).

2.8 Incidence of veterinary drug residues outside Africa

Pavlov *et al*. (2008) conducted a study to determine the residue concentration of antimicrobials in edible tissues of chicken slaughtered in two Abattoirs in Bulgaria. A four-plate agar diffusion test using *Bacillus subtilis* and *Bacillus mycoides* as the test microorganisms was evaluated for the determination of the presence of antibiotics. Out of the 75 samples taken the first abattoir, 2 tested positive for antibiotic residues while samples from the second abattoir registered no positive samples for breast muscles. Kidney and liver tissues recorded the highest number of samples containing residues of antimicrobials. It was concluded that, probably in some instances chicken meat producers did not adhere to regulations regarding withdrawal periods of veterinary medical products.

A work carried out to establish the presence of antibiotic residues in 130 samples of raw and ready to eat chicken and beef was taken from markets in Bangkok and neighbouring areas. It was also aimed at determining*Salmonella* 24 contamination rate and also the resistance profiles of *Samonella* isolates from the samples.The screening for penicillin, tetracycline, and sulphonamide clsss of antibiotics was conducted using drug residue assessment test kits. Fifty-one out of 130 samples constituting 39% tested positive for at least one of the antibiotic residues. The results showed that 28% tested for tetracycline, 23% for sulfonamide residues and 20% tested positive for penicillins.

In Bursa and Turkey, Cetinkaya *et al*. (2012) analysed chicken meat for residues of tetracyclines (oxytetracycline, chlortetracycline, doxycycline and tetracycline) by means of LC-MS/MS method. Doxycycline residues were detected in 60 samples in the range of 19.9-35µg/kg. Tetracycine (17.2µg/kg) was found in only one sample. Chlortetracycline and oxytetracycline were not detected in any of the matrices tested.A work which involved the collection of 127samples of chicken and 104 sampes of beef meat from markets of Ankara (Turkey) established the presence of quinolone residues using ELISA technique. It was detected that 118 (51.1%) out of the 231 chicken and beef samples were positive for quinolone residues. Fifty-eight (58) samples which constituted 45.7% of beef samples tested positive for quinolones, while 60 samples (57.7%) of the 104 beef meat also contained quinolone residues. The mean concentrations of quinolone residues were found to be 30.81 ±0.45µg/kg and 6.64 ±1.11µg/kg in chicken and beef samples respectively (Bucket *et al*., 2013). In the case of Rawalpindi/Islamabad, 30 samples each of breast chicken meat and eggs were collected from different farms and sale locations for analysis for sulfonamide residues. The study detected the contamination of samples with sulfonamide residues in poultry as a result of the indiscriminate application of sulfonamide in commercial broilers and layers without adhering to withdrawal periods. It was found that 43% of meat samples were contaminated with sulfonamide residues in the range of 0.02-0.8 µg/g and 30% of egg samples had sulfonamide contaminats in the range of 0.2-0.8µg/mL (Mehtabuddin *et al*., 2012).

2.9 Methods used in antibiotic residue analysis

Methods for determining the presence of residues of antibiotics in foods usually fall into one of two categories, screening or quantitative/confirmatory procedures. A wide range of analytical techniques exist for the analysis of antibiotic residues in edible animal products. They are the biosors, microbial inhibition test, charm test, Liquid Chromatography-Tandem mass spectrometry (LC-MS/MS), high performance liquid chromatography (HPLC), Utra performance liquid chromatography-mass spectrometry (UPLC-MS),and Liquid chromatography with mass spectrometry(LC-MS) are widely utilized for confirmation and quantification of drug residues in meat, milk and eggs(Jank*et al*., 2017; Hakem *et al*., 2013; Rinken and Riik, 2006).

2.9.1 High performance liquid chromatography (HPLC)

HPLC stands out as one the popular and sensitive analytical devices used in detecting antibiotic residues in foods. It is composed of a variety of mobile phases, a library of column packings and a difference in the mode of operations (Jank *et al*., 2017). In the determination of oxytetracycline and penicillin G residues from Ethiopia, samples were collected from Nazareth farms. The total samples collected were 400. Out of these samples, 48 had detectable levels of oxytetracyclines and penicillin G in the range of 45-192 and 0-28 µg/l respectively. Also, the analysis of chloramphenicol, quinolones, sulphonamides and tetracyclines using HPLC-Diode array detection in samples of milk collected from individual farms revealed residue concentration of 13.5-147.9µg/kg, 0.6-0.22.0µg/kg and 17.4-149.1µg/kg for sulphonamide, quinolones and tetracyclines, respectively. None of the samples contained chloramphenicol residues above the MRL (Senyuva *et al*., 2000; Elizabeth *et al*., 2011).

Various edible tissues of cattle including glutal muscles, diaphragm, kidney, triceps muscles and liver were sampled from different markets in Iran and examined for tetracycline (oxytetracycline, tetracycline and chlortetracycline) by the HPLC technique. The results showed that tetracycline concentration was 405.3 ng/g, 96.8ng/g, 672.4ng/g, 176.3ng/g and 651.3ng/g for glutal muscles, diaphragm, kidney, triceps muscles and liver, respectively.The level of detection in tetracycline residues were higher in liver and kidney samples in comparism with the other samples (Abbasi *et al*., 2012) and was also foundto be higher in cured meat products (Senyuva *et al*., 2000).

In the evaluation of the levels of the residues of 13 veterinary antimicrobials, an HPLC technique equipped with a photodiode array detector (HPLC-PDA) was used. The drugs concerned included ethopabale, sulphadiazine, clopidol, sulphathiazole, sulphamerazine, carbadox, sulphamethazine, sulphamonomethoxine, ormethoprim, sulphamethoxazole, furazolidone, sulphaquinoxaline and sulphadimethoxine in swine and chicken meat. The samples to be examined were extracted using acetonitrile and filtered. This was followed by partitioning the fitrate with acetonitrile-saturated n-hexane for taking out the interference. After evaporating to dryness, the residue was passed through a Sep-Pak C18 catridge for sample clean up before HPLC determination. In this work, analysis of veterinary drug residues was carried out by (HPLC-PDA) using a Luna 5µC18 (2) 25 cm*4.6 mm internal diameter of 5µm analytical column and a gradient elution of acetonitrile and 0.05Msodium dihydrogen phosphate. The 13 veterinary drugs from swine and chicken were recovered at levels of 0.1, 0.2 and 0.4 ppm in the range of 71.9-96.9% and 71.1-99.6%, respectively, with coefficients of variation less than 8%. The limits of detection (LOD) were 0.2 ppm for 12 drugs apart from sulphathiazole which had an LOD of 0.4 ppm. The work was conducted in Taipei in which 25 samples each of swine and chicken muscles were collected from local markets in Taipei and were analysed for antibiotic residues (Kao *et al*., 2001). It was found that one sample recorded a residue concentration of 1.23 mg/kg which was above the recommended MRL.

2.9.2 Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

This is one of the most commonly employed analytical methods for detection of a greater number of multidrug residues in food (Martins *et al*., 2012; Layada *et al*., 2016). The determination of antibiotic residues in milk using LC-MS/MS is more reliable and specific. An analysis of 14 antibiotics from different antibiotic classes included four sulfonamides, five beta-lactams, one macrolid, one cephalosporin and three tetracyclines by means of liquid chromatography with electro spray ionization (LC-ESI) and triple quadrupole mass spectrometry (MS/MS)(Martins-Junior,2007). In another work, a tetracycline and enroflavin residue in pork and chicken was determined using LC-MS (Kim *et al*., 2013). Aminoglycoside residues were found in animal tissues like bovine kidney and poultry liver (Plozza *et al*., 2011). In all, out of the 72 samples, 12 samples showed detectable concentrations of residues of aminoglycosides including neomycin, dihydrostreptomycin, and streptomycin were found to be above the recommended MRL. The residue concentration of neomycin, dihydrostreptomycin and streptomycin were 10000µg/kg, 300µg/kg and 300 µg/kg (Plozza *et al*., 2011) and doxycycline was found in poultry muscles at levels of 847.7µg/kg (Jank *et al*., 2017).

Utra-performance liquid chromatography in combination with time-of-flight mass spectrometry (UPLC-ToF-MS) was employed in screening and quantification of veterinary antimicrobials in milk (Nielen *et al*., 2008).The veterinary drugs belonged tovaried groups of antimicrobials such as macrolids, benzimidazole, ionophores, transquillizers, quinolones, penicillins, pyrimidines, sulphonamides, tetracyclines, amphenicols, nitromidazoles and non-steroidal anti-inflamatory agents (NSAIDs).The processes involved the precipitation of proteins, centrifugation and solid-phase extraction. The extracts were analysed for residues by UPLC-ToF-MS and a general full scan data generated. From this data, the drug specific ions were extracted for construction of the chromatograms and evaluation of the results. The analytical technique was validated using the EU guidelines (2002/657/EC) for a quantitative screening method.At the residue concentration of interest (MRL level), the outcome for repeatability (% RSD<20% for 86% of the compounds), reproducibility (%RSD<40%for 96% of the compounds) and the accuracy (80-120% for 88% of the compounds) were satisfactory. A set of 100 samples of raw milk were screened for residues. No suspected (positive) results were recorded except for the included blined reference samples containing sulphamethazine (88µg/l) that tested positive for this compound. The UPLC-ToF-MS was very powerful for the multi-drug determination of veterinary antimicrobials. The method was also useful in the analysis of organic residues like mycotoxins, pesticides and plant toxins in a single determination.

During the confirmatory mechanism for determining veterinary drug residues, Chrusch *et al*. (2008) established the performance activity of anup-to-date multi-stage residue analysis for non-steroidal anti-inflamatory drugs, anabolic steroids and corticosteroids in edible tissues of aniamals. A new LC-MS/MS technique was developed for the determination of 29 veterinary residues including three different cases of drugs, in animal tissues. The process used, measured the performance factors of the technique and theresults found using blank bovine kidney tissues and muscles. For a quantitative and confirmatory method, the typical performance parameters analysed were the limits of quantification, recovery, actuality, accuracy, selectivity, stability and roughness. The distinctive performance parameters outlined for the technique was confirmed during a validation study by an independent experienced analyst to decide whether the process used was appropriate for adoption in a regulatory monitoring and control program for residues of the 29 analytes.

Comparism of varied extraction mechanisms for multiclass determination of antibiotic drugs in eggs using ultra-high pressure liquid chromatography-tandem mass spectrometry (UHPLCMSMS) was carried out as a detection mechanism for veterinary drugs (Frenich *et al*., 2010). This work related four extraction methods for the simultaneous analysis of macrolids, tetracyclines, sulfonamides, quinolones and anthihelmintics (including avermectins and benzimidazoles) in eggs. Solvent extraction, solid-phase extraction matrix, solid-phase dispersion and modified procedure were likened interms of recovery and number of veterinary antimicrobials extracted. The solvent extraction process was confirmed, recording recoveries ranging from 60% (sulphaquinoxaline) to 119(LM) with repeatability values (expressed in relative standard deviation (RSDs)) lesser tha 20% at two levels of concentrations excluding ivermectin, erythromycin and emamectin. A limit of quantification (LOQ) is at all time equivalent or subordinate to 5g/kg. To conclude, the technique was used in egg samples and enrofloxacin, erythromycin, thiabendazole, difloxacin, emamectin and fenbendazole were determined in four samples.

The HPLC procedure ws used for the analyses of cyromazine and melamine remains in pork and mik as described by Wei *et al*. (2009). The method applied NH_2 column and 97% aceto nitrile eluate to determine the insecticide cyromazine and the metabolite melamine residues in pork and milk. Samples were treated with NaOH and extracted with acetonitrile possessing 20% NH_4OH. Target analytes of samples were cleaned up and concentrated by C18 column solid-phase extraction. A separation for cyromazine and melamine was produced and the respective retention times were 8 and 12 min. The calibration curves for cyromazine and melamine were linear in a concentration range of 0.01-1.0 µg/mL, with correlation coefficients of 0.9999 and 0.9997, respectively. Recoveries of cyromazine and melamine at fortified hights of 0.02, 0.05 and 0.1 mg/kg ranged from 84.5-090.8% and 83.6-91.3% respectively, with coefficient of variation of 3.1-7.8%.

2.9.3 Integrated approach for veterinary drug residue analysis

Mor *et al.* (2012) aimed at analyzing sulphonamide residues (sulphadiazine, sulphanilamide, sulphamerazine, sulphathiazole and sulphadimethoxine) in cattle meat by means ofcharm II method followed by confirmation of sulphonamide concentration by high performance liquid chromatography with florescent detector HPLC-FLD. Charrm II method was used in detecting 9 samples (5.73%) that were positive out of a total of 157 meat samples. In order to ascertain the validity of the finding, a quantative confirmation of sulphonamide content of positive samples, an HPLC-FLD was employed and four were confirmed as positive.In the HPLC determination, the LOD was in the range of 8-15µg/kg and LOQ was 13-25 µg/kg. The average recoveries of sulphonamides ranged from 44.6% to 81% with RSDs less 6% (n=6). It was established that, the results from field screening by means of the Charm II technique as is routinely practiced in Turkey and worldwide lacked insufficiency and therefore the results should be validated by sensitive mehods such as HPLC.

Schneider *et al.* (2009) compared various screening techniques for cattle meat, kidney juice and serum. Speedy screening test can be utilized as part of an effective program designed to monitor antibiotic drug residues in cattle. In an investigation designed to screen samples for residues of antibiotcs, different rapid tests were used, the Premi®, Kidney Inhibition Swab Test and Fast Antimicrobial Screen Test were likened using beef kidney juice and serum samples.In order to offer an accurate assessment, 235 samples of beef kidney juice and serum collected were procured from carcasses of cattle which had been retained by meat inspectors in a processing plant for further examination and testing. An LC-MS/MS method was used to determine the presence of anbiotic residues and their levels of concentration. The results obtained from the rapid sceening test were compared with that of the LC-MS/MS analysis relative to their sensitivity as well as the valuation of information regarding false positive and negative responses rates.

