

**SCHOOL OF PUBLIC HEALTH, COLLEGE OF HEALTH
SCIENCES**

UNIVERSITY OF GHANA, LEGON

**ANTIMICROBIAL DRUG RESIDUE IN POULTRY PRODUCTS IN
DORMAA MUNICIPALITY OF THE BRONG AHAFO REGION OF
GHANA**

BY

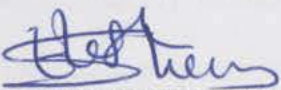
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**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA,
LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENT
FOR THE AWARD OF MPHIL APPLIED EPIDEMIOLOGY AND
DISEASE CONTROL DEGREE**

JULY, 2011

DECLARATION

This work is the result of an independent investigation under the supervision of Dr K.A. Bugyei and Dr Priscillia Nortey. Where my work is indebted to the work of others, I have made acknowledgements. I declare, therefore that this dissertation, either in whole or part has not been presented elsewhere for another degree.

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ACKNOWLEDGEMENT

I am heartily thankful to my supervisors, Dr. Edward Baggio and Rosalinda Nantayo, for their encouragement and supervision from the start to the end of this work. Their

This work is dedicated to my husband, Eugene and children, Baaba, Eugenia and Dela Johnson, who offered me unconditional love and support throughout the course of my study. It is also dedicated to my mother, Dr Rebecca Ganusah, and niece Benedicta Asembi, without whose caring support it would not have been possible to come this far.

Discipline Officer, for their technical and moral support throughout the study period.

I am grateful to CDC, AFENET and Ghana FERTF for the financial assistance during the program. I thank especially Dr. B. Williams and Professor H.A. Aducci for their constant support through the preparation and completion of this work.

Special thanks to the Director, Veterinary Services Directorate, Dr. Mark Hahner and staff, for their invaluable support during the laboratory investigation. I am indebted to Dr. A. Asong and Jerry Obed for assistance in conducting the serological work at the Animal Care Unit.

I am grateful for the support received from IAH's Technical Staff and District staff of the Veterinary staff in Dambou, who assisted me during the sample collection.

I am indebted to Mr. Ernest Ofori, the District Manager, District of Dambou, for his support during the field work in the District animal party.

Finally, I want to thank to Mr. David Mawusi of IAH for his moral support through my work. Several thanks to thank that it was so smoothly done.

Lastly, to my family and friends, who are always in my difficult moments. I say thank you.

ACKNOWLEDGEMENT

I am heartily thankful to my supervisors, Drs Kwasi Bugyei and Priscillia Nortey, for their encouragements and supervision from the start to the end of this work. Their preparedness to go through my thesis, even at their inconvenience sometimes, was most touching. I am grateful!

I would like to thank faculty and staff of the Department of Applied Epidemiology and Disease Control for their technical and moral support throughout the study period.

I am grateful to CDC, AFENET and Ghana FELTP for the financial assistance during the program. I thank especially Dr .F Wurapa and Professor E.A. Afari for their immense support through the preparation and completion of this work.

Special thanks to the Director, Veterinary Services Directorate, Dr Mark Hansen and staff, for their invaluable support during the laboratory investigation. I am indebted Dr J. Awuni and Jerry Oddoye for assisting me with the bench work at the Accra Vet Lab.

I am grateful for the support received from Ms Scholastica Delle and Cudjoe, staff of the Veterinary staff in Dormaa, who assisted me during the sample collection.

I am indebted to Ms Florence Idrissah, the Dormaa Municipal Director of health, for her support during the field work in the Dormaa Municipality.

I offer my deepest gratitude to Mr David Mensah of NMIMR who read through my work several times to ensure that it was statistically sound.

Lastly, to my family and friends, who stood by me during difficult moments, I say thank you.

ABSTRACT

Background: Antimicrobial drugs are important tools in the management of infectious diseases in both animals and human beings. Their use in food animal production, could lead to the occurrence of antimicrobial residues in the animal products such as meat and eggs. Antimicrobial residue in animal food products has been associated with adverse effects such as hypersensitivity reactions, development of resistant strains of microorganisms, carcinogenic effect and potential harmful effect on human intestinal microflora. In Ghana, there is no monitoring program for antimicrobial residue in foods of animal origin. This study determined the types of antimicrobial drugs used in poultry production in the Dormaa municipality and the levels found in poultry products.

Method: A cross sectional study was conducted between December 2010 and June 2011. A structured questionnaire was administered to managers, caretakers and owners of randomly selected farms which covered demographic characteristics, educational status, and type of antimicrobial drugs used, knowledge and adherence to withdrawal periods. Laboratory detection and quantification of residues in poultry products was conducted using microbial inhibition plate assay.

Results: A total of 132 farmers, mostly males (97.7%) were interviewed from 115 farms. Almost half (40.9%) of the farmers had a junior high school education. Ninety eight (74.2%) had not had any training in antimicrobial usage. Most farms, (86%) administered drugs to the birds for prophylactic purposes. Eighty one (61.4%) farmers did not know what withdrawal period meant. A total of 28 (24.3%) farms had tetracycline residue in eggs, 25 (21.7%) in kidney and 14 (12.2%) in liver.

Mean (\pm SD) tetracycline concentration ($\mu\text{g/g}$) found in poultry products were: eggs 0.02 ± 0.003 ; kidneys 0.02 ± 0.01 ; and liver 0.01 ± 0.008 . Twenty six farms (92.9%; $p>0.05$) that had residues in eggs had sold eggs during treatment of the laying birds.

A disinfectant, omnicide® (44.3%) and 2 antibiotics in human formulations namely, tetracycline 20 (17.4%) and Chloramphenicol 3 (2.6%) capsules were administered to the birds on an extra-label basis.

Conclusion: Tetracyclines were the main drugs used in poultry farming in the Dormaa municipality with little or no regards for withdrawal periods. Tetracycline residue was found in poultry but was below the Maximum Residue Level set by Codex Alimentarius. Extra-label use of disinfectant to water-medicate birds pose health hazard to farmer and consumer. Training of poultry farmers and other stakeholders in the correct use of drugs in poultry farming is recommended.

Keywords: antimicrobial residue, poultry products, extra-label use, Dormaa municipality, Ghana

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LIST OF ABBREVIATIONS

| | |
|--------|--|
| ELU | Extra-label use |
| ADI | Admissible Daily Intake |
| MRL | Maximum Residue Limit |
| FDA | Food and Drug Administration |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| WHO | World Health Organization |
| US FDA | United States Food and Drug Administration |
| CDC | Centers for Disease Control and Prevention |
| VSD | Veterinary Services Directorate |
| NCTC | National Collection of Type Cultures |
| ATCC | American Type Culture Collection |

DEFINITIONS OF TERMS

Chevon: Goat meat (meat of the domestic goat)

Mutton: Meat from a mature domestic sheep

Broilers: Type of chicken raised specifically for meat (a chicken slaughtered for meat while it is still young and tender).