An antimicrobial screening assay has been developed which combines either a physical fluid extraction or a solvent extraction technique with a commercially available Premi® Test (Stead *et al*., 2005). In order to eliminate biases of the visual endpoint measurement with this microbial inhibition assay, work was carried out to connect the Premi® Test to a scanner technology. The utilization of thesolvent extraction influenced the detection capability for a broad range of antimicrobials at or below one-half of the MRL levels in different matrices as confirmed by dosage response curve data. Secondary class-specific assays, for the detection of sulphonamides and beta-lactams subsequent to the primary screen had hitherto been developed and confirmed by means of the scanner technology. The method of validation using both fortified and cured tissues had been carried out to confirm the roughness of the method. The false negative and positiverates have been established at below 5% for a range of drug/matrice combination.

A method was developed for the determination of sulphonamide residues (sulphaquinoxaline sulphadimethoxine and sulphamonomethoxine) in eggs without the application of organic solvents (Furusawa, 2003) but made use of a HPLC method fortified with a photodiode array detector (HPLCPDA).A determining technique of sulphonamides (sulphamonomethoxine, sulphadimethoxine and sulphaquinoxaline) in eggs, without use of organic solvents, was developed (Furusawa, 2003) utilizing a HPLC method equipped with a photodiode array detector (HPLCPDA). The sample preparation involved homogenization with perchloric acid solution using a handy ultrasonic-homogenizer followed by a centrifugal ultra-filtration unit. An analytical column and an isocratic mobile phase for HPLC are a reversed-phase C4 column (150× 4.6mm internal diameter) and 0.18 mol^{-1} citric acid solutions respectively. The average recoveries of the sulphonamides ranged from 80.3 to 88.4%, with RSDs 3.4% and 5.8%. In all the determination process, no organic solvents were applied at all.

An immunochemical screening assay with a surface plasmon resonance was established for the determination of chloramphenicol and chloramphenicol glucuronide remains in honey, prawn, cow's milk and poultry meat using a sensor chip with an antibody and a CM derivative (Ferguson *et al*., 2005). It was found that, the antibody cross-reacted with chloramphenicol glucuronide at these respective levels in 69.2% (honey), 75.7% (prawn), 84.8% (milk) and 73.8%(poultry). No cross-reaction was observed in commonly used antibiotics aswell as in drugs with similar physiochemical properties. The assay permitted direct determination of bovine milk (fat content~3.5%). Honey, prawn and poultry samples were extracted using ethyl acetate followed by determination on biosensor. Between run accuracy (n=3) conducted at identical levels produced the following outcomes: 4.7% (honey), 5.5% (prawn), 7.6% (milk) and 3.0% (poultry). This screening strategy though integrated has provided a reliable platform for the monitoring and survailance of antimicrobial residues.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Nature of research

The research consisted of two parts: a survey and labouratory analysis. The survey was carried out to determine the knowledge and handling of antibiotics by ruminant livestock farmers/veterinary officers in Sunyani Municipality through questionnaire administration and personal interviews. Beef and chevon samples were randomly collected from three (3) different market locations (Abattoir, Abisin and Central) in the Sunyani Municipality. Laboratory analysis was carried out to determine the antibiotic residues in beef and chevon at the Pesticide Residues Laboratory of the Ghana Standards Authority.

3.2 Study area

The study was carried out in the Sunyani Municipality of Ghana. The Sunyani Municipality covers an area of 829.3 square kilometers (320.1sq. miles) and lies between latitudes 7 degrees 20’N and 7 degrees05’N and longitudes 2 degrees 30’W and 2 degrees 10’W (Ghana Statistical Service (GSS), 2010). The Municipality is highly densed with a projected population of about 151,378 as at September, 2019 (GSS, 2019). The Municipality shares borders with Dormaa Municipality and Dormaa East District to the West, Tano North District to the East, Asutifi District to the South and Sunyani West District to the North. Sunyani is the capital of the Municipal Assembly and also doubles as the capital of the Bono Region. The capital is approximately 432 kilometers from the national capital, Accra. Figure 10 shows the Sunyani Municipality.

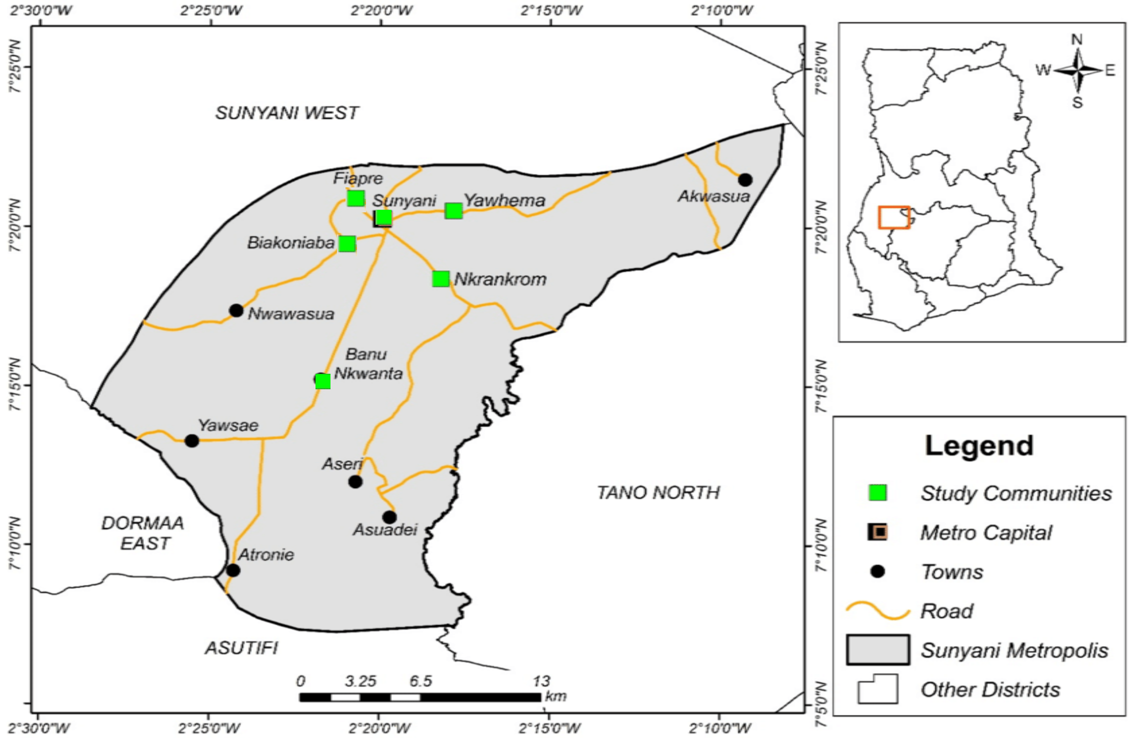


Figure 10: Map of Sunyani Municipality (Ghana Statistical Service (GSS), 2010)

3.3 Quesionnaire administration

One hundred and fifty farmers and two veterinary officersin the Sunyani Municipality wereselected through stratitified random sampling, andinformation regarding the demographic/socio-economic characteristics, biosecurity management practices implemented, antibiotic drug dispensation in ruminant animals, and the level of knowledge concerning the dispensation of antibiotics in animals were asked. Each farmer answered one questionnaire and it was a face to face interaction. The approval of the selected farmers to partake in the study was sought and obtained before the survey. The respondents were individually interviewed using semi-structured questionnaire made up of both open and close ended questions. Responses were also collated from veterinary officers. Out of the five veterinary officers available two agreed to answer the questionaire. Details of collated responses were presented in tables.

3.4 Determination of antibiotic residues

3.4.1 Sample collection procedure, preparation and storage

Beef and chevon were purchased between the times of October and December 2018 from three different market locations in the Sunyani Municipality. In all 36 samples were purchased from the three locations. The locations where the meats were purchased are Sunyani Central Market, Sunyani Abattoir and Abisim Market.These purchasing points were purposively selected based on their high meat production and consumer patronage. At each purchase point, six (6) samples each of beef and chevon were obtained using random sampling. Due to financial, time and logistical contrains, the study was limited to beef and chevon. Each of the meat samples was packaged in a neat sterile plastic packaging rubber bags, and marked using a permanent marker stating the date of sampling, the purchasing point and numbered. These samples were conveyed to the Pesticide Residues Laboratory of the Ghana Standard Authority in an ice chest containing ice. All samples were blended with a high speed blender to obtain a homogeneous sample and preserved in a deep freezer at -18 degrees Celsius until the time for the analysis.

3.4.2 Procedure for antibiotic drug residues analysis

3.4.2.1 Overview

The method involved a rapid and efficient protein precipitation extraction by acidified acetonitrile, followed by the use of EMR-Lipid dSPE and a polish kit. The samples were homogenized with Ultra Turrax homogenizer and centrifuged. The concentration of histamine was analysed by liquid Chromatography-Mass Spectrometry (Agilent Technologies, 2015).

3.4.2.2 Chemicals and reagents

All solvents and reagents were HPLC or analytical grade. Acetonitrile (CAN) was from Honeywell (Muskegon, MI, USA). Veterinary drug standards were from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Reagent-grade formic acid (FA) was from BDH Laboratory Supplies, England. Ammonium acetate ($NH_4CH_3CO_2$) was from Fisher Chemicals (Fair Lawn, NJ, USA).

3.4.2.3 Extraction

Extraction was carried out by means of Agilant Bond Elut EMR-Lipid for the determination of veterinary drugs in bovine samples (5991-6096EN), modified and adopted by the Pesticide Residues Laboratory of the Ghana Standards Authority. The raw meat samples (beef and chevon) were ground in adomestic blender and homogenized. Two (2) g of homogenised samples were weighed and transferred into 50mL centrifuge tubes. After which 10mL of acidified acetonitrile (containing 5% FA) were added. The samples were then homogenized using ultra Turrax and mixed on a mechanical shaker for 2 minutes. They were then centrifuged at 5,000 rpm for 5 minutes and 5mL ammonium acetate buffers (5mM) were added to 15mL EMR-Lipid dSPE tubes. Five (5) mL of the supernatants obtained were transferred into the EMR-Lipid tube and immediately vortex and mixed for 60 seconds on a multi position vortexer table. The samples were then centrifuged at 5,000 rpm for 3 minutes and 5mL of the supernatants were transferred into a 15 mL dSPE -Lipid polish tube containing 2 g salts (1:4 NaCl: $MgSO_4$) and vortex for 1 minute. They were centrifuged at 5,000 rpm for 3 minutes. After these, there were combinations of 500µL of upper CAN layer and 500µL water in a 2mL sample vial. The entire sample preparation flow path is shown in Figure 10.

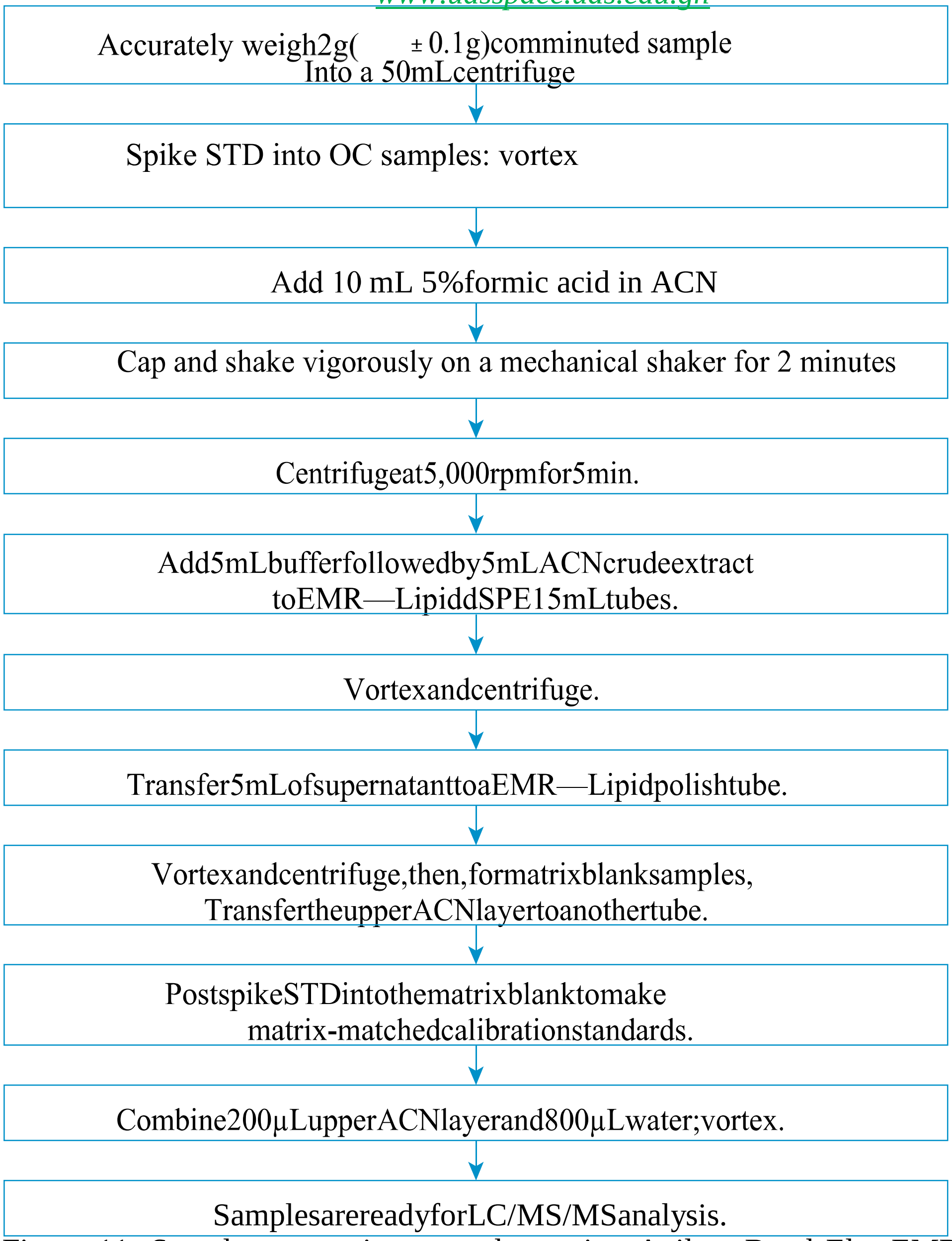


Figure 11: Sample preparation procedure using Agilent Bond Elut EMR-Lipid for the analysis of veterinary drugs in bovine samples (Agilent Technologies, 2015).

3.4.3 Preparation of standard solutions

Formic acid (5%) in CAN was freshly prepared by adding 5mL formic acid to 95mL CAN. Ammonium acetate stock solution (1 M) was made by dissoving 19.27g ($NH_4CH_3CO_2$) in 250mL Milli-Q water. The solution was stored at 4^{o}C. Also 5mM ammonium acetate in water solution was made by adding 5mL of 1 M ammonium acetate stock solution to 1 L of milli-Q water. Standard stock solutions were made in DMSO at 2.0 mg/mL except for danofloxacin stock solution in DMSO at 1.0 mg/mL and ciprofloxacin stock solution in DMSO at 0.25 mg/mL. All stock solutions were prepared in amber glass vials and stored at -20^{o}C. A combined 10 and 1 µg/mL standard working solutions were prepared in 1/1 ACN/water.

3.4.3.1 Preparation of stock solution of veterinary drug standards (100 µg/mL)

Veterinary drug standards (0.0025g) were weighed into 25mL volumetric flask and the volume topped up to the 25mL mark to prepare 100µg/mL.

3.4.3.2 Preparation of working standard (10 µg/mL)

A 2.5mL of the 100µg/mL solution was pipetted into 25mL volumetric flask and topped to the mark with 1/1 CAN/water to prepare a working standard solution of histamine 10µg/mL.

3.4.3.3 Preparation of working standard (1 µg/mL)

2.5 mL of the 10µg/mL solution was pipetted into 25 mL volumetric flask and topped to the mark with 1/1 CAN/water to prepare a working standard solution of histamine 1 µg/mL.

3.4.3.4 Preparation of calibration standard solution

Volumes of working standard solutions required to prepare the various concentrations of the calibration curve standard solution are shown in Table 1.

Table 1: Calibration Standard solution preparation

A calibration curve was prepared by injecting the calibration standard solutions.

Initial concentration (µg/mL)	Aliquot (ml)	Final volumetric flask (mL)	Final concentration (ng/mL)
0.1	0.01	1	1
0.1	0.05	1	5
0.1	0.1	1	10
0.1	0.2	1	20
0.1	0.5	1	50
1	0.1	1	100
1	0.2	1	200

The concentrations of veterinary drug residues in the beef and chevon samples were expressed as ng/mL. The peaks were identified by comparing the retention times of standard solutions.

3.4.4 LC/MS Analysis

Instrument conditions: Agilent 6420 Triple Quad LC/MS

LC conditions

Column: Agilent Poroshell 120 EC-C18, 2.1× 150 mm, 2.7µm

Agilent Poroshell 120 EC-C18UHPLC Guard, 2.1 × 5 mm, 2.7µm

Mobile phase: A) 0.1% FA in water

B) 0.1% FA in acetonitrile

Flow rate: 0.2 mL/min

Column temp: 40 °C

Autosampler temp: 4 °C

Inj vol: 6µL

Needle wash: 1:1:1 ACN: MeOH: H_2O with 0.2% FA

MS conditions

Posittive/negative mode

Gas temp: 300 ^{o}C

Gas flow: 13 L/min

Nebulizer: 40 psi

Capillary: 3,000 V

3.5 Data Analysis

The data gathered from the survey was analysed using Statistical Package for the Social Science (SPSS) Version 23. Results obtained in the form of frequencies and percentages were used to draw Tables. Antibiotic residues were analysed using ANOVA of GENSTAT 12th Edition and differences were separated at 5% significant level.

3.6 Health risk assessment associated with consumption of beef and chevon in the Sunyani Municipality

Food safety experts have developed the concept of maximum residue limits (MRLs) as a means of determining the levels at which substances could be hazardous to human health when present in food. MRLs are dependent on acceptable daily intakes.

The health threat posed by the consumption of beef and chevon from Abattoir, Abisim and the Central markets for ractopamine was assessed, using the following parameters.

Level of Concern (LOC) = $\frac{Acceptable\ Daily\ Intake\ (ADI)}{Rate\ of\ Meat\ consumption\ (RMC)}$……………….Equation 1

The level of concern (LOC) is a threshold concentration of drugs above which a health hazards can be encounted. LOC is calculated as the ratio of Acceptable Daily Intake to the Rate of Meat Consumption (RMC).

Acceptable Daily Intake (ADI) represents an estimate of a quantity of substance in food expressed in body-weight basis ingested daily over a life time with no observable adverse effects. The unit of ADI is reported in µg per kg of body weight.

The values of the Risk Quotients were calculated as the ratio between the mean concentrations of antibiotic in beef and chevon at each purchasing point to the level of concern (LOC) for the antibiotics.

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

The rate of beef and chevon consumption (RMC) was 65g/person/day or 0.065kg/person/day obtained from the Empirical Economic Letters which estimates daily food supply in Ghana (Osei- Asare, 2014).

CHAPTER FOUR

4.0 RESULTS

4.1 The knowledge and practices of antibiotic usage by ruminant livestock farmers and veterinary officers in Sunyani Municipality

4.1.1 Sex and educational level of farmers

The demographic/socio-economic characteristics of the 150 respondents showed that most of the farmers were males (83.3%) and had non-formal education (39.3%).

Table 2: Sex and educational level of ruminant livestock farmers in the SunyaniMunicipality

VARIABLE	FREQUENCY	PERCENTAGE
SEX		
Male	125	83.3
Female	25	16.7
EDUCATION		
Non-formal	59	39.3
Primary	29	19.3
JHS	26	17.3
SHS	11	7.3
Tertiary	14	9.3
Others	11	7.3

4.1.2 Ruminant breeds, years of rearing experience and husbandry practices

Out of the 150 respondents/farmers, 30% kept only goat, followed by 28% who kept goat/sheep. Those who kept cattle/goat were 1.3% and 2% minority reared cattle/sheep. In terms of years of experience on the job, the results indicated that 36.0% of respondents had 6-10 years' experience, followed by 28.7% who had 3-5 years' experience (Table 2). Those engaged in rearing of animals 10 years and above were 23.3%, and those with 1-2years experience were 9.3%.

The assessment of the type of farm husbandry practiced by farmers is shown in Table 2. The majority (96%) of farmers practiced semi-intensive system, 2.7% practiced intensive system, and 1.3% kept their animals extensively.

Table 3: Ruminant breeds, years of rearing experience and husbandry practices by ruminant farmers in Sunyani Municipality

Variable	Number of farmers	Percentage
Animal ownership		
Sheep only	34	22.7
Goat only	45	30.0
Cattle only	5	3.3
Goat/Sheep	42	28.0
Cattle/Goat	2	1.3
Cattle/sheep	3	2.0
Cattle/Sheep/Goat	19	12.7
Experience		
1-2 years	14	9.3
3-5 years	43	28.7
6-10 years	54	36.0
Above 10 years	35	23.3
No response	4	2.7
Husbandry systems		
Intensive	4	2.7
Semi-intensive	144	96
Extensive	2	1.3

4.1.3 Biosecurity measures adopted and diseases observed by farmers

Assessment of biosecurity measures adopted by ruminant livestock farmers in the Sunyani Municipality is presented in Table 4. The most common biosecurity practiced is fencing which recorded 98.7%, followed by adopted sanity of drinking water which had 80%

respondents. The least practiced biosecurity measures were the use of netting (2%) and quarantine (2%) of incoming animals.

Table 4: Biosecurity measures adopted and diseases observed by ruminant farmers in Sunyani Municipality

Variable	No of farmers	Percentage
Biosecurity measures		
Fencing	148	98.7
Netting	3	2
Sanitary of drinking water	121	80.7
Quarantine of incoming animals	3	2
Use of protective clothing	4	2.7
Regular vaccination	11	7.3
Regular fumigation	7	4.7
Farm away from water bodies	120	80.0
Farmers encounter of infections on their animals		
Yes	149	99.3
No	1	0.7
Farmers ability to identify the kind of infection based on their animals		
Yes	96	64
No	54	36
Farmers consultation of veterinary officers to know what kind of infection it was upon failure to identify		
Yes	47	87
No	7	13

Assessment of disease occurrence and identification in food animals revealed that 91.3% had ever experienced disease conditions in their animals (Table 4). Four (2.7%) farmers did not experience any disease condition on the farms and attributed the reasons to good and proper farm management. Of the 54 farmers that were unable to identify the kind of infections their animals experienced based on their own experience, 87.0% consulted veterinary officers to know the type of infections but 13% farmers did otherwise and bemoaned that they could not afford veterinary services and therefore sought the assistance of friends.

4.2 Assessment of antibiotic usage in ruminant livestock

4.2.1 Veterinary consultation for prescription

Table 5 shows the assessment of antibiotic drug usage in ruminant livestock by farmers. The results revealed that 78.7% of farmers consulted veterinary officers for prescriptions and recommendations to treat their animals, and 21.3% did not but depended on the advice of colleagues.

4.2.2 Reasons for antibiotic usage by farmers

Majority (68%) of the farmers use antibiotics for chemotherapy purposes, 31.3% used antibiotics for prophylactic purposes and 0.7% declined to answer that question. No farmer used antibiotics for growth promotion (Table 5).

The common clinical signs or conditions that necessitated the use of antibiotics for medication included diarrhea, nasal discharge, profuse salivation, lameness, mastitis and cough. Diarrhea was the highest and recorded 65.3% and the least reported condition was cough (12%) (Table 5).

Table 5: Prescription, purpose of antibiotic usage and disease/clinical signs observed in ruminants by ruminant farmers in Sunyani Municipality

Variable	**Number of Farmers**	**percentage**
Veterinary officer prescription		
Prescription	118	78.7
No prescription	32	21.3
Purpose of antibiotic use		
Chemotherapy	102	68
Prophylaxis	47	31.3
Growth promotion	0	0
No response	1	0.7
Clinical Signs		
Mastitis	19	12.7
Diarrhea	98	65.3
Nasal discharge	54	36.0
Cough	18	12.0
Lameness	31	20.7
Profuse Salivation	40	26.6

4.2.3 Types of antibiotics used

The majority consisting of 56.7% used tetracycline, followed by gentamycin (18%), amoxycilin/clavanic (26%), penicillin (24%), Tylosin (5%) and sulfamethoxazole (5%) (Table6). Chloramphenico l(1.3%) was used by the farmers, and no farmer used ceftriaxone (0%) and ciprofloxacin (0%).

Table 6: Types and reasons for antibiotic usage by ruminant livestock farmers in Sunyani Municipality

Variable	Number of Farmers	percentage
Type of antibiotic		
Tetracycline	85	56.7
Gentamycin	28	18.7
Amoxycline/clavanic	26	17.3
Sulfamethoxazole	5	3.3
Penicillin	24	16.7
Ciprofloxacin	0	0
Tylosin	5	3.3
Chloramphenicol	2	1.3
Ceftriaxone	0	0
Reasons for antibiotic usage		
Effectiveness	126	84
Easily accessible	93	62
Ease to use	51	34
Cost effective	23	15.3
Colleagues advice	26	17.3
No response	1	0.6

4.2.4 Antibiotic choice factors

The reason that accounts for the farmer's preference/choice of drugs for treatment of their animals is shown in Table 6. Most (84%) of the farmers patronized these drugs because of their effectiveness in the treatment and prevention of diseases, followed by 62% for farmers who said easy accessibility as a factor which influenced their choice of antibiotics usage. The choice of antibiotic drugs was least influenced by advice from colleagues.

4.2.5 Knowledge of farmers on antibiotics and accessibility

The knowledge of farmers on antibiotics and it accessibility is shown in Table 7. The results indicated that majority of farmers denoting 56% do not have knowledge about the use of antibiotics. Furthermore, 56% of the farmers' secure information on antibiotics from veterinary officers whiles 0.7% obtained information from NGOs. Also 62.7% purchased antibiotics from veterinary shops/clinics and 2% obtained the drugs from friends.

Table 7: Knowledge on antibiotics and accessibility byruminant farmers in Sunyani Municipality

Variables	Response	No of farmers	Percentage
	Yes	57	38
Knowledge on the antibiotic	No	84	56
	No response	9	6
	Extension	9	6
	officer	1	0.7
	NGOs	42	28
Source of information	Colleague	84	56
	Veterinary	2	1.3
	officers	12	8
	Others		
	No response		
	Vet shop/clinic	94	62.7
	Friends	3	2
Source of antibiotic	Market	34	22.7
	Others	11	7.3
	No response	8	5.3

4.2.6 Antibiotic administration

The frequency of antibiotics administration amongst farmers showed that 52.0% administered treatment whenever their animals are sick, 26.0% dispensed antibiotics on animals every 1-3 months, 10.0% affirmed that they administer antibiotics every 4-6 months, 2.0% said they do that every 7-9 months and 10.0% offered no response (Table 8).

Table 8: Frequency of treatment/admiration/dosage/ observance of withdrawal period by ruminant farmers in the Sunyani Municipality

Variables	Response	No of farmers	Percentage
Frequency of treatment	1-3 months	39	26
	4-6 months	15	10
	7-9 months	3	2
	Whenever animals are sick	78	52
	No response	15	10
Antibiotic administration	Self	57	38
	Veterinary officer	70	46.7
	Both	22	14.7
	No response	1	0.7
Observance of safety and dosage instructions	Yes	97	64.7
	No	47	31.3
	No response	6	4
Observance of withdrawal period	Yes	52	34.7
	No	80	53.3
	No response	18	12
Periodic sensitization/ education	Yes	22	14.7
	No	119	79.3
	No response	9	6.0

4.2.7 Consequences of misuse of antibiotics

The knowledge of farmers about the promotion of the emergence of antibiotic resistant pathogens through the misuse and improper dispensation of drugs in animals and its zoonotic ability (animal to human transfer) via the food chain was also assessed. The results showed that 76.5% were ignorant about antibiotic resistant strains, 38.7% had no knowledge that improper administration could worsen the animals condition and 21% knew about antibiotic resistance and the adverse health impacts it imposes on the human population whiles 2.0% farmers declined to give any answer (Table 9).