Layers: Birds that lays eggs, or one that is used for laying eggs as opposed to one raised to be fattened and eaten

Extra-label use of drugs: The use of a drug product in an animal in a manner that is not consistent with what is indicated on the label.

Withdrawal period / Withholding time: Period between the last administration of a drug and the collection of edible tissues or products from a treated animal that ensures the residue in the food is within acceptable limits.

Food-producing animal: Animals raised for the purpose of providing food for humans. They include poultry, swine, cattle and sheep, but does not exclude other domestically managed animals.

Antimicrobial agent: Any substance of natural, synthetic or semi-synthetic origin which at low concentrations kills or inhibits the growth of microorganisms but causes little or no host damage.

Antimicrobial resistance: The ability of a microorganism to continue to multiply or persist in the presence of therapeutic levels of an antimicrobial agent.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Antimicrobial drugs are chemical compounds that kill or inhibit the growth of susceptible microorganisms and are powerful tools for the management of infectious diseases in animals and humans (Tripathi, 2008). Antimicrobial drugs have been used in human and veterinary medical practice for more than 50 years, with tremendous benefits to both human and animal health. They have contributed to the treatment or prevention of many infections that otherwise were fatal or left individuals with severe disability (FDA, 2004).

Antimicrobial drugs used in food animal production are identical and / or related to drugs used in human medical practice, eg penicillins, tetracyclines, cephalosporins and fluoroquinolones, among others (Tollefson and Miller, 2000). In food animal production, such as poultry, antimicrobial drugs are used to control, prevent and treat infection. They are also used to enhance animal growth through feed efficiency. The prophylactic use of antibiotics has had a positive impact on intensive production of livestock and poultry. The use of these drugs have facilitated confinement housing and allowed higher densities of animals to be maintained (Tollefson and Miller, 2000 FDA, 2004).

In poultry, antibiotic usage has facilitated their efficient production, and also enhanced the health and wellbeing of poultry by reducing the incidence of diseases. Unfortunately however, poultry tissues may be contaminated with harmful concentrations of drug

residues as a result of such practice (Sharref *et al*, 2009, Reig and Tondra 2008, Ezenduka *et al*, 2010).

According to Lee *et al*, (2001), approximately 80% of all food-producing animals receive medication at sometime of their lives. Antibiotics are widely used in developing countries due in part, to the high incidence of infectious diseases in many areas (Al-Mustafa and Al-Ghamdi, 2002)

Treatment of animals reared for food, especially poultry, is generally directed at groups or herds but not individual animals (Pavlov *et al*, 2008). With intensively reared stock, such as poultry, individual treatment usually is not feasible and mass oral medication (in feed or water) is the only practical method of treatment, as individual handling and injections are stressful for them. Sick cattle and sheep are usually treated individually (Amstrong, 2008)

The use of antimicrobial drugs in food-producing animals has become a public health issue, due to the fact that these agents are increasingly being used in farm animal production (AL-Ghamdi *et al*, 2000). The administration of antimicrobial drugs to food producing animals could lead to drug and / or their metabolites remaining in their products as residues. (Sasanya *et al*, 2005; Pikkemaat, 2009)

Alhendi *et al*, (2000) indicated that administration of drugs to food-producing animals requires not only consideration of effects on the animal but also the effects on humans who ingest food originating from these animals. Doyle, (2006), Tollefson and Miller, (2000) suggested that the main factors contributing to antimicrobial residues occurring in foods of animal origin are non-adherence to withdrawal period and the extra- label use of

these chemicals. Extra-label use (ELU) is the use of a drug product in a manner that is not consistent with what is indicated on the label, package insert, or product monograph approved by a Regulatory Body by (FDA,1994).

Accordingly, national and international legislations have been established to ensure that consumers are not exposed to potentially harmful residues of these drugs in food. International bodies such as Codex Alimentarius, Joint FAO/WHO Expert Committee on Food Additives (JECFA), Veterinary Medicines Directorates (VMD) of the European Union as well as Food and Drug Administration of the USA have set tolerance or Maximum Residue Limits (MRLs). In addition, Acceptable Daily Intake (ADIs) for humans and withholding times for pharmacologically active substances including antimicrobial agents prior to marketing animal products prior have also been established (JECFA, 1994; WHO, 2000).

The withdrawal period, also known as withholding time is the period between the last administration of a drug and the collection of edible tissues or products from a treated animal that ensures the residue in the food is within acceptable limits (Codex Alimentarius JECFA, 2004).

Maximum Residue Limit, as defined by the Codex Alimentarius Commission, is the maximum concentration of residue resulting from the use of an antimicrobial drug (expressed in mg/kg or µg/kg on a fresh weight basis) that is recommended to be legally permitted or recognized as acceptable in or on a food.

Acceptable Daily Intakes is an estimate of the amount of a veterinary drug, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk (standard man = 60 kg).

Antimicrobial residue in animal food products are of public health importance because of the adverse effects they have on human health. Some of the effects include hypersensitivity reactions, development of resistant strains of microorganisms, carcinogenic effect and potential harmful effect on human intestinal microflora. (Ezenkuda *et al*, 2010; Reigh and Tondra, 2008; Adesiyun *et al*, 2005; Anderson *et al*, 2003 and JECFA 2004). Studies in humans have confirmed chloramphenicol to cause aplastic anaemia, which is not dose dependent and as such, very little amount of chloramphenicol, in a form of residue in foods of animal origin could be hazardous to human health (JECFA, 2004). This is the main reason why its use in food animals is strictly regulated or even banned in the European Union (Reig and Tondra, 2008).

Additionally, the prevalence of antimicrobial sensitivity in the general population has been estimated to be between 3–10% (Doyle, 2006); thus, exposure of sensitive people to antimicrobial residues such as penicillin could lead to the development of an allergic reaction in such individuals.

The effect of using antimicrobial drugs in food animals has been associated with transfer of resistant pathogens to humans through consumption of contaminated food or direct contact with animals. (Salehzadeh, 2006). Transfer of resistant genes from bacteria to bacteria causing human disease through the food chain with concomitant increase in incidence of human infections has also been documented. (WHO, 2000)

Globally, the continuing emergence and spread of resistant pathogenic organisms are of increased concern. Resistance has emerged in zoonotic enteropathogens, notably *Salmonella* spp., *Campylobacter* spp, commensal bacteria like *Escherichia coli*, *Enterococci* and animal bacterial pathogens such as *Pasteurella* and *Actinobacillus* spp (Doyle, 2006).