Table 9: Consequences of antibiotic usageamong ruminant farmers in Sunyani Municipality

Variable	Frequency	Percentage
Knowledge of improper administration of antibiotics on animal can worsen animal health		
Yes	89	59.3
No	58	38.7
No response	3	2
Knowledge that misuse of antibiotics can promote development of resistant pathogens		
Yes	32	21.3
No	115	76.5
No response	3	2
Knowledge that improper adherence to withdrawal period can lead to accumulation of residues in animals		
Yes	38	25.3
No	108	72.0
No response	4	2.7
Knowledge that consumption of residues of antibiotics in animal edible tissues can be harmful to humans		
Yes	90	60.0
No	55	36.7
No response	5	3.3

4.3 Common bacterial infections encountered by veterinary officers

Veterinary officers agreed having encounted bacterial infections in their daily work. They indicated that they come across the clinical signs of these infections in goats, sheep and cattle and administer antibiotics accordingly. Bacterial infection, causal agent, treatment process and type of antibiotic administered by veterinary officers in their daily routine work are thus indicated in Table 10.

Table 10: Bacterial infection, causal agent, treatment process and type of antibiotic used

Species	Bacterial agent suspected	Treatment provided	Type/types of antibiotics used	Reason
Bovine	Mycoplasmosis m.	Tylosin and oxytetracycline injection	Tylosin and oxytetracycline	Not given
Avian	Collibacillosis Mycoplasma syreviaviae	Enrofloxacin, Tylosin and oxytetracycline injection	Enrofloxacin, Tylosin and oxytetracycline	Not given
Canine	*Staphylococcus intemedius*	Topical ointment and oxytetracycline injection	Oxytetracycline	Cures wound suspected to be infectedby bacteria

4.3.1 Veterinary officers' knowledge on dosage and withdrawal periods

The results regarding veterinaryofficers' compliance to dosage recommendations and withdrawal periods is presented in Table 11.

Table 11: Dosage and withdrawal periods of antibiotics by veterinary officers in Sunyani Municipality

Name of antibiotic	Dosage recommended by manufacturer	Dosage administered	Withdrawal period
Oxytetracycline	1 ml /10kg body weight	1 ml/10kg body weight	14 days
Enrofloxacin	1 ml/ 10kg body weight	1 ml/10kg body weight	21 days
Tylosin	0.5-1 ml/10kg body weight	1 ml/10kg body weight	8 days
Sulfadiazine	3 ml / 10kg body weight	3 ml /10kg body weight	10 days
Penstrep	1 ml / 10kg body weight	1 ml/10kg body weight	10 days
Gentamycin	0.5 ml/10kg body weight	0.5ml/12kg body weight	7 days

4.3.2 Farmer sensitization and other relevant questions

Veterinary officers affirmed that, they sensitize farmers on the residual effects of antibiotics. Their response also showed that, farmers do not often bother to observe withdrawal periods in order to minimize residues in animals. They denied the presence of untrained personnel offering veterinary services to farmers but admitted the existence of community livestock workers. They lamented over the absence of a tracking system to identify and monitor farmers who sell their animals without observing withdrawal periods (Table 12).

Table 12: Farmers' sensitization and other relevant information

Variable	**Frequency**	**Percentage**
Advice of farmers on the residual effects of antibiotics		
Yes	2	100
No	0	
Presence of community livestock workers in your area of jurisdiction		
Yes	2	100
No	0	
Regular training of community livestock workers on antibiotic administration		
Yes	0	
No	2	100
Presence of monitoringsystems for tracking farmers who sell their animals without observing withdrawal periods		
Yes	0	
No	2	100
Regular sensitization of ruminant livestock farmers		
Yes	2	100
No	0	

4.4 Prevalence of antibiotic residues in beef and chevon in the Sunyani Municipality

The prevalence of antibiotic residues in beef samples collected from different selling points (Abattoir, Abisim Market and Central Market) is shown in Table 12. Significant differences (P>0.05) did not exist among all the antibiotic residues in beef except ciprofloxacin which recorded significant levels (P<0.05). Ciprofloxacin was highest in beef samples (16.57µg/kg) obtained from the Abattoir. There were no significant differences (P>0.05) in residue levels of all the antibiotics detected in chevon except chlortetracycline and norfloxacin which were significantly different (P<0.05) (Table 13). Chlortetracycline and norfloxacin were highest in chevon samples collected from the Central Market. Also there were no traces of resides of chloramphenicol andmetronidazole in both beef and chevon samples. Similarly, diminazen were not detected in all the chevon and beef samples except chevon obtained from the Central Market.

Table 13: Prevalence of antibiotic residues in beef samples collected from the Sunyani Municipality

Antibiotics (µg/kg)	Abattoir	Abisim Market	Central Market	Sem	P-value
Amoxycilin	17.16	19.27	17.58	2.33	0.192
Chlorotetracycline	5.96	5.93	5.98	0.13	0.76
Ciprofloxacin	16.57^{a}	15.18^{b}	15.31^{b}	0.97	0.022
Danofloxacin	9.74	9.70	9.73	0.05	0.193
Doxycycline	8.57	8.61	9.02	0.59	0.26
Norfloxacin	13.49	13.41	13.46	0.08	0.138
Oxytetracycline	8.36	10.74	10.16	4.62	0.572
Ractopamin	17.67	17.45	17.29	2.46	0.952
Sulfadiazine	1.28	1.28	1.27	0.02	0.842
Tylosine	17.32	17.53	17.35	0.26	0.264
Chloramphenicol	ND	ND	ND	ND#	ND#
Diminazen	ND	ND	ND	ND#	ND#
Metronidazole	ND	ND	ND	ND#	ND#

ND, not detected; ND#, not done; Means with different superscript along the columns are different at $P < 0.05$.

Table 14: Prevalence of antibiotic residues in chevon samples collected from the Sunyani Municipality

Antibiotics (µg/kg)	Abbatoir	Abisim Market	Central Market	Sem	P-value
Amoxycilin	21.60	21.90	22.60	7.68	0.965
Chlorotetracycline	6.03^{b}	6.27^{b}	7.54^{a}	0.83	0.005
Ciprofloxacin	15.72	16.46	16.26	1.21	0.474
Danofloxacin	9.72	9.75	9.77	0.06	0.331
Doxycycline	8.77	9.16	10.94	1.89	0.079
Norfloxacin	13.47^{b}	13.67^{b}	14.85^{a}	0.73	0.004
Oxytetracycline	8.32	8.93	9.49	0.94	0.075
Ractopamin	17.46	15.81	17.90	2.75	0.305
Sulfadiazine	1.27	1.28	1.30	0.02	0.174
Tylosine	17.35	17.33	17.37	0.05	0.368
Chloramphenicol	ND	ND	ND	ND#	ND#
Diminazen	ND	ND	2.95	ND#	ND#
Metronidazole	ND	ND	ND	ND#	ND#

ND, not detected; ND#, not done; Means with different superscript along the columns are different at $P < 0.05$.

4.5 Health risk assessment associated with consumption of beef and chevon in the Sunyani Municipality

Ractopamine was the only antibiotic whose levels were detected above it's MRL of 10µg/kg, which could have a negative effect on human healthand thus its risk quotient was calculated. The U.S Food and Drugs Administration established an ADI for ractopamine of 1.25 µg/kg of body weight per day.

Level of Concern (LOC) = $\frac{Acceptable\ Daily\ Intake\ (ADI)}{Rate\ of\ Meat\ consumption\ (RMC)}$.................Equation 1

The values of the Risk Quotients were calculated as the ratio between the mean concentrations of antibiotic (Ractopamine) in beef and chevon at each purchasing point to the level of concern (LOC) for the antibiotics.

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

The rate of beef and chevon consumption (RMC) was 65g/person/day or 0.065kg/person/day obtained from the Empirical Economic Letters which estimates daily food supply in Ghana (Osei- Asare, 2014).

Level of concern (LOC) = $\frac{1.25\mu g/kg}{0.065/kg}$ = 19.231 (µg) calculated as the ratio of the ADI to RMC.

Risk quotients (RQ) was 0.9079, 0.8221 and 0.9309 for chevon samples from the Abattoir, Abisim and the Central markets, respectively calculated from the ratio of mean concentrations and LOC. The beef samples recorded RQ of 0.9188, 0.9074 and 0.8891 for Abattoir, Abisim and the Central markets, respectively.

Table 15: Risk analysis of the mean concentrations of ractopamine present in beef samples from Abattoir, Abisim and Central markets

Antibiotics	LOC (µg/g)	RQ (Abattoir Market)	RQ (Abisim Market)	RQ (Central Market)	ADI µg/kg	RMC kg/person/day
Ractopamine	19.231	0.9188	0.9074	0.8891	1.25	0.065

Table 16: Risk analysis of the mean concentration of ractopamine present in chevon samples from Abattoir, Abisim and Central markets.

Antibiotic	LOC (µg/g)	RQ (Abattoir Market)	RQ (Abisim Market)	RQ (Central Market)	ADI µg/kg	RMC kg/person/day
Ractopamine	19.231	0.9079	0.8221	0.9309	1.25	0.065

CHAPTER FIVE

5.0 DISCUSSION

5.1 The knowledge and practices of antibiotic usage by ruminant livestock farmers and veterinary officers in Sunyani Municipality

5.1.1 Sex and educational level of farmers

The socio-demographic assessment of the respondent farmers revealed that, the male gender constituted the highest population engaged in ruminant livestock production as compared to females. In a work to determine antimicrobial usage in livestock management in north- Eastern Nigeria, it was revealed that the majority of the respondents were males constituting 54% of the farmers while the female farmers were 46% (Mamza, 2017) which is similar to the finding in this work. In Ghana,Food and Agriculture O rganization (FAO), (2012) reported that majority (80%) of household heads were males. This probably explains why there is male dominance of ownership of ruminant livestock in the Sunyani Municipality than females.

The educational level indicates that 60% of ruminant livestock farmers had formal education (Table 2) ranging from primary to tertiary education. It is expected that, this level of education should therefore help them comprehend and make informed decisions on the choice, dispensation, dosage and withdrawal periods regarding the usage of antibiotics than their counterparts with non-formal eduction.Yasin *et al*. (2019) reported a relationship between educationallevel and antibiotic knowledge level of participants in a survey conducted in Bingol Provence in eastern Turkey to determine the knowledge, attitudes, and behaviors of farmers working in the livestock sector on antibiotics and antimicrobial resistance. The report concluded that, antibiotic knowledge level of the participants with a postgraduate degree was found to be significantly higher than high school and primary school graduates or uneducated ($p < 0.001$). It also saidparticipants with associate degrees and/or bachelor degrees had significantly higher antibiotic knowledge when compared to those without education ($p < 0.001$).

5.1.2 Ruminant breeds, years of rearing experience and husbandry practices

From this study, the farmers kept either single or combined species of ruminant animals. The factors which influenced the type of ruminants kept included financial capability of the farmer, religion, tenancy status, management experience, availability of rearing space owned by the farmer, the easy marketability of the animal, the selling price of the animal and the animals' ability to resist disease attack among many other factors which in relative terms are cheaper for sheep and goat than for cattle.

From the data gathered, majority of the farmers in the Sunyani Municipality considered keeping more of sheep and goats perhaps as a result of the lucrativeness and unique biological attributes such as shorter conception period, higher productiveness, high feed conversion efficiency and faster growth rate as compared to cattle. This fact is supported by Nwanta *et al*. (2011) who reported that 84% of farmers in Enugu State, South eastern Nigeria, engaged in livestock production especially goats and sheep due to the profitability, procreative potentials and shorter gestation period of the animals.

The marketability of goats, sheep and cattle without religious and cultural restrictions gave way for the rearing of these livestock or consumption of their meat and other products within the municipality guaranteeing farmers' confidence of the sustainability of their farming enterprise. Apart from these considerations Devendra and Chantalakhana (2002) reports the invaluable contribution this farming enterprise in alleviating economic vulnerability including ensuring food security, employment, improving social and cultural relationships and poverty reduction as the reason farmers got involved in ruminant keeping.Otte. (2004) also confirmed the aforementioned facts in the promotion of sustainable livelihood in arid regions of sub-Sahara Africa, such as northern Ghana. Also improvement in soil fertility, transport and the world's food supply in general are highlighted (Kitalyi *et al*., 2005; Randolph *et al*., 2007).

The rearing of cattle was largely hindered due to non-availability of land and therefore few farmers kept cattle alongside sheep and goats especially those who lived in the outskirts and in the 'Zongos' of the study area. This finding agrees with the report of Olafadehan and Adewumi (2010) who found that, the lack of grazing land in urban centers was a major constraint to the rearing of large ruminants such as cattle.

The investigation also established that management experience in the matter of livestock keeping was a vital factor which drives the farming business. The farmers/ respondents interviewed had various years of livestock management experiences ranging from one to more than ten years. Olurotimi (2014) reported a similar finding that the majority of livestock farmers from Oyo metropolis in Nigeria had management experience in livestock production of more than five years. It seems the economic and social hardships being experienced in the country over the last decade have informed urban dwellers to engage in ruminant production either to boost their animal protein source intake or increase their household income. Oluwatayo and Oluwatayo (2012) opined that the keeping of ruminants has contributed to improving the nutritional and protein requirements of both rural and urban households.

Aboagye *et al*. (2014) found that farmers adopted the semi-intensive or extensive systems of keeping animals and mostly kept more than one animal species under small farm size holdings. Also Turkson and Naandam (2006) confirmed the predominance of these two management systems practiced by ruminant farmers in northern Ghana. Similarly, this work found the adoption of these husbandry systems in the Sunyani Municipality than the intensive husbandry system. In most cases, it was observed that sheep, goats and a few cattle were usually left freely and at the mercy of the weather in search for food on their own and supplemented in the evening on their return to their housing with cut-and-carry forage, household food waste, crop residues and crop by-products consolidating a similar finding by Duku *et al.* (2010). The intensive husbandry system was limited in the study area. Out of the one hundred and fifty respondent population, only four farmers practiced the intensive system. This finding is quite better than the report of Umunna *et al*. (2014) who surveyed one hundred ruminant farmers in three Local Government Areas of Ilorin metropolis, Nigeria and found that no farmer practiced the intensive management system.

This work also revealed that, farmers who kept cattle also kept sheep, goats or both in addition. In many farms, animals were kept together in one kraal under unhygienic conditions especially those who kept all types of ruminants under one confinement. This condition predisposed animals to diseases which will demand constant prophylactic practices to minimize the spread of diseases (Flowers *et al*., 2013; Bow, 2013) and also reduce the effect of zoonosis. There were evidences of bottles of veterinary drugs and these ranged from antibiotics, fungicides, anthelmintics, multivitamins and many more establishing the fact that these drugs are used for disease prevention (Flowers *et al*., 2013; Callens *et al*., 2012). In such farms, it was noticed that farmers self-treated their livestock with the excuse that, it was difficult meeting the financial demands of veterinary doctors when their services are sought. Boamah *et al*. (2016) made a startling finding that, farmers who kept more than one category of animal species in a single confinement did not count it as an obligation to seek veterinarian intervention regarding treatment of their animals and mostly resorted to use of disinfectants, fumigants and antibiotics without prescription as compared to farmers who kept only one type of animal species. It is quite clear that under such practices, farmers are likely to violate the drugs labeled instructions which could pose deleterious effects on animals' wellbeing in the matter of drug residue accumulation in the tissues of edible products.

5.1.3 Biosecurity measures adopted and diseases observed by farmers

Housing of animals was mostly poorly done with insecure biosecurity measures. It was noticed that, the majority of the farmers utilized poor wood and corrugated iron sheets as fencing without protective sheds, poor vaccination, no fumigation, no use of protective clothing whiles on duty, and no quarantining incoming animals before they are released into their mainstream flock was also not practiced among the farmers. Even in the very few intensively kept housing units, such conditions of poor biosecurity existed. These indiscriminate bad practices predispose the animals to diseases which could otherwise have been prevented. It has been reported that, animal death rate is high owing to poor housing, congestion, insufficient supply of veterinary medicines and poor ventilation, allowing the incursion of ailments and parasites such as pneumonia and diarrhea, particularly during the rainy seasons (Turkson *et al*., 2004).

One startling observation which was made was that, some farmers had their pens sited right in the middle of their houses. They virtually lived with the animals in the same compound with its attendant unhygienic conditions. These adversities impose high disease impacts in food animal production which reduces productivity and lucrativeness of the farming enterprise and poses a key hazard to public health as the transmission of zoonotic pathogens in the animals to humans through the food chain could be predominant as established by Alexander *et al*. (2008) and Martins da Costa *et al*. (2013). An attempt to salvage the situation demands the subtherapeutic administration of antibiotics to animals as a measure to curb the onset of diseases which comes along with its attendant consequences or the treatment of clinically infested animals which are already under disease attack.

Greater proportion of ruminant livestockfarmers (99.3%) in the Municipality confirmed the intermittent attack and sometimes invasion of their farms by various kinds of disease conditions. Although about 63.3% of the farmers gave indication that they were able to identify diseases which infected their animals upon reliance on their own experience, the finding of this work discovered that the majority of farmers possessed inadequate knowledge on the etiology of animal diseases and therefore depended on the disease clinical signs for the identification. It was observed that most of the farmers lacked specific protocols for determining the infections of individual ruminants and whether there was any need for antibiotics. Therefore, the methods of identification used often offer no definitive opportunity for proper diagnosis and curative treatments of such infections. In such situations antibiotic therapy in the farms could fail due to wrong diagnosis. Antibiotics administered based on these lines of action do not achieve the desired result and potentially leads to residue accumulation and selection of bacterial resistance (Hughes and Heritage, 2001). A critical consideration of the situation affirmed the prevalence of diarrhea, nasal discharge, profuse salivation, lameness, mastitis, coughing and skin infections in the study area and these formed the basis for which antibiotic medications were dispensed as a targeted response to curtail these diseases. From the observations made, adopting prudent farm management practices such as improved hygienic practices, regular vaccinations, effective nutrition and implementation of vigorous biosecurity practices could be a potent and cost-effective alternative to dependence on prophylactic antibiotic application in controlling such diseases in ruminant animals (Martins da Costa *et al*., 2013).

Disease diagnosis is an important index in finding a cure to animal maladies. For instance, a precedent work concluded that bacterial toxins in the gastrointestinal track can cause diarrhea (Talaro and Talaro, 2002) and therefore using antibiotics for therapy of such conditions could cause bactericidal activity on the bacteria but not the toxins and in this case combining antibiotic treatments with toxin binders could potentially ameliorate the diarrhea. In the same way, not all coughs may be bacteria related and using antibiotics in such diagnoses implies misuse which may consummate in selection of antibiotic resistant strains. Furthermore, rashes could as well be of fungal or bacterial origin contracted by means of scratches on objects, bruises due to fight and transportation or ectoparasite settlement which influences itches on the body of ruminants forcing them to find relief by scratching themselves against all manner of objects thus damaging the skin and issuing infections of different etiologies (Talaro and Talaro, 2002). In this case proper diagnosis needs to be carried out before treatment is effected in order not to misapply antibiotics. Mention must be made that 47 (80.0%) respondents out of the 54 farmers who could not identify the type of infections on their animals using their own experience did not circumvent veterinary consultation and this is highly commendable, it must be encouraged and reinforced by legislation in order to guarantee the welfare of livestock.

5.2 Assessment of antibiotic usage in ruminant livestock

5.2.1 Veterinary consultation for prescription

The finding of this research established that majority (78.7%) of farmers consulted veterinary officers for prescription and recommendation for the therapy of their animals. The veterinary drugs recommended mainly included antibiotics, anthelmintics, multivitamins, fungicides and antivirals. It was also found that almost all the farmers interviewed used antibiotics on their animals either in co-administration with other drugs as stated or as a single dose dispensation. The rest of the (21.3%) farmers who did not depend on veterinary prescriptions relied on their experienced colleague farmers and drug vendors on how to apply the antibiotics they secured. Some also conceded mixing antibiotic treatments with ethno veterinary herbals to treat diarrhea and worm infestations. Kamini *et al.* (2016) re-counted an analogous outcome in poultry where majority (75.5%) of farmers in Cameroon depended on veterinary Doctor's recommendations for antibiotic use while a minority (24.5%) administered antibiotics without prescription.

5.2.2 Assessment purpose of antibiotic use and clinical signs

Beyene and Tesega (2014) found that, the relevance of antibiotics in veterinary medical practice as applied in therapeutics, prophylactics and growth promoters was far reaching and has become a subject of prudent and imprudent use. However, no ruminant farmer livestockin the Sunyani Municipality applied antibiotics for growth promotion purposes, and this discovery offers a relief due to the associated consequences of drug resistance and residue build-up in animal products which could be passed on to human food chain (Chee-Sanford, 2008). The findings in this work revealed that, majority 68% of the respondents used antibiotics mainly for chemotherapy to treat clinically sick ruminants while 31.3% agreed using the drugs on fit animals for prophylactics. No farmer in the Municipality admitted using antibiotics for growth enhancing purposes. This discovery concurs with a work conducted in Kampala, Uganda which could not verify the usage of antimicrobials for growth enhancement purposes, but reported the confirmation of therapeutic and prophylactic administration of antimicrobials by mostly 58 out of 60 farmers (Sasanya *et al*., 2005). It has however, been established that, the practice of prophylactics trigger antibiotic resistance and therefore, regulatory efforts should target discouraging farmers' reliance on such practices due to its reliance on subtherapeutic prescriptions to achieve such intended purpose (WHO, 2011). The clinical signs of the disease which necessitated the medication of antibiotics included mastitis, diarrhea, nasal discharge, coughing, lameness, profuse salivation among others according to the findings in the study area.

5.2.3 Type of antibiotics used by farmers

It was also discovered that tetracycline was the commonest antibiotic mostly patronized by the farmers followed by gentamycin, amoxycilin, penicillin, sulfamethoxazole, tylosin and chloramphenicol but no use was made of ceftriaxone and ciprofloxacin for clinical therapy of most diseases (Table 14). In a survey on antimicrobial resistance surveillance in feedlot cattle, tetracyclines were found to be one of the most common antimicrobial drugs used (Katharine, 2011). They are considered as benign drugs and have several positive properties such as wide range of action, low injuriousness, less cost and possess in most cases stress-free dispensation (Jeong *et al*., 2010). Alo and Ojo (2007) reported a high application of tetracycline, gentamycin, quinolones, neomycin, tylosin and streptomycin in poultry farms in Ekiti State, Nigeria. Similarly, the use of tyosin, colistin, oxytetracycline and enrofloxacin has also been proven in Khartoum, Sudan (Sirdar *et al*., 2012). These reports do not conflict with the observations in the Sunyani Municipality and also gives credence to the fact that these antibiotics and several others have been broadly employed in curtailing infections of bacteria origin in Ghana and other parts of the world as a whole in human and veterinary medical practice.

The indiscriminate investment and misuse of these antibiotics creat a problem of food security and safety issues especially on public health due to the potentiality of cross-contamination and cross resistance placing a limitation in their wider use on farms (Page and Gautier, 2012).It must be noted that in Ghana and elsewhere in the world, there are reports of treatment encumbrances and infectiveness in persons or farmers infested by *Salmonella, Staphylococcus* and *Chlamydia* based diseases contracted by means of cross contamination with resistant bacteria from ruminants and pigs (Chang *et al*., 2014;Emerson *et al*., 2001). It has been reported that, majority (61%) of the multi-drug resistance coagulase-negative *Staphylococcus* (CoNS) isolates was extracted from humans including farm workers, owners and managers of farms, whereas the bedding material had 39% of the multi-drug resistance isolates. These were attributed to the fact that farm workers were more unprotected from multi-drug resistant *Staphylococcus* strains owing to their direct contact or interactions with animals (Boamah *et al*., 2017).

5.2.4 Antibiotic choice factors

Various reasons were assigned by respondents as to why they preferred using these veterinary drugs. These reasons include the efficacy of the antibiotics in the control of diseases, the easy administration and handling of the drug, readily accessibility and affordability in terms of the cost of the antibiotics, while others cited colleagues' recommendations. These were the main verifiable determinants which influenced the patronage and use of antibiotics in the Sunyani Municipality. The effectiveness of the drugs had an immense influence on their use and application by livestock farmers in the Municipality than the other factors. The necessity for all countries to preserve the effectiveness of vital antibiotics has been reported, in specific case, those that are of outmost importance in animal husbandry due to the global surge in antimicrobial resistance (WHO, 2010; WHO, 2014; Tomson and Vlad, 2014).

5.2.5 Knowledge on antibiotics and accessibility

In the assessment of farmers' knowledge regarding antibiotics and their application, it was established that almost all the farmers were ignorant about the pharmacology of antibiotics except for their clinical usage. They knew nothing regarding the pharmacodynamics and pharmacokinetics of these drugs which govern the design of treatment regimens veterinarians use as a guide for drug administration. This critical finding is in conformity with a research result conducted in Ashanti Region of Ghana, which established that pig farmers had very little concerning antibiotic pharmacology apart from their awareness on clinical application of antibiotics (Sekyere, 2014). During the interview session of this study, majority offarmers conceded not possessing any knowledge about the antibiotics they used whiles some intimated that they had considerable knowledge about the drugs. However, the observation and conclusion drawn through interactions with those farmers who had knowledge about the antibiotics was that, they rather knew about how to apply the antibiotics and not about the drugs' pharmacology which takes into account disease etiology before the drug's administration.

Due to the lack of adequate knowledge by the majority of the farmers on antibiotics, theycould hardlydistinguish between different antibiotics with similar or same bioactive ingredients and because they often administered them without veterinary advice, consequently treatment failure arising out of treatment errors coupled with unimproved animal's conditions occurs. It was however discovered that, some of the farmers normally made effort to follow the products regimental instructions than those with non-formal education. Continual misapplication of antibiotics in the manner as stated above could lead to residue accumulation and introduction of resistant strains in animals. According to the responses in this work, 76% of the farmers lacked knowledge about the promotion of the development of antibiotic resistant pathogens through the misuse and inappropriate dispensation of antibiotics in animals and its zoonotic ability via the food chain (Eagar *et al*., 2012).

Ruminant farmers in the study area did not have challenges as regards to sourcing information on antibiotics or access to antibiotics. Enquiries confirmed farmers were introduced to antibiotic drugs by veterinary staff, friends, extension officers and NGOs. Some of the farmers obtained their knowledge from other sources such as drug vendors. It was found that majority of farmers purchased antibiotics from pharmacy shops/clinics which were mostly visited, others bought the drugs from the open market from animal drug vendors while some secured the drugs from other sources including friends and family members. Eight farmers (5.3%) declined answering the question. Oluwasile *et al.* (2016) reported a similar outcome from Nigeria among poultry farmers. They reported that, 91.4% of poultry farmer's acquired their antibiotics from pharmacy shops and 8.6% bought them from vendors or drug hawkers.

5.2.6 Frequency of antibiotic treatment, who administers the drug and other relevant information

On the issue of frequency of drug dispensation, it was discovered thatfarmers dispensed drugs to animals at varied periods. For instance, the study revealed that 52.0% of farmers administered antibiotic treatments to animals whenever they are clinically sick, whiles others sited different range of times. A critical apprehension of the frequency of antibiotic administration in the Municipality revealed that there is no properly regulated mechanism guarding the medication of livestock thus giving way for their discretional use by farmers.

A work which was undertaken in Sudan on the use of antibiotics cited the absence of a clear and active role of authorities to govern and regulate the use of these hazardous substances and also lamented over the absence of clear protocols to treat food animals. It concluded that this deficit opens the door broadly for the development of antibiotic resistance and various hazards to be prevalent (Eltayb *et al*., 2012) endorsing the situation in Sunyani Municipality.