1.2 Problem Statement

In Ghana, poultry products are consumed by almost everybody, especially on festive occasions and at public functions such as conferences and workshops. Poultry products are among the major source of protein in Ghana, and a review of Ghana's poultry industry by Aning, (2008) indicated a steady increase in poultry production over the years. Donkor *et al*, (2011) reported that weekly egg consumption topped the various animal source foods consumed by Ghanaians.

Just as occurs in most developing countries, the Ghanaian poultry industry battles with viral, bacterial, parasitic and other diseases, with consequent increase in the use of chemicals including antibiotics and coccidiostats (Turkson, 2008). Since the privatization of the sale and distribution of veterinary drugs in 1999, poultry farmers have had free access to all classes of drugs, with or without veterinary prescription. Thus there is uncontrolled distribution and sale of antimicrobial drugs on the market. Additionally, there are no designated slaughter facilities for chicken in Ghana, as established for ruminants like sheep, goat and cattle, where meat inspection is carried out prior to marketing to the public. And since the slaughter of birds is carried out on farm premises at different times convenient to the farmer, monitoring inspection for residues can be a daunting task.

There is no antimicrobial drug residue monitoring program in place for locally produced or imported poultry products in Ghana. Furthermore, Maximum Residue Level (MRL) and Acceptable Daily Intake (ADI) of antimicrobial residues are yet to be established in Ghana. The implication of the absence of residue monitoring program means that the Ghanaian consumer of poultry products could be at risk of consuming foods of animal origin contaminated with drug residue.

It is known that antimicrobial agents are widely used in the poultry industry in Ghana (Turkson, 2008) but to date the prevalence and quantity of residues in poultry products have not been documented. The protection of public health against possible harmful effects of veterinary drug residues is an important problem that cannot be overlooked.

The aim of this study was to determine the antimicrobial residue levels in locally produced poultry products.

1.3 Justification

There is paucity of information on antibiotic residue in poultry products in Ghana, especially with regards to the quantity of residue in the products. A study carried out by Donkor *et al*, (2011) in Ghana, determined the risk of exposure to antibiotic residues in meat and eggs in Accra and Kumasi, but the study did not quantify specific drugs in the food samples.

There is a need to therefore to detect and quantify residue of animal origin, which will be useful in establishing a baseline data on drug residues in foods of animal origin in Ghana. The detection of antimicrobial drug residues in poultry products will be useful for further

studies which could culminate in policy formulation by regulatory bodies, such as Food and Drugs Board, on rational antimicrobial use.

This will assist the Veterinary Services Directorate to better define the context, contributing factors, and implications on public health, food safety, and animal health. Training needs of farmers with regards to husbandry practices and use of antimicrobial drugs in animal farming would have also been determined.

1.4 Study Objectives

Main objective

To determine the type of antibiotics used in poultry production in Dormaa municipality and the antibiotic levels found in poultry products.

Specific Objectives,

1. To detect and quantify the type of antimicrobial agents in poultry products.
2. To determine the prevalence and factors contributing to the occurrence of drug residue in products of animal origin
3. To assess poultry farmers' knowledge and practices of antimicrobial drugs usage with regards to adherence to withdrawal periods.

CHAPTER TWO

2.0 LITERATURE REVIEW

The use of antimicrobials drugs for the treatment and prevention of disease in animals closely followed their uses in humans (Gustafson, 1993), and they were first employed in veterinary medicine for the treatment of mastitis in dairy cows (Spencer, 1950).

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

2.1 Antimicrobial usage in food animals

Antibiotics are among the few classes of drugs used in food animals for both therapeutic and sub-therapeutic purposes. Therapeutically, they are used for treating existing disease conditions and sub-therapeutically they are used when pathogens are known to be present in the environment or when animals are subjected to stressful conditions, which make them prone to infections. Sub-therapeutic doses of antimicrobial drugs are doses less than

that required to treat established infections (Toffelson and Miller 2000, Doyle 2006, Salehzadeh *et al*, 2006).

Anderson *et al*, (2003) noted that a substantial portion of antibiotic given to food producing animals is for non-therapeutic purposes (used in the absence of disease) such as growth promotion, a practice that is becoming increasingly a public health concern. This was corroborated by Mellon *et al*, (2001) when they estimated that between 60–80% of antibiotics produced in the United States were administered to healthy livestock at non-therapeutic levels. These same antibiotics were administered to humans in clinical settings, and they include tetracyclines, macrolides, streptogramins, and fluoroquinolones (Anderson *et al*, 2003; FDA, 2004). Antibiotic usage has facilitated the efficient production of poultry, allowing the consumer to purchase, at a reasonable cost, high quality meat and eggs. Antibiotic usage has also enhanced the health and well-being of poultry by reducing the incidence of disease (Tollefson and Miller, 2000; Donoghue, 2003). Al Mustafa and Al Ghamdi, (2002) suggest that antibiotics are widely used in developing countries, due in part to the high incidence of infectious diseases in many areas.

Several routes of drug administration of antimicrobial drugs to food producing animals have been employed and they are; by injections (intravenously, intramuscularly, or subcutaneously), orally in feed or water, topically on the skin and by intramammary and intrauterine infusions (Mitchell *et al*, 1998). In theory, all of these routes may lead to residues appearing in foods of animal origin such as milk, meat and eggs (Johnston, 1998).

Food animals, such as poultry, that are raised under intensive type of production rely on the use of antimicrobials to promote growth and control diseases (Tollefson and Miller, 2000).

Alhendi *et al*, (2000), in a study on antimicrobial residue in broilers in the Saudi poultry industry, found that birds were frequently raised in conditions where there was high level of stress, and as such different types of antibiotics are used to control infections. The use of antimicrobials on farms depends on many factors such as the type of husbandry, the environmental pressure on a farm, and the standard of stockmanship (the knowledge and skillful management of livestock) (Johnston, 1998). Good farm management practices such as sanitation, housing, feeding practices, space utilization could reduce the use of antimicrobials. However, even if well managed, the increased density of livestock or poultry in intensive rearing operations requires an aggressive approach to disease control, which can lead to heavy prophylactic and therapeutic antimicrobial use (Tollefson and Miller, 2000).