The consequences of contaminants of antibiotics in foods of animal origin can be daring to public health and therefore any opportunity afforded to analyze and seek redress must be handled with the attention it deserves. Judging from the periods of drug administration as indicated in the results, one can clearly see that, there is less possibility for those animals that receive antibiotic medication every 4-6 months or at the beginning of every season to accumulate and develop resistance as compared to those who receive it every 1-3months. The aspect which presents a very precarious and undetermined mode of tracking is those who do administer antibiotics whenever livestockis clinically ill. Since this work lacked the capacity at the time of collation of data to determine how frequent the animals become sick, there was considerable constraint in coming to a definitive conclusion regarding the contribution of this to residue buildup and resistance. The probable explanation is that drug administration under this is determined by how frequent livestock become clinically ill. Invariably the more frequent the animals become sick, the more antibiotic drugs are dispensed and vice versa. This presupposes that farmers, who administer antibiotics to their animals anytime a sign of indisposition is detected, do so with no regular pattern of antibiotic administration. This is more likely to predispose animals to develop resistance and build-up residues if antibiotics are frequently dispensed without proper regimental procedures. This practice constitutes an abuse or imprudent use of antibiotics. Research works confirm that irresponsible use of antibiotics in food animals expedite occurrence and spread of antimicrobial-resistant organisms (Eagar *et al*., 2012; Donkor *et al*., 2012; Marshall and Levy, 2011) and also facilitates the accumulation of antimicrobial drug residues in edible animal tissues (Landers *et al*., 2012).

Many farmers in the study were implicated in some violations regarding antibiotic administration in animals although the majority of them averred that they sought veterinary services anytime they noticed a sign of infection. As many as 38% of respondents did admit self-medicating their animals instead of veterinary doctors whenever infections arise and sometimes also they depended on their fellow experienced farmers for such activities. This finding is in tandem with work conducted in Sudan which revealed that self-medication was very common among farmers due to the acquisition of drugs sometimes without authorized prescriptions (Eltayb *et al*, 2012). It is also in conformity with studies conducted in Ogun, southwest Nigeria (Oluwasile *et al*., (2014) which reported that 50% of poultry farmers in their study medicated their livestock with antibiotics based on the prescription of veterinary doctor's while 43% depended on self-medication, and in Ghana (Boamah *et al*., 2016) also found a respondent population of 80% of poultry farmers following veterinary Doctor's recommendations for antibiotic administration.

In this work, the awareness of the farmers about withdrawal period and dosages was found to be very low (Table 8). In this case, taking self-decision to medicate afected animals without recourse to trained practitioners could lead to improper use of antibiotics and non-adherence to antibiotic withdrawal periods, which could culminate into the buildup of high concentration of drug remains in animal products (Guetiya *et al*., 2016). It could also pave way for bacteria resistant gene spread and potential dominance (Kirbis and Krizman, 2015). The non-compliance to label directions including, failure to administer the right dosage, use of improper measuring equipment to take the right dosages are reported as introducing errors in antibiotic dispensation in livestock farming in northern Ghana (Addah *et al*., 2009) thus increasing residue deposition in animal products.

The outcome in this work also disclosed that a total of (53.3%) respondents had no idea about withdrawal times. Owing to the inadequate sensitization by the requisite organizations, the farmers lacked the requisite knowledge and turned out to be ignorant transmitters of residues and virulent bacterial strains to the public through the food supply chain by imprudent handling practices involving antibiotics. Again majority of thefarmers had no idea that administration of antibiotics close to slaughter time and not adhering to withdrawal periods could result in accumulation of residues in edible tissues. The farmers revealed that, government lacks vigilance and desire required to safeguard food quality control which is a bench mark to guarantee public health (WHO, 1998).

In terms of adherence to the manufacturers labeled protocol, 64.7% showed preference in the observance of safety and dosage instructions for the antibiotics; while 31.3% did indicate that, they could not follow the labeled rules mostly due to ignorance. This result shows a perturbing trend where non-observance of manufacturer's labeled guidelines recorded in this study is attributable to the lack of sensitization/education by the requisite organizations. This finding concurs with a work which concluded that, farmers who received less/no education or sensitization from trained veterinary professionals face constraints in assimilating and utilizing scientific knowledge and skills required to increase agricultural productivity (Epeju, 2010). Moreover, there are inadequate efforts deliberately initiated in Ghana, either by the government on non-governmental organizations, to instruct livestock farmers on the need for proper utilization of antimicrobials in food-producing animals. This far reaching conclusion was confirmed by 79.3% of the farmers who agreed without mincing words that periodic sensitization/education regarding the administration of antibiotics in livestock production conducted for them by government or other organizationsis inadequate. An observation made during this study was that, ruminantlivestock farmers in the Municipality have not formed associations to fight for a common goal in the matter of boosting their business.

5.2.7 Assessment of knowledge of farmers about misuse of antibiotics and emergence of resistance and health consequences in livestock farmers

Respondent's knowledge concerning the consequences of not following proper antibiotic treatment regimen was also assessed. The majority of the livestock farmers interviewed conceded being aware that improper administration of antibiotics in livestock had the potential to worsen the animals' health condition while (38.7%) had no idea. In addition, many farmers were ignorant that the misuse of antibiotics could promote the development of antibiotic resistant pathogens and its zoonotic ability (animal to human disease transfer) via the food chain. This finding is supported by Ojo *et al*. (2016) who indicated that indiscriminate application of antibiotics in food-animals imposes an undue pressure on the gut microflora and favours the persistence of antibiotic-resistant bacteria for onward spread to individuals through the foodstuff chain. In the same vain Adesokan *et al*. (2014) bemoaned that, there were reports of increasing emergence and extent of resistant strains of bacterial pathogens due to haphazard usage of antibiotics in food animals, which pose a severe encounter to both animal and human fitness in Nigeria.

From the findings 72% of respondent had no idea about the contribution of the non-adherence to withdrawal periods or the administration of antibiotics close to slaughter time and its resultant effect on accumulation of residues in edible tissues whereas 25.3% were informed in the matter. It has been established that the non-compliance of farmers to abide by recommended withdrawal times, result in the recurrent determination of antibiotic residues in animal and likewise contribute to the increasing resistant strains of pathogenic bacterial agents aside causing irritation, hypersensitivity and resistance in humans (Annan-Prah, 2012; Darwish, 2013;Hakem, 2013).

Finally, efforts were made to find out farmers' awareness regarding the consequences of consumption of residual antibiotics in edible animal tissues and its associated health challenges it can exact on humans. To this, (60.0%) farmers responded in the affirmative, (36.7%) showed indications of non-awareness while (3.3%) provided no answer. Resistant bacteria and antibiotic residues contaminate livestock and farm animal products during slaughter and processing, and this is consequently transmitted to humans who consume these food products. Common examples of such potentially food-poisoning organisms are *Salmonella*, *Campylobacter* and *E. coli*. Findings from research on antibiotic resistant strains give conclusive evidence that the food chain is the main mode of transmission. Contamination of meat generally results from faecal material getting onto the carcasses during the slaughter and evisceration process during which time gut of animals are removed. Infected meat can also contaminate other foods in domestic, restaurant or catering kitchens by contact. The European Food Safety Authority (EFSA) concluded in 2010 that live chickens colonized with *Campylobacter* are 30 times more likely to result in polluted meat than are uninfected birds (European Food Safety Authority, 2010).

The associated hazards incurred by the populace due to the imprudent use of antibiotics only reflects a compromised food safety and security system and as a result the need for this survey as part of a concerted measure to finding solutions to this lasting misfortune.

5.3 Common bacterial infections encountered by Veterinary Officers

Diseases are infirmities that stall the health of livestock, diminish animal performance and undermine the safety, quality and security of food derived from animal. More dangerously is its zoonotic potential, infecting humans with communicable diseases. Veterinarians contacted revealed that, the common bacterial infections they encounter in the field include *Mycoplasmos*, *Collibacillosis*, *Mycoplasma syreviaviae,* and *Staphylococcus intemedius* based infections.

They indicated that Mycoplasmas pay secondary roles in infections most often exacerbating pre-existing diseases, but it has been shown that *Mycoplasma bovis* (*M. bovis*) can play a fundamental role. Practical field work identifies *M. bovis* as one lethal pathogenic species and is the most frequent *Mycoplasma* pathogen of mastitis, arthritis and pneumonia in cattle (Ruffin, 2001). *Mycoplasma* has complex mechanisms of antigenic variation that allow them to evade the immune system (Ruffin, 2001). It is also recognized as the cause of bovine respiratory disease, arthritis and other enteric disorders. Diseases associated with mycoplasmas are found in the respiratory and urogenital tracts, mammary glands, joints and eyes. Some of these species of mycoplasma are highly pathogenic, causing diseases of major importance, namely, contagious bovine pleuropneumonia, contagious caprine pleuropneumonia and contagious agalactia (Gourlay, 1981). Regarding the treatment of these infections, veterinary doctors used tylosin and oxytetracycline injections.

Staphylococcus intemedius is an inhabitant of the canine hair cover and often isolated bacteria and systematic treatment with ampicillin, amoxicillin, tetracycline dihydrostreptomycin and sulphonamides are ineffective. Commonly recommended antibiotics for treatment based on susceptibility results and site of infection (Atalay *et al*., 2005) include topical antibiotics (polymyxin, neomycin, ofloxacin), of penicillin, amoxicillin-clavulanic acid, vancomycin (Kelesidis and Tsiodras, 2010).

Collibacillosis is an infection of *Escherichia coli* (*E. coli*) and is a key source of mortality among young calves. *E. coli* is one of the common resident microbiota of the intestinal tract of calves, and certain strains of this organism that are pathogenic. The pathogenic strains of *E. coli* are the cause of severe diarrhoea, fatigue, dehydration, fever, malaise and depression that results in economic losses in both beef and calf production (Hirsh *et al*., 2004; Radostits *et al*., 2007).

5.3.1 Veterinary officers' knowledge on dosage and withdrawal periods

The veterinary officers noted that there are a number of therapeutic medications that deals with infections in food producing animals. Mostly infections such as metritis, peritonitis, diarrhea and other enteric infections are the popular conditions they normally encounter. They added that they normally follow the recommended manufacturers' dosage except in rare cases where they need to go beyond and administer higher/lower doses and in some instances co-administer with other antibiotics to achieve desired results depending on the type and extent of infection. They were fully aware that improper dosages/discontinued course of treatment with antibiotics results in the presence of high level of residues of drugs used in animal treatment origin in the finished products. Literature confirms that in selecting drug combinations, their respective dosage regimen and drug interaction should be considered in view of the pathophysiological status of the animal (Rahal *et al*., 2013). Table 10 gives evidence that veterinary offers duly adhered to the recommended dosages and withdrawal licensed veterinary including topical ointments, ophthalmic and aural preparations, intra-uterine pessaries and tablets. They complained that many farmers violate their instructions that accounted for some level of prevalence of antibiotic residues in livestock products in the locality which included failure to observe drugs with-holding periods, poor records of treatment, no attempt to detect treated animals and extra-label application of antibiotics. Similar findings have been long-established (Akinynka *et al*., 2012). Brahma *et al*. (2012) gave a report that inappropriate drug use are characterized by over-prescription (prescribing drugs when non are required clinically), the employment of inappropriate dosage (too low or too high), omission (when required drugs for clinical indications are not prescribed), unnecessary risk (use of injection or intravenous antibiotic when oral preparations would be suitable), incorrect duration (too short or too long) and inappropriate selection (misalliance) of drugs and organisms.

5.3.2 Farmer sensitization, antibiotic residue monitoring and other relevant information

Antibiotic residues inflict public health with daring consequences as agreed by the veterinary officers interviewed. They noted that, they do their best even within the logistical constrains they are saddled with to sensitize farmers though not regular basis on the residual effects of wrongful dispensation of antibiotics and admonish them not to engage in overdosing, but adhere to withdrawal times. Although they admitted the presence of community livestock workers in their areas of jurisdiction, they do not offer any training to them since they are not licensed to undertake veterinary functions. They also stated the lack of equipment and tracking system for identifying antibiotic residues in the Municipality and Ghana as a whole is a worrying situation that should be tackled by authorities. Many farmers in the study area only request for veterinary services when their animals are not responding to their self-treatments.

5.4 Determination of Antibiotic Residues in Chevon and Beef

Thirty-six (36) samples of beef and chevon, from three different markets (Abbatoir, Abisim and Central Market) in the Sunyani Municipality of Ghana were analysed for antibiotic drug residues including amoxycilin trihydrate, danofloxacin, sulfadiazine, norfloxacin, oxytetracycline, tylosin, ciprofloxacin, chlortetracycline hydrochloride, doxycycline and ractopamine. The concentration of the several residues in each sample was calculated in μg/kg sample. The mean concentration ranks of contaminants were documented. In the present study, chloramphenicol and metronidazole were not detected in all sample types from the three markets. diminazen aceturate was present in a single chevon sample with a residual concentration of 2.95 μg/kg. It was found that residue levels of all antibiotics under study were below the recommended MRLs except in ractopamine.They are thus not likely to cause any health threat to consumers. As a result, this work did not assess any risk of consumption except for ractopamine. Darko*et al*. (2017) gave a similar report during the analysis of residues of chloramphenicol, sulfathiazole, sulfamethoxazole and oxytetracycline in dairy products in the Kumasi metropolis of Ghana.

5.4.1Amoxycilin trihydrate residue in beef and chevon

Amoxycilin trihydrate remains were detected in chevon and beef samples from the three (3) markets. Amoxycilin registered the highest residue concentration of all the residues detected in samples obtained from the three markets. A study on antibiotic drug residues in livestock from Ghana showed high concentration of amoxycilin (30 μg/kg) (Osei-Asare and Eghan, 2009). This observation supports the findings of the current study, which indicated highest mean concentration of amoxycilin among the antibiotic drugs investigated in the chevon samples from the markets. The average residues detected in chevon were 21.60 μg/kg, 21.90 μg/kg and 22.57 μg/kg for Abattoir, Abisim market and Central market, respectively. Concentration of amoxycilin in chevon was highest in samples taken from the Central market (22.57μg/kg) as compared to that of Abisim and Abattoir in numerical terms. The residue levels of amoxycilin in both chevon and beef were however, lower than the recommended joint FAO/WHO expert committee on food and additives (JECFA) maximum residue limits (MRLs) of 50 µg/kg given by Codex Alimentarious Commission (Commission, 2012). Amoxycilin in beef samples from the Abattoir (17.16 µg/kg) registered the lowest level of detection when compared to samples mean value from Central market (17.58 µg/kg) and Abisim (19.27 µg/kg) (Table 13). Although, all samples tested positive for amoxycilin, the mean residue levels in both chevon and beef from the three markets were not significantly different (P>0.05). These residual concentrations were lower than the mean levels reported by Shafaqat*et al*.(2014). In their study to estimate amoxicillin residues in commercial meat and milk samples in Pakistan, they detected greater levels of amoxycilin residues 46 µg/kg, 84 µg/kg, 21µg/kg, 9 µg/kg and 80 µg/kg in beef samples 1, 4, 6 8 and 10, respectively. The detection of amoxycilin residues in samples as found in Central, Abisim markets and Abattoire may possibly be the consequence of the frequent usage of veterinary drugs as regularly practiced among livestock producers and marketers (Olufemi and Agboola, 2010).

5.4.2 Chlorotetracycline residue in beef and chevon

The three (3) market locations in the Sunyani Municipality gave the mean concentration of chlortetracycline residues from the beef and chevon samples analysed (Table 13 and 14) respectively. For beef samples, the Central market had the highest mean residue level of 5.98 µg/kg followed by Abattoir (5.96 µg/kg) and Abisim (5.93 µg/kg). The differences in mean residue levels were however not significant (P > 0.05) for the three locations for beef. All the values obtained for both beef and chevon were less thanthe recommended maximum residue limit (MRLs) by JECFA for chlortetracycline for beef and chevon which is 200µg/kg (Codex Alimentarious Commission, 2012). Mean variations in chlortetracycline concentrations were found in chevon samples obtained from the three locations. The level of residue recorded in samples from the Central market was 7.54 µg/kg and was significantly different (P > 0.05) from the mean concentrations in samples from the Abattoir (6.03 µg/kg) and Abisim market (6.27 µg/kg). Olusola *et al*. (2012) determined the levels of chlortetracycline in frozen meat sourced from major markets in Lagos and Ibadan. The mean concentrations of chlortetracycline residue levels in the samples spanned from 1.1589 mg/kg-1.0463 mg/kg which was higher than the residue concentration recorded in the present study. Olusola *et al*. (2012) found no significant differences (P > 0.05) in levels of chlortetracycline from the two markets as observed in beef samples from the three markets in this study.

5.4.3 Ciprofloxacin residue in beef and chevon

Ciprofloxacin residue was detected in all samples from the three meat purchasing locations. The mean residue levels of ciprofloxacin in beef were 16.57 µg/kg, 15.18 µg/kg and 15.31µg/kg for Abattoir, Abisim market and Central market, respectively. Abattoirs registered the highest mean concentration (16.57 µg/kg) which was found by analysis to be significantly different (P < 0.05) from residue levels of ciprofloxacin detected in beef samples from Abisim and Central markets. There was no significant difference (P > 0.05) between the latter two markets in beef residue levels. The concentration of ciprofloxacin residue in chevon was highest in samples obtained from Abisim market (16.57 µg/kg) (table 14) followed by Abattoir (16.26 µg/kg) and then central market which had 15.72 µg/kg. The overall analytical residue values were lesser than the recommended JECFA (MRLs) of 100 µg/kg as quoted by Gouvêa *et al*. (2015). In a work to investigate the occurrence of antibiotic residues in chicken meat, Ramakant and Poornima (2014) reported a higher concentration of 64.59 µg/kg in chicken liver from Old Delhi in India while its muscle tissue contained 6.03 µg/kg of ciprofloxacin. Buket *et al.* (2013) randomly sampled 127 chicken and 104 beef meat samples from markets of Ankara (Turkey) and investigated the levels of quinolones using the ELISA technique. Out of 231 chicken and beef samples, 118 (51.1%) were positive for quinolone residues. Of the 127 chicken meat samples tested, 58 samples (45.7%) and 60 samples representing 57.7% of 104 beef meat samples tested positive for quinolones respectively. The mean concentrations (±SE) of quinolones were found to be 30.81 ± 0.45 µg/kg and 6.64 ± 1.11 µg/kg in chicken and beef samples, respectively. Like the present study, the reported concentrations in the two literatures referenced were also below the JECFA MRLs. High levels of ciprofloxacin above the adopted MRL for this drug was recorded in chevon (345.62 ± 796.35) µg/kg by Omotoso and Omojola, (2015) in Nigeria. Naeem *et al*. (2006) analysed poultry products in Pakistan and reported that 58-85% of the samples contained ciprofloxacin residues.

5.4.4. Danofloxacin residue in beef and chevon

There were no significant differences (P>0.05) in the mean concentrations of danofloxacin detected in samples from all the locations. The average mean chevon residues of danofloxacin were 5.72 µg/kg, 9.75 µg/kg, 9.77 µg/kg for Abattoir, Abisim market and Central market, respectively. The maximum residue limit for danofloxacin is 200 µg/kg according to JECFA which is evidently far above the average mean residues in both beef and chevon in all the three locations (Codex Alimentarious Commission, 2012). Numerically, chevon samples in the Central market registered mean concentration of 9.77 µg/kg, while Abattoir documented the lowest mean concentration of 9.72 µg/kg**.** For beef, samples from Abattoir recorded a higher value of 9.74 µg/kg while Abisim had the least value of 9.70 µg/kg. According to a report by the European Agency for the Evaluation of Medical Products (Veterinary Medicines Evaluation), (1997), a study in cattle given a 5-daily intramuscular injection of unlabeled danofloxacin at a dose rate of 1.25 µg/kg mg/kg bw, residues of danofloxacin in liver declined from 372±63 µg/kg to 13±3 µg/kg, 5 days after treatment. This reported outcome is above the finding in this present study.

5.4.5 Doxycycline residue in chevon and beef

Table 14 indicates the average concentration of doxycycline drug residues in the chevon and beef samples from Abattoir, Abisim market and the Central market. The Central market registered the highest mean concentration of 9.02 µg/kg for residues detected in beef samples which was highest nonetheless not significantly different (P>0.05) from Abattoir (8.57 µg/kg) and Abisim (8.61 µg/kg). Chevon samples from the same market (Central market) recorded the highest doxycycline residue concentration, followed by samples from the Abisim market (9.16 µg/kg) and Abattoir (8.77 µg/kg). Equally no significant differences (P>0.05) existed among residue levels of the chevon samples. All residue levels were below the JECFA tolerance limits of 100 µg/kgset by Codex Alimentarious Commission 2016). Cetinkaya *et al.* (2012) found doxycycline in four of the 60 samples in the span of 19.9 µg/kgto 35.6 µg/kg during an analysis of chicken meat in Bursa and Turkey for antibiotic class of tetracyclines using the LC-MS/MS method. These reported mean values that were higher than the values observed in this current work.

5.4.6 Norfloxacin residue in beef and chevon

Norfloxacin residue in chevon samples from the Central market registered significance levels (P < 0.05), the highest mean concentration was 14.85µg/kg (Table 14). The difference in norfloxacin mean concentration in chevon samples from Abattoir (13.47 µg/kg) and Abisim market (13.67 µg/kg) were not significant (P>0.05). Residues concentrations of both chevon and beef samples from the three purchasing points were established below the JECFA tolerance limits (MRLs) for norfloxacin (50 µg/kg) (Codex Alimentarious Commission, 2016). Norfloxacin residue was detected in all beef samples from the three locations. Samples from Abattoir recorded the highest mean concentration of 13.49 µg/kg. The differences in mean residue levels among the locations were however, not significantly different (P>0.05). In a work to examine the residue concentration of fluroquinolone in raw meat from open markets in Ibadan, Nigeria, Omotoso and Omojola (2015) found that, the mean residue levels of norfloxacin (173.40±154.73µg) were above the maximum residue limit of 100 µg/kg set by WHO (2011). Despite a swift rate of elimination, detectable residues of norfloxacin can have prolonged persistence in the body for as long as a week or even more after treatment, due to inhibition of one or more enzyme(s) concerned with metabolism of norfloxacin (Sally and Mona, 2017).

5.4.7 Oxytetracycline residue in beef and chevon

The determination indicates that Abisim market samples for beef recorded the highest average residue levels of 10.74 µg/kg, followed by the Central market (10.16 µg/kg). Abattoir recorded the least oxytetracycline residue level for the beef samples (8.36 µg/kg). From the total of 36 samples analysed during the study, all three locations contained measurable concentration of oxytetracycline residues from which none violates the levels of JECFA residue limits (200 µg/kg) (Codex Alimentarious Commission, 2015). The mean residues were 8.32 µg/kg, 8.93 µg/kg and 9.49 µg/kg for Abattoir, Abisim market and Central market, respectively for chevon. This outcome designates that consumers are not subject to health consequences and does not hinder international meat trade from Ghana. Residues of oxytetracycline values detected in both chevon and beef samples from the three locations remained insignificant (P>0.05) from each other. Oxytetracyclines are broadly applied in veterinary medical practice as feed additives for growth promotion and prophylactics in livestock production oweing to their wide range of efficacy against pathogenic organisms coupled with their low cost (Fritz and Zuo, 2007). Levels detected in this study were below the values record in meat samples in a work conducted by Olufemi and Agboola (2010) from Akure metropolitan (Nigeria) abattoir using HPLC for oxytetracycline contaminants. The mean residues for positive samples in their study were 51.8 µg/kg, 372.7µg/kg and 1197.7 µg/kg for muscle, kidney and liver respectively.

5.4.8 Ractopamine residue in beef and chevon

All the values registered for ractopamine residues in this work are greater than the suggested JECFA MRLs of 10 µg/kg (JECFA, 2006), which may predispose consumers to health consequences.Abattoir had the highest mean concentration of 17.67 µg/kg in beef samples for ractopamine residues which was found to be insignificant compared to figures of Abisim and Central market given as 17.45 µg/kg and 17.25 µg/kg respectively (Table 13). Ractopamine residue in chevon samples from the Abattoir, Abisim and Central markets had average concentrations of 17.46µg/kg, 15.8µg/kg and 17.90µg/kg, respectively. Central market recorded the greatest mean concentration (17.90 µg/kg) of ractopamin in the chevon samples though insignificant when compared to values of the other two purchasing points. The high level of residues of antibiotics in meat for human consumption at violative concentrations could be attributed to the haphazard and abusive use of these drugs as frequently practiced among livestock farmers and marketers without adhering to withdrawal periods prior to slaughter (Olatoye and Ehinmowo, 2010). The high prevalence (100%) of ractopamin in this study may be a signal of extensive usage of this antibiotic drug by food animal producers throughout the Sunyani Municipality, in so much as these samples were sourced from different parts of the city. This may perhaps be owing to the point that the cure of larger section of cattle and goats reared in Ghana are conducted by ruminant farmers or herdsmen who have unrestricted access to these veterinary drugs and always purchase them over the counter for administration to their animal without veterinary prescription and supervision. These inflict abundant menaces and dangers to human health that could bring about allergy and antimicrobial resistant effects on the consumers (Abavelim, 2014). It could also be due to the misuse of the drug pre-slaughter, coupled with non-adherence to safe withdrawal periods and has steered the build-up of antibiotic drug residues in most meats in developing countries (Khatun *et al*., 2018).

5.4.9 Sulfadiazine residue in beef and chevon

There were measureable levels of sulfadiazine residues in chevon and beef from samples from Abattoir, Abisim market and the Central market in the Sunyani Municipality. The mean concentrations of sulfadiazine were 1.27 μg/kg, 1.28 μg/kgand 1.30 μg/kg for chevon samples from Abattoir, Abisim market and Central market, respectively. Beef samples from the three (3) locations recorded same residue levels of 1.28 μg/kg for both Abattoir and Abisim market differing non-significantly ($P>0.05$) from Central market which recorded a figure of 1.27 μg/kg. No significant differences ($P>0.05$) existed among sulfadiazine concentrations detected between the locations in both chevon and beef. Mor *et al*. (2012) determined sulphonamide residues in beef by high performance liquid chromatography with florescent detector (HPLC-FLD). All samples in their study were positive for sulfadiazine. All values obtained from the three markets were lower than the recommended JECFA Maximum residue limits of 100 μg/kg (JECFA, 2000).

5.4.10 Tylosine residue in beef and chevon

The prevalence of the tylosin residues in beef and chevon samples from Abattoir, Abisim and the Central markets in the Sunyani Municipality was confirmed in this work (Tables 13 and 14). Abisim market registered the highest mean concentration of 17.53 µg/kg in the beef. The concentration detected was, however, not significantly ($P > 0.05$) from the levels detected in the beef samples from the Abattoir (17.32 µg/kg) and Central market (17.35 µg/kg). Mean concentration of tylosin detected in the chevon samples was highest in Central market (17.37 µg/kg) as compared to Abattoir (17.35 µg/kg) and Abisim market (17.33 µg/kg). Lower outcomes were acquired by Hyo-Ju *et al.* (2018) who determined the average value of tylosin in chicken meat at 0.07µg/kg in Korea. Birhan and Mulugojjam (2018) in a study to detect the incidence of antibiotic residues and its public health risk in beef in Ethiopia published a higher value of 50µg/kg. The results obtained in this study were less than the JECFA tolerance limit of 100 µg/kg set by Codex Alimentarious Commission (Commisson, 2012). It appears that the current standing of this antibiotic drug in beef on the market is not at risk of the public, but notwithstanding; the outcomes do not discard the possibility of misapplication of this drug in the impending and meaningful exposure of humans which may harmfully distress health. There is, therefore, the requirement to consistently monitor this chemical as a food quality control measure.

5.5The health Risk assessment associated with the consumption of beef and chevon in the Sunyani Municipality.

When antibiotics present in beef and chevon exceed the MRLs, there is a health menace related to the consumption of such meat (Kang'ethe *et al.*, 2005). Ractopamine residue in the beef and chevon sampled from the Abbatoir, Abisim and Central markets had average concentrations higher than the recommended JECFA MRLs quoted as 10 µg/kg (MacNeil *et al*., 2004) and highly likely to pose a health risk to consumers, therefore its risk factor was calculated and assessed.