2.2 Sources of antimicrobial residues in animal product

Several factors have been mentioned to be the cause of antimicrobial residues in foods of animal origin, however, it is suggested that the most likely cause of violative drug residues is the failure to observe withdrawal times (Paige and Kent, 1987; Van Dresser and Wilke, 1989; Guest and Paige, 1991; Paige, 1994). Other factors are improper maintenance of treatment records, failure to identify treated animals adequately, (Sundlof, 1989) as well as improper use of a licensed product or the illegal use of an unlicensed substance. The latter include dosages and use of drugs which have not been approved for the species in question (Papich *et al*, 1993; Kaneene and Miller, 1997; Higgins *et al*,

1999). Drugs, including unapproved and unregulated compounds that may have no Admissible Daily Intake (ADI), can often be easily purchased from veterinary shops and informal vendors (Dipeolu and Alonge, 2002; Keyyu *et al*, 2003). Secondly, there may be no quality control of the substances supplied and concentrations and dosage rates may be incorrectly recorded (Turkson, 2001). Additionally, there is often a lack of veterinary advice regarding withdrawal periods and this compounded by illiteracy, renders labeling and printed instructions for drugs of limited use. Drugs are frequently administered by unqualified farmers or para-veterinary field staff and extended usage or excessive or multiple dosages of the compounds are common (Cannavan, 2004). According to Oboegbulem and Fidelis, (1996), there is no national program for routine monitoring of residues in food animals in most developing countries. They suggested that it was highly probable therefore that an undetermined proportion of cattle and poultry slaughtered in developing countries and much of the meat for export contains significant antimicrobial residues.

2.3 Extra – label use of antimicrobials

Extra-label use (ELU), defined as the use of a drug product in a manner that is not consistent with what is indicated on the label can take many forms (Kirkpatrick, 2002). Both prescription and nonprescription drug products, for example may be used in a manner that is not indicated, such as at an increased dosage, a greater or lesser frequency, different indications, longer or shorter duration of treatment, or different route of administration (oral, injectable). It may also be used for different animal species or age groups, in a different form, or in combination with other drugs. It may be a human drug administered to an animal (Haggin *et al*, 1999).

Extra-label use is a public health and food safety issue for several reasons. Firstly, drug residue may be present in food products derived from ELU-treated animals consumed by humans. (Reig and Tondra, 2008; Doyle, 2006) Secondly, adverse reactions may arise in treated animals, since these products have not been tested appropriately for their new conditions of use. Thirdly, drugs used in this manner may have a reduced or uncertain efficacy for the conditions being treated. Finally, ELU may be a potential contributing factor in the development of antimicrobial resistance (AMR) (Kirkpatrick, 2002).

Under the US Food and Drug Administration (FDA, 2004), conditions under which extra-label use of drugs could be carried are firstly, a careful diagnosis or evaluation of the conditions to be treated must be made. Secondly, a substantially extended drug withdrawal period that is supported by scientific evidence should be established. Thirdly, steps be taken to ensure that the withdrawal period is met and no illegal drug residues occur in food from the treated animals and lastly, that proper record and identification of the treated herds / flock be kept properly.

2.4 Effects of Antimicrobial residue on human health

In a consumer survey, Resurreccion and Galvez, (1999) reported that 77% of consumer respondents considered animal drug residues in food of animal origin to be an extreme health concern. Two of the effects antimicrobial drug residue could have on human health are the following:

2.4.1 Effects on the gastrointestinal flora

The effects of antimicrobial residue on human gastrointestinal flora have been identified as follows: modification of the metabolic activity of micro flora in the gastrointestinal

tract, changes in bacterial populations, selection of resistant bacteria, and perturbation of the barrier effect (Boisseau, 1993; Gorbach, 1993; Sundlof *et al*, 2000).

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

2.4.2 Hypersensitivity

Drug hypersensitivity is defined as an immune-mediated response to a drug agent in a sensitized patient (Riedl and Casillas, 2003). Immunologic reactions may manifest in forms ranging from life-threatening anaphylactic reactions to mild reactions, such as rashes. They include anaphylactic shock, asthma and angioneurotic oedema; haemolytic anaemia and agranulocytosis; serum sickness and allergic vasculitis and allergic dermatitis (Dayan, 1993; Riedl and Casillas, 2003).

Antimicrobial drug residues in animal products may cause hypersensitivity reactions in humans and this may be triggered by antimicrobial residues in a previously sensitized individual. In the literature, only a few cases of hypersensitivity have been reported as a result of exposure to residues in animal products (Woodward, 1991), however, failure to associate minor reactions, e.g. urticaria, with exposure to allergenic residues may be one reason for the lack of reported cases, although it may also be due to a genuine dearth of reactions. Aplastic anemia can occur in susceptible individuals exposed to concentrations

of chloramphenicol that might remain as residues in edible tissues of chloramphenicol-treated animals (Settepani, 1984). The WHO has recommended the prohibition of use of chloramphenicol in all food producing animals, particularly lactating cows and laying birds (Settepani, 1984; WHO, 1988). Aminoglycosides can produce damage in urinary, vestibular and auditory functions (Clark, 1977; Shaikh and Allen, 1985). Toxic and allergic reactions in humans and animals caused by tetracyclines have been observed at therapeutic doses (Berends *et al.*, 2001).

2.5 Prevention of residues

Potential components of prudent use strategies include defining the need for the treatment and ensuring the appropriate therapeutic doses and regimens (Angulo *et al.*, 2000). To ensure that drug residues have declined to a safe concentration following the use of drugs in animals, a specified period of drug withdrawal must be observed prior to providing any products for human consumption. Withdrawal period is the time which passes between the last dose given to the animal and the time when the concentration of residues in the animals tissues: (muscle, liver, kidney, skin/fat) or products such as milk, eggs and honey is lower than or equal to the Maximum Residue Limit (MRL) (Jackson, 1980).

Other preventive measures recommended by the FDA is identifying and tracking animals to which drugs were administered, in order to preclude the sale of animal tissue, milk, or eggs containing illegal residues. Identification may be by specific animal identification, pen or lot (such as for poultry) or quarantine/segregation, among others. It is also important to maintain a system of medication/treatment records that, at a minimum, identifies the animals treated (individual animals, pens, lots, etc.), dates of treatment, the

drug(s) administered, and the withdrawal time prior to slaughter. Other measures include properly storing, labeling, and accounting of all drug products and medicated feeds as well as obtaining and using (as much as possible) veterinary prescription drugs prior to purchase of drugs.

In spite of these several studies have found the use and misuse of antimicrobial drugs in a manner that have left residues in animal products. AL-Ghamdi *et al*, (2000) reported a widespread misuse of antimicrobial drug such as tetracycline including multiple use of drugs belonging to the same pharmacological group and non-adherence to recommended withdrawal times in a study conducted in Eastern province of Saudi Arabia. Ezenduka *et al*, (2010) found antimicrobial residues in raw table eggs from farms and retail outlets in Nigeria. Oxytetracycline residue was reported found in chicken tissues that were above Maximum Residue Levels in Iran (Salehzadeh *et al*, 2006). In an investigation into the risk of exposure to antibiotic residues contaminating meat and egg in Accra and Kumasi, Ghana, it was found the prevalence rates of drug residues in the various animal source foods as follows; 30.8% (beef); 29.3% (chevon); 28.6% (pork); 24% (mutton); and 6.8% (egg) (Donkor *et al*, 2011).

The public health hazards of antimicrobial residues appears to be a major problem in developing countries, where there may be no local legislations regulating MRL for marketed products (Oboegbulem and Fidelis, 1996).

2.6 Antimicrobial Use and Development of Resistance

The use and sometimes misuse of antimicrobials in food animal production has resulted in the emergence and dissemination of resistant pathogens and resistance genes. Antimicrobial resistant bacteria in food animals can affect not only animal health, but

also public health when they enter the food chain. Antibiotic-resistant bacteria can be transmitted from animals to humans in several ways (Tollefson, 2000). It can be passed on through food, when people consume animal products that contain antibiotic residues or have been contaminated with the resistant bacteria during slaughter (Wilson, 2004), through direct contact when farmers and farm workers become infected by the animals and pass it on to the family and community and the environment when animal manure contaminates local waterways and groundwater.

According to WHO (WHO, 2000), antimicrobial resistance develops when a microorganism mutates or acquires a resistance gene. Selective pressure resulting from widespread antimicrobial use is the underlying force in the development of resistance. Through international trade and travel, resistant microbes can spread quickly worldwide (FDA, 2004).

The association between increased antimicrobial use and resistance has been documented for nosocomial infections as well as for community-acquired infections (Baquero *et al*, 1991). For foodborne pathogens, especially for those species that are rarely transferred from person to person in industrialized countries such as *Salmonella*, the most likely source of antibiotic resistance is use of antimicrobials in food-producing animals. (Tollefson and Miller, 2000)

Sackey *et al* (2001) in a study in Ghana concluded that imported and locally produced chicken were a potential source of multiple-antibiotic-resistant enteropathogenic bacteria. They isolated *Campylobacter*, *Salmonella*, *Shigella* species and 12 *Escherichia coli* serovars from live and dressed birds which were found to be resistant to antibiotic

cefotiam, penicillin, cefadroxil, the commonly used drugs for the treatment of such infections.

2.7 Detection and identification of antimicrobial residues

Screening of food products from animal origin for the presence of antimicrobial residues started soon after the introduction of antibacterial therapy in veterinary medicine (Myllyniemi, 2004). Initially it mainly concerned process monitoring in the dairy industry to prevent problems in fermentative dairy production, but from the early 1970s regulatory residue screening in slaughter animals also became more commonly introduced (Pikkemaat, 2009). Several methods have since been developed for the detection of residue in foods of animal origin. They include: microbiological, immunological and physicochemical methods, although some are a mixture of these methods (Moats, 1997)

2.7.1 Microbiological methods

Microbial inhibitions assays were the earliest methods used for the detection of antibiotic residues (Mitchell *et al*, 1998) and they are still widely used. This method employs the interaction between residue of interest and a bacterium used as the test organism. They are very cost-effective and in contrast to, for example, immunological or receptor-based tests have the potential to cover the entire antibiotic spectrum within one test (Pikkemaat, 2009).

Two main test formats can be distinguished: the tube test and the multi- plate test. A tube (or vial, or ampoule) test consists of a growth medium inoculated with (spores of) a sensitive test bacterium, supplemented with a pH or redox indicator. At the appropriate temperature, the bacteria start to grow and produce acid, which causes a color change.

The presence of antimicrobial residues prevents or delay bacterial growth, and thus is indicated by the absence or delay of the color change. This format is commonly applied in routine screening of milk (Suhren and Heeschen, 1996), but it is also increasingly used for analysis of other matrices (Stead *et al*, 2004; Kilnick *et al*, 2007).

A plate test consists of a layer of inoculated nutrient agar, where samples are applied on top of the layer, or in wells in the agar. Bacterial growth turns the agar into an opaque layer, which yields a clear growth-inhibited area around the sample if antimicrobial substances are present.

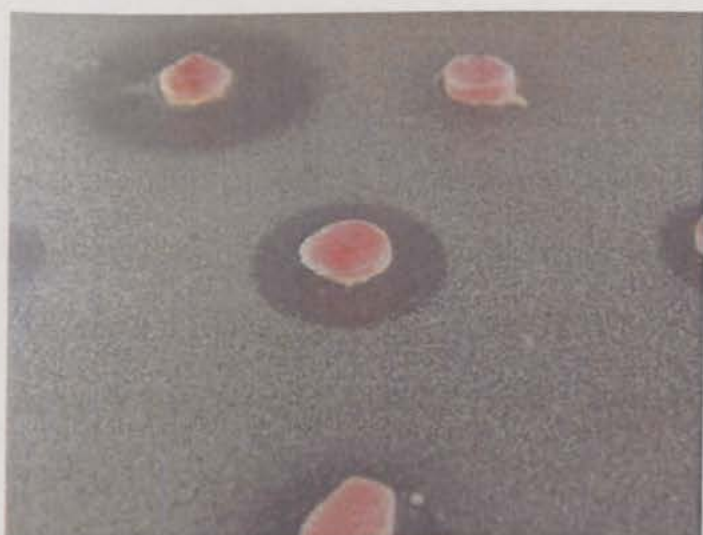


Figure 2.1: A photograph of microbial inhibition assay for muscle samples. Courtesy of Pikkemaat, (2009)

2.7.2 Diffusion methods

This method depends on the ability of the residue to diffuse from the site of sample application throughout the agar medium and inhibit growth of a test organism seeded in the layer (Katz, 1986). The diameter of the zone of inhibition is measured, after a predetermined incubation period, and comparing the diameter of the zone of inhibition with a standard curve the concentration of residue is determined (Katz, 1986)

Test organisms commonly used for the plate assays include *Bacillus subtilis* and *Bacillus cereus*. *Bacillus subtilis* has been widely used (Nouws *et al*, 1988; Koenen-Dierick *et al*, 1995) because of its sensitivity to a wide range of antimicrobials, and because of a commercially available spore suspension. *Bacillus cereus* strains have also been used to screen for tetracycline residues (Bugyei *et al*, 1994; Okerman *et al*, 2001 and 2004). The highest free drug concentrations of most antimicrobials are found in kidney and renal pelvis (Nouws, 1981; Aerts *et al*, 1995). Oxytetracycline concentrations in edible tissues could be predicted from kidney cortex concentrations (Myllyniemi, 2004).