It is worthy to note that the evaluation of the risk quotient (RQ) for ractopamine provided a convenient way of examining the danger related to the consumption of beef and chevon from the three markets to determine whether it exacts hazard to human consumers. For instances where RQ<1, the antibiotics are not likely to cause danger to human consumers (Commission of the European Communities, 2005). The RQ's for all the samples were below 1 (thus RQ<1) in beef and chevon sampled from the Abattoir, Abisim and Central markets. Thus, the consumption of these meats is unlikely to cause harm to its consumers.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The misuse of antibiotics is capable of generating residues in foods obtained from animals. Residues of the antibiotics under study were detected at varied levels. The ignorance of farmers about antibiotic residues and the emergence of resistance and spread make them disregard treatment regimen, and this consequently exposes farm animals and humans to all manner of health hazards. There is therefore, the need for stake-holder effort in educating farmers as regards the potential hazard posed by imprudent dispensation of antibiotics in farm animals, proper farm management and hygiene practices, veterinarian engagement in keeping animal health, the proper and rational administration of antibiotics by ruminant livestock farmers cannot be overemphasized.

6.2 Recommendations

- Farmers should ensure strict adherence to recommended withdrawal periods and drugs labeled instructions.
- Veterinary sensitization of farmers and other stakeholders about antibiotic usage and application should be enhanced.
- Alternative remedies that can replace the application of antibiotics in food producing animals should be explored.
- Antibiotic monitoring and testing programs ought to become a constituent of the quality control process in producing high quality meat products.

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APPENDICES

Appendix I: Antibiotic drugs residues (µg/kg) in Chevon samples from Central Market (CM), Abisim Market (AM) and Abattoir Market (AB)

Sample	[illegible]	Chlorotetracycline	Ciprofloxacin	Danofloxacin Mesylate	Doxycycline	Norfloxacin	Oxytetracycline	Ractopamine	Sulfadiazine	Tylosine
CMD1-CHEV	[illegible]	9.476	17.666	9.765	14.734	16.898	11.375	16.967	1.270	17.419
CMD2-CHEV	[illegible]	8.423	15.847	9.802	14.014	15.238	9.954	24.831	1.270	17.313
CMD3-CHEV	[illegible]	7.558	17.863	9.792	9.293	14.413	9.223	14.723	1.305	17.398
CMD4-CHEV	[illegible]	6.962	14.483	9.825	9.437	14.379	9.008	17.488	1.303	17.379
CMD5-CHEV	[illegible]	6.530	14.504	9.662	8.978	14.078	8.871	15.045	1.336	17.338
CMD6-CHEV	[illegible]	6.315	17.185	9.749	9.185	14.069	8.496	18.333	1.298	17.343
AMD1-CHEV	[illegible]	6.414	16.278	9.681	10.216	13.776	8.491	16.417	1.292	17.300
AMD2-CHEV	[illegible]	6.132	16.507	9.840	8.712	13.788	8.744	15.307	1.273	17.388
AMD3-CHEV	[illegible]	6.032	15.360	9.766	8.273	13.632	8.278	16.883	1.271	17.321
AMD4-CHEV	[illegible]	6.489	17.084	9.753	8.541	13.705	8.817	15.079	1.327	17.318
AMD5-CHEV	[illegible]	6.095	17.065	9.682	10.312	13.610	10.804	14.813	1.267	17.303
AMD6-CHEV	[illegible]	6.447	16.447	9.801	8.926	13.503	8.441	16.359	1.259	17.336
ABD1-CHEV	[illegible]	5.977	15.425	9.694	8.872	13.466	8.272	14.762	1.268	17.313
ABD2-CHEV	[illegible]	6.104	16.867	9.771	8.479	13.515	8.286	17.200	1.261	17.303
ABD3-CHEV	[illegible]	6.102	14.802	9.743	8.797	13.337	8.391	16.532	1.278	17.454
ABD4-CHEV	[illegible]	5.861	16.047	9.715	9.050	13.550	8.450	18.665	1.269	17.359
ABD5-CHEV	[illegible]	6.193	15.242	9.720	8.371	13.414	8.244	19.347	1.289	17.322
ABD6-CHEV	[illegible]	5.972	15.963	9.677	9.027	13.515	8.289	18.254	1.2742	17.319

Appendix II: Antibiotic drugs residues (µg/kg) in Beef samples from Abisim Market (AM), Abattoir Market (AB) and Central Market (CM)

Sample	Amoxycilin	Chlorotetracycline	Ciprofloxacin	Danofloxacin Mesylate	Doxycycline	Norfloxacin	Oxytetracycline	Ractopamine	Sulfadiazine	Tylosine
ABD1-BEEF	16.904	5.927	15.413	9.692	8.739	13.630	8.277	14.507	1.285	17.313
ABD2-B	[illegible]	6.049	16.052	9.689	8.394	13.493	8.269	15.986	1.286	17.297
ABD3-B	[illegible]	5.884	16.299	9.785	8.705	13.539	8.236	18.860	1.268	17.296
ABD4-B	[illegible]	5.972	16.178	9.729	8.538	13.384	8.450	19.679	1.276	17.301
ABD5-B	[illegible]	6.014	18.678	9.786	8.340	13.465	8.529	19.325	1.276	17.346
ABD5-B	[illegible]	5.922	16.776	9.745	8.704	13.404	8.399	17.680	1.259	17.378
CMD1-B	[illegible]	5.808	14.458	9.683	10.347	13.415	8.196	19.025	1.2704	17.313
CMD2-B	[illegible]	6.034	15.299	9.701	8.566	13.501	8.206	15.061	1.264	17.318
CMD3-B	[illegible]	6.049	15.077	9.758	8.336	13.429	8.236	15.641	1.284	17.328
CMD4-B	[illegible]	5.943	16.030	9.751	8.549	13.405	8.279	18.714	1.302	17.403
CMD5-B	[illegible]	5.802	16.469	9.710	9.693	13.580	19.814	18.873	1.262	17.359
CMD5-B	[illegible]	6.255	14.523	9.779	8.651	13.457	8.243	16.398	1.263	17.353
AMD1-B	[illegible]	5.892	14.738	9.670	8.612	13.400	10.362	18.156	1.299	17.389
AMD2-B	[illegible]	5.897	15.360	9.711	8.378	13.399	8.322	16.479	1.269	17.316
AMD3-B	[illegible]	5.945	15.888	9.715	8.454	13.471	20.934	15.052	1.287	17.385
AMD4-B	[illegible]	5.974	15.455	9.756	8.792	13.375	8.252	22.090	1.285	17.441
AMD5-B	[illegible]	6.036	14.781	9.663	9.113	13.370	8.221	16.014	1.271	17.311
AMD6-B	[illegible]	5.860	14.869	9.664	8.305	13.416	8.366	16.933	1.262	18.313

Appendix III: Survey Questionnaire 2018 used to collect information from ruminant farmers in the Sunyani Municipality.

UNIVERSITY FOR DEVELOPMENT STUDIES
FACULTY OF AGRICULTURE
DEPARTMENT OF ANIMAL SCIENCE

A SURVEY ON USAGE OF VETERINARY ANTIBIOTICS IN RUMINANT PRODUCTION IN THE SUNYANI UNICIPALITY

This study is to identify the most frequently used antibiotics, their dose, period of use and the withdrawal times prior to market or slaughter. Please, information given will be treated with high level of confidentiality. Please fill the questions below as best as you can. Tick where appropriate { }

A. PERSONAL DATA

1. Gender: Male { } Female { }
2. Age group. A. 20-29 { } B. 30-39 { } C. 40-49 { } D. 50-60 { } E. 61& above { }
3. Marital Status: A. Married { } B. Single { } C. Divorced { } D. Others { }
4. Religion A. Christianity { } B. Islamic { } C. Traditional { } D. Others { }
5. Educational level. A. Non Formal { } B. Primary school { } C. Junior High School{} D. Senior High School { } E. Tertiary { } F. Others { }

B.FARMER

6. What type of ruminants do you rear? A. Cattle { } B. Goat { } C. Sheep { }
7. How many years have you been engaged in this business? A. 1-2 years { } B. Between 3-5years{ } C. 6-10 years { } D. above 10 years
8. What type of Management System do you keep? A. Intensive B. Semi-intensive C. Extensive
9. Which of these biosecurity measures do you adopt? A. fencing B. Netting C. Sanity of drinking water D. Quarantine of incoming animal E. Use of protective clothing on duty F. Regular vaccination G. Regular fumigation H. Farm away from water bodies
10. Have you ever encountered any infection in the animals on your farm? A. Yes {} B. No{}
11. Were you able to identify the kind of infection based on your own experience? A. Yes {} B. No {}

12. If No, did you consult veterinary officer(s) to know what kind of infection it was?
13. If Yes or No did you consult veterinary officers for prescription? A. Yes { } B. No {}
14. If No, Give reason why?..
15. If yes, what kind of veterinary medication did he/she recommend for you to treat the animals? A. anthelmintics B. antibiotics C. fungicides D. Multivitamins
16. What was the purpose of the medication? A. Chemotherapy B. Chemoprophylaxis C. Growth promotion
17. What clinical indications necessitated the drug administration? A. Mastitis B. diarrhea C. nasal discharge E. coughing F. lameness G. profuse salivation
18. If your medication included antibiotics, what class of antibiotics did you use? A. Gentamicin { } Tetracycline { } C. Penicillin { } D. Amoxicillin/Clavulanic { } E. Trimethoprim/Sulfamethoxazole { } F. Ciprofloxacin { } G. Ceftriaxone { } H. Chloramphenicol { } I. Teicoplanin { } Others……………………….
19. Why do you prefer this/these antibiotic/s? A. it is effective { } B. it is less costly { } C.it is easy to use { } D. easily accessible { } E. Colleague advice { } F. others { }
20. *Did* you have knowledge on the antibiotic *that was used*? A. Yes { } B. No { }
21. **If yes**/*No*, ***how did you get to know about it***? A. extension officers { } B.NGOs{} C. Colleague farmers{ } D. veterinary staff E. others{ }
22. Where do you purchase the antibiotics from A.Veterinary clinic/shops { } B.Friends{} Market{} others (specify).
23. How often do you treat your animals with antibiotics?..........................
24. Who administers the antibiotics to the animals? A. Self { } B. veterinary officer { } C. Both D. Others specify ……………...
25. If self or both do you observe safety and dosage protocols for the antibiotic? A. Yes {} B. No { }
26. If No, why?.....................................

27. If yes, what are the dosage and withdrawal periods of the antibiotics you have ever used for treating ruminants?

Species	Name of Antibiotic	Dosage	Frequency of admin	Duration of Treat	Withdrawal Period

28 . In case the treated animal is not recovering, what do you do to the animal? A. sell to butchers{ } B. home consumption { } C. market { } D. others specify…………………………………

29 In case you are going to sell or consume a treated animal, how long does it take from the time of last administration of a treatment dose to the time of sale or consumption?...

30 Do you observe withdrawal periods? A. Yes B. No

31 Do you receive periodic sensitization/education on the use of antibiotics? A. Yes B. No

32 .Are aware that non-adherence to label instructions/treatment protocols regarding the administration of antibiotics in livestock can worsen the animal's condition? A. Yes B. No

33 Are you aware that the administration of antibiotics without recourse to treatment instructions can promote the development of antibiotic resistant pathogens in animals which may be passed on to humans through the food chain? A. Yes B. No

34 Are you aware that non observance of withdrawal periods or administration of antibiotics close to slaughter time can lead to accumulation of residues in the animal's edible tissues? A Yes B No

35 Are you aware that consumption of residual antibiotics in edible animal tissues can predispose the human population to health challenges? A. Yes B. No

Appendix 4: Survey Questionnaire 2018 used to collect information from veterinary officers in the Sunyani Municipality.

UNIVERSITY FOR DEVELOPMENT STUDIES
FACULTY OF AGRICULTURE
DEPARTMENT OF ANIMAL SCIENCE

A SURVEY OF ANTIBIOTIC USAGE IN A RUMINANT PRODUCTION IN THE SUNYANI MUNICIPAL ASSEMBLY

This study is to identify most frequently used antibiotics, their dose, time of use and the withdrawal times prior to marked slaughter. Please, information given will be treated with high level of confidently.

A.PERSONAL DATA

1. Gender a. { } b. Female { }
2. Age group (Years) a. 20 - 29 { } c. Divorced { } d. others { }
3. Marital status: a Married { } b. Single { } c. Divorced { } d. Others { }
4. Household size a. 1 – 4 { } b. 5-9 { } c. 10 – 14 { } d. 15 and above { }
5. Educational level. A. Non formal { } b. Primary school c. Junior / Senior High { }

Tertiary { } e. Others specify ………………………………………………………….

Veterinary officers

6. How many years have you been in this work?

……………………………………………………………………………………………

7.Do you encounter bacterial infections in animals you have ever treated? a.Yes b. No

8.If yes in which animal species did you encountered this? A. Cattle b. sheep c. Goat d. All

9.In every species that you encountered the bacterial infection indicate be casual suspected, the kind of treatment processed and antibiotic / antibiotic used.

Species	Bacterial Agent Suspected	Treatment	Type / Types of antibiotics used	Reasons

10. Where do you obtain your antibiotic from?

…………………………………………………………………………………………

11. What are the dosages of the five most common antibotics used for treating remnants?

Antibiotic Name	Dosage Recommended by Manufacturer	Dosage Administered	What are the withdrawal periods for	Reason for below or above recommended dosage

			theses antibiotics	

12. Do you advice farmers on residual effect on antibiotics? A. Yes { } b. No { }
13.If yes, what are their responses?
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14. If No, Give reasons why?...
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15. What are the constraint's you encounter when advising the farmers?
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16. Do you have community Livestock workers operating in your area of jurisdiction? A. Yes { } b.
No { }
17. If yes, do they get training regarding the administration of antibiotics? A. Yes { } b. No. { }
18. If No give reason why?
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19. Do you encounter untrained personal offering veterinary service on the field? a. Yes { } b. No.{ }
20. If yes, what action did you take?
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21. Do you have any way of tracking animals which have just been treated with antibiotics but are being sent to be slaughtered? a. Yes { } b. No. { }
22. If No why give a reason?
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23. If yes, how is that done and how is the success rate?
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24. What action do/ did you take against farmers selling a treated animal whose withdrawal period has not elapsed?
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25. Do you conduct regular sensitization to farming regarding the use of antibiotics? a. Yes { } b. { }