Other confirmatory methods commonly used procedures for the detection of veterinary drug residues include High Performance Liquid Chromatography (HPLC), gas chromatography (GC), thin layer chromatography (TLC) and mass spectrometry (MS) (Aerts *et al*, 1995; McCracken *et al*, 2000). Chemical methods generally were considered too specific and expensive to be applied as an initial screening. Their use in developing countries therefore is very limited.

CHAPTER THREE

3.0 METHODS

3.1 Study Area

The study was conducted in the Dormaa municipality, which is one of the 22 districts of the Brong Ahafo region. It is located in the Mid-western part of Ghana. Dormaa municipality is bound on the north by Jaman and Berekum Districts, on the east by the Sunyani Municipality, in the south and southeast by Asunafo and Asutifi Districts, respectively. It is also bound on the southwest by Western region and in the west and northwest by la Cote d'Ivoire. The municipal capital is Dormaa Ahenkro, located about 80 kilometres west of the regional capital, Sunyani. The mainstay of the municipal economy is agriculture, which is estimated to employ about 60% of the districts economically active labor force. The Dormaa municipality was chosen for being the leading producer of poultry in Ghana.

The Dormaa municipality has 2 million birds spread in over 320 farms scattered over a total land area of 1,368 square kilometers (Veterinary Services Directorate, 2009). Poultry products from this municipality are retailed in almost all regions of Ghana, and a study here could give a good representation of the type of antibiotic residue in the poultry products. Samples were collected from 24 communities (Figure 2).

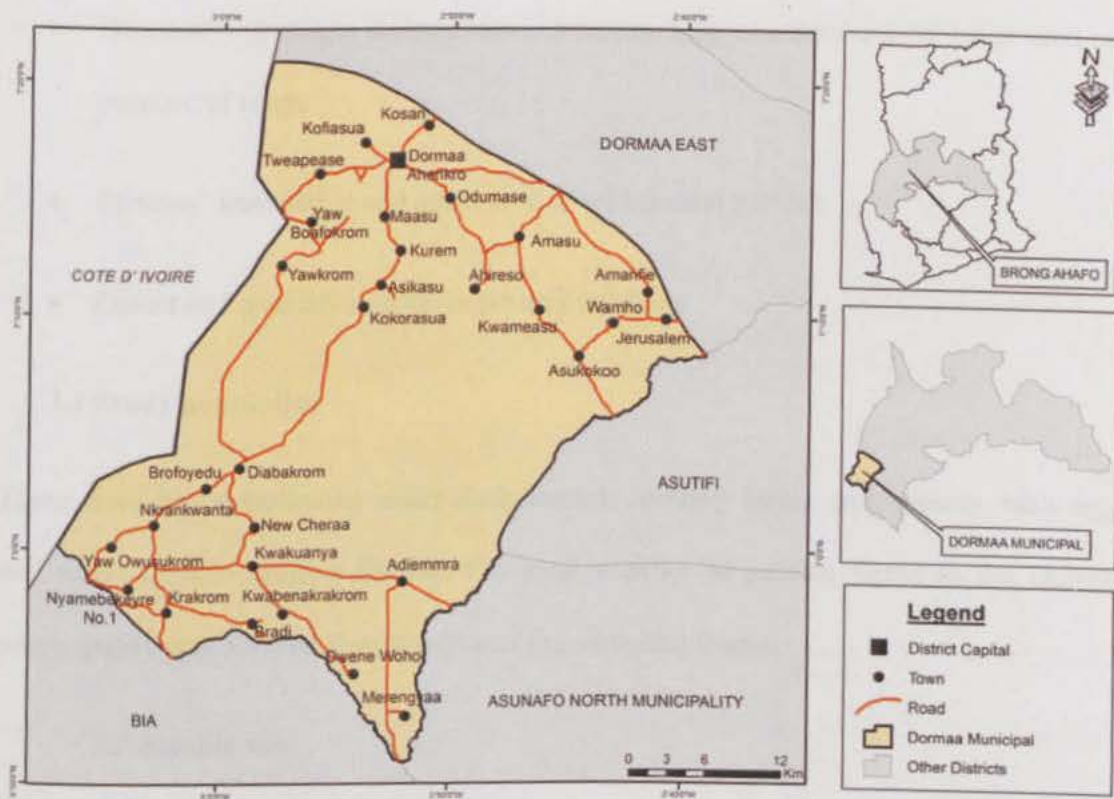


Figure 3.1: Map of Dormaa municipal showing some sampling sites, Brong Ahafo region.

3.2 Study Design

It was a cross sectional study involving poultry farmers and poultry products from the Dormaa municipality. The study was carried out from December 2010 to June 2011.

3.3 Variables

Variables under study were limited to the following:

- Socio-Demographics: age, sex, location, educational level, farming experience of respondents.

- Husbandry practices include record keeping, type of antimicrobial drugs used and purpose of usage
- Farmers' knowledge and adherence to withdrawal periods
- Detect and quantify residue in poultry products

3.4 Study population

There were four populations under study namely, poultry farms, respondents, table eggs and ready-for-sale broilers (birds). The total number of poultry farms in the Dormaa municipality was 320 and that constituted the sampling frame.

3.5 Sample size

Farms: In the absence of the prevalence of antimicrobial residue on poultry farms at the time of the study, a maximum prevalence of 50% was used to calculate the sample size. Therefore, at a 95% confidence level, and a maximum allowable error of 10%, the minimum sample size was arrived at 96 farms. In all a total of 115 farms were sampled.

Respondents: A total of 132 farmers were interviewed from the selected farms. Where available, an owner, caretaker (farm hand) and a manager were interviewed per farm. In seven farms, more than one person was interviewed.

Broilers and Eggs: With minimum and maximum farm sizes of 200 and 40,100 birds respectively, eggs and broilers were selected on probability- proportional- to- size basis as per Table 3.1. Each group of birds and eggs taken from a farm constituted a farm

sample. Eggs from each individual farm were pooled together to serve as a egg sample / farm.

Table 3.1. Selection of birds and eggs in the Dormaa municipality, December 2010 – June 2011.

| Bird Population | No. of Birds | No of Eggs | No of farms per stratum |
|------------------------|---------------------|-------------------|--------------------------------|
| < 1000 | 2 | 2 | 29 |
| 1000-10,000 | 3 | 3 | 69 |
| > 10,000 | 4 | 4 | 17 |
| Total | 9 | 9 | 115 |

Identification numbers were assigned to questionnaires, tissues and egg samples. This enabled the matching of questionnaire data with laboratory data on each farm to be done for purposes of analysis.

3.6 Sampling procedure

3.6.1 Selection of farms

A list of poultry farms in the Dormaa municipality was obtained from the Dormaa Veterinary Office and this constituted the sampling frame. Farm selection was conducted as follows: each farm on the sampling frame was allocated a number and all the numbers were put together in a bag. Farms were selected as the numbers were picked from the bag.

3.6.2 Selection of respondents

At the premises of the selected farm, people who were directly involved in the administration of drugs to the birds became the target of our interview. Three categories of respondents (where available on farm), who consented to be interviewed were selected, namely Owners, Managers and Caretakers and those who owned and also managed their farms, (i.e. Owners / Managers).

A structured interviewer-administered questionnaire was administered to each consenting respondent from the selected farms. The questionnaire assessed factors that could account for the occurrence of drug residues in products of animal origin, and it covered demographic characteristics, educational status, and type of antimicrobial drugs used, knowledge and adherence to withdrawal periods. (Appendix VI)

3.6.3 Selection of birds and eggs

Broilers and eggs were purchased a day or two after the same farms from which respondents were interviewed. The purchase of the birds and eggs was carried out by a research assistant other than the one who conducted the interview on any given farm. This was to ensure that it was done blindly to avoid interviewer-related bias.

3.6.4 Pre-laboratory processing of samples

A workstation was set up at the Dormaa veterinary office for the slaughter and harvesting of tissues. Purchased ready-for-sale broilers were slaughtered at the work-station, whole kidneys and livers were harvested, packaged and labeled. They were immediately frozen

at -20, until laboratory analysis. Eggs were also refrigerated at between 4°C and 8°C until lab processed.

Kidneys and livers were selected because of the sensitivity of the plate assay method used for the detection. An earlier test run had indicated that detecting antimicrobial residue in muscle using the antimicrobial plate assay developed was less sensitive compared to the excretory organs. The excretory organs were used as marker to determine what could be in edible tissues (Bugyei *et al*, 1994). Eggs were also purchased from the same farms as the broilers and tested.

3.6.5 Eligibility criteria

The criteria for selecting a farm were the availability of ready-for-sale broiler birds and table-eggs as well as the willingness of the farm to participate.

3.7 Data Collection Techniques and Tools / Instruments

3.7.1 Training of interviewers

Three research assistants were trained by the researcher in the questionnaire administration and basic communication skills. They were drawn from Dormaa municipality due to their familiarity with the research area. All 3 research assistants were veterinary field staff of the Ministry of Food and Agriculture. They were trained on the correct interpretation of each questionnaire in order to ensure consistency of responses, and also reduce interview-related errors.

3.7.2 Pre-Testing and Review of Instruments / Tools

Questionnaires were pre-tested in Cape Coast in the Central Region and an informal focus group discussion held with some members of the poultry farmers association. The outcome was used to improve the data collection tools before data collection begun.

3.7.3 Data Quality

All questionnaires were submitted at the end of each day and were reviewed by team members before submission in order to ensure completeness of data. Data was double entered, validated and checked for inconsistencies.

3.7.4 Data storage and analysis

All data collected were coded and securely stored to ensure privacy and confidentiality.

Exploratory data analysis was carried out to obtain descriptive statistics. Categorical variables, such as adherence to withdrawal period, level of education were compared using Chi square test and student t-test were used in analyzing quantitative variables like residue levels found in the poultry products. Comparison of means across more than two groups was done using ANOVA test. Binary logistic regression was used to express the relationship between binary dependent variable (such as presence or absence of antimicrobial residue level) and independent variables such as knowledge and adherence to withdrawal periods. Data entry and analysis was done in Epi Info (version 3.4.1).

3.7.5 Ethical clearance

The proposal was reviewed and approved by the Ethical Review Board of the Ghana Health Service (Ethical clearance ID No: GHS-ERC 08/01/11). Permission was sought and obtained from the District Directorate of Agriculture and the Local Poultry Farmers Association.

Verbal and written consent was duly sought from respondents individually. Participation was voluntary and confidentiality maintained.

3.8 Laboratory Methods

Laboratory processing of samples was conducted at the Accra veterinary laboratory. Frozen samples from Dormaa were transported chilled to Accra in a coldbox, in order to prevent thawing. Samples were sent to Accra veterinary laboratory within 7 days after collection. The assessment of antimicrobial residues in liver, kidney and eggs samples was carried out using microbial inhibition plate assay (Bugyei *et al* 1999); Pikkemaat (2009). The plate assay is based on the growth inhibition of *Bacillus cereus* and *Bacillus subtilis* on an agar medium. The test organism *Bacillus cereus* NCTC 7464 / ATCC® 10876 and *Bacillus subtilis* NCTC 10400/ ATCC 6633 were supplied by Becton Dickson International, Belgium. Oxytetracycline hydrochloride and Chloramphenicol were purchased from Letap Pharmaceutical Company, Ghana.

Bacillus subtilis and *Bacillus cereus* were used due to their sensitivity to a wide range of antimicrobials and commercial availability of spores (Nouws *et al*, 1988; Koenen-Dierick *et al*, 1995). *Bacillus cereus* strains have been used to screen for tetracycline residues (Bugyei *et al*, 1994; Okerman *et al*, 2001 and 2004).

3.8.1 Preparation of Plates for Antimicrobial Assay

Muller-Hinton agar (Liofilchem Bacteriology Products, Italy) was prepared according to the manufacturers instruction. Thirty six grams of agar was dissolved in 1000 milliliters of distilled water. The solution was autoclaved at 121°C for 15 – 20 minutes prior to use. Five milliliters of the prepared agar was poured into sterile petri dish of dimension 100 x 5mm to form a base layer. The agar was allowed to cool and solidify for about 4 hours. The plates were prepared in the morning, maintained at room temperature, and used in the afternoon of the same day.

The test organism of *Bacillus cereus* was prepared by diluting 1ml of spore suspension with 1ml of 0.9% physiological saline. Twenty microliters of the diluted spore suspension was added to test tubes containing 5ml of Muller-Hinton agar medium maintained at a temperature of 60°C. The contents of the tubes were rotated to ensure thorough mixing and poured onto the previously prepared base layers.

3.8.2 Preparation of Antimicrobial Standards

A stock solution of 10µg/ml was prepared by diluting 10³µg each of tetracycline hydrochloride and chloramphenicol in 1000ml of distilled water. These were further diluted to obtain 5µg/ml as a working solution.

Two grams of kidney or liver from non-medicated birds (blank tissues) were weighed out, ground in a mortar with pestle, and homogenized with 4 ml of distilled water to make a homogenate of 1:2 tissue dilutions. Blank tissues were harvested from 6-8 weeks old birds that were raised without antimicrobials.

A standard tissue concentration of 0.5µg/ml was prepared by adding 1ml of each working solution to 9 ml of the homogenate. The standard concentration was serially diluted to obtain concentrations of 0.25, 0.125, 0.0625, 0.03125, 0.015 and 0.0018 µg/ml. Twenty microliters of supernatants of each standard, was pipetted onto a 6mm filter paper disc. The filter paper discs were picked up with 20 G 2.5cm injection needle and gently placed on the prepared base agar.

Plates for each standard concentration point were prepared in duplicate, giving a total of 4 filter paper discs (2 per plate) for each concentration. The plates were incubated at 37°C for 18-20 hours. Zones of inhibitions around the paper disc were measured using a caliper to the nearest millimeter, and the mean zones of inhibition (\pm SD) value of 4 discs calculated.

A regression analysis of zones of inhibition (X-axis) versus drug concentration (Y-axis) was carried out using the standard formula

$$Y=Ae^{\alpha z}$$

Where

Y=standard concentration

A= intercept on the concentration Y-axis

e=natural logarithm

α =slope

z=measured zone of inhibition

The y-intercept (A), slope and correlation coefficient r was calculated using a regression analysis.

3.8.3 Sample tissue analysis

Two grams of kidney and liver from bird was weighed out and homogenized with 4ml of distilled water to make a homogenate of 1:2 tissue:dilution. One ml of the homogenate was then added to 2mls of distilled water and 20 μ g of the supernatant was pipetted on a filter paper disc and placed on the seeded Muller-Hinton agar. The same procedure was used for the egg samples.

Plates for each standard concentration point were prepared in duplicate, giving a total of 4 filter paper discs (2 per plates) for each concentration. The plates were incubated at 37°C for 18-20 hours. After incubation, each plate was examined and the diameters of the zones of inhibition were measured including the diameter of the filter paper. A positive sample was one that a clear and measurable zone of inhibition. Zones of inhibition were measured using sliding calipers, which were held on the back of the inverted petri plate. The mean zones of inhibition were plotted against tissue homogenate drug concentration to establish the tissue drug concentration / zone of inhibition relationships.

CHAPTER FOUR

4 RESULTS

4.1 Description of farms

A total of 115 farms were sampled. The total bird population ranged from 250 to 40,100 birds (Table 4.1). Forty (34%) farms sold their produce to the Ivorian market and the rest in Ghana.

Table: 4.1: Farm size and age of birds, Dormaa municipality, 2011

| Farm characteristic | Range (birds) | Median (birds) |
|---------------------|---------------|----------------|
| Total flock size | 250-40,100 | 2150 |
| Broiler population | 100-31,000 | 400 |
| Layer population | 200-40,000 | 1500 |
| Age of birds | Age (weeks) | Mean (SD) |
| Broilers | 6-12 | 7.5±1 weeks |
| Layers | 20-72 | 36±13.2 weeks |

4.2 Socio-demographic of respondents

A total of 132 respondents were interviewed from the 115 farms. In 7 farms, more than 1 person was interviewed. There were three categories of respondents namely, farm managers, owners and caretakers. Seven of the farm owners also doubled as managers.

Table 4.2: Total samples obtained per farm in the Dormaa municipality, December 2010 - June 2011

| Bird Population | No. of Birds / farm | No of Eggs / farm | No of Farms | Total samples | |
|-----------------|------------------------|----------------------|-------------|---------------|------------|
| | | | | Birds | Eggs |
| <1000 | 2 | 2 | 29 | 58 | 58 |
| 1000-10. 000 | 3 | 3 | 69 | 207 | 207 |
| >10.000 | 4 | 4 | 17 | 68 | 68 |
| Total | 9 | 9 | 115 | 333 | 333 |

An average of three birds and three eggs were were sampled per farms (Table 4.2)

Almost all (97.7%) the respondents were males. The mean (\pm SD) age of respondents was 33 ± 10 years with a range of 17 - 64 years. Ninety two (69%) of the farmers were aged between 20 to 39 years.

Table 4.3 shows the educational status of farmers, responsibility on farm and number of years in poultry farming (experience). Fifty four (40.9%) of the farmers had had a Junior High School (JHS) education, and only 6 (4.5%) did not have any formal education.

Fifty four (41%) farmers owned and also managed their farms. Fifty nine (44%) farmers have been in the poultry business for between 2 to 6 years and only 2 (1.5%) have been running their farms for more than 22 years.

Table 4.3: Socio-demographics of poultry farmers in the Dormaa municipality, December 2010 - June 2011.

| Socio-demographics | No (%) (n=132) |
|---|-------------------|
| Highest education Level attained | |
| Primary school | 16 (12.1) |
| JHS ¹ | 54 (40.9) |
| Secondary school | 42 (31.8) |
| Tertiary ² | 14 (10.6) |
| None ³ | 6 (4.5) |
| Responsibility on farm | |
| Caretaker ⁴ | 45 (34.1) |
| Manager ⁵ | 18 (13.6) |
| Owner | 15 (11.4) |
| Owner & Caretaker | 54 (40.9) |
| No of years in poultry farming | |
| ≤1 | 29 (22.0) |
| 2-6 | 59 (44.7) |
| 7-11 | 25 (18.9) |
| 12-16 | 10 (7.6) |
| 17-21 | 7 (5.3) |
| 22-25 | 2 (1.5) |

¹JHS: Junior High School

²Tertiary: farmers with polytechnic education and beyond

³None: individuals who never had formal education

⁴Caretaker: hired hands who were directly involved in administering antimicrobials to the birds.

⁵Manager: some farms had managers who were not the owners of the farm. They were in charge of the daily activities of the farm, including supervising caretakers.

Table 4.4, shows a relationship between highest level of education attained by the farmers and their responsibility on the farm. All 45 caretakers but two had received formal education. Half of the managers 9 (50%) had JHS education. None of the 15 owners was uneducated. Only 3 (5.6%) of the 54 farmers who owned and managed their farm, had no formal education. There was no statistical association ($p>0.05$) between the level of education and responsibility on farm.

Table 4.4: Highest educational level and responsibility of farmers in the Dormaa municipality, December 2010 - June 2011

| Highest Education level attained | Responsibility on Farm | | | | Total (%) |
|----------------------------------|--|------------------|------------------|--------------------------|--------------------|
| | Caretaker ¹ n (%) [*] | Manager n (%) | Owner n (%) | Owner & Manager n (%) | |
| Primary school | 9 (56.3) | 2 (12.5) | 1 (6.3) | 4 (25) | 16(12.1) |
| JHS ^o | 19 (35.2) | 9 (16.7) | 7 (13.0) | 19 (35.2) | 54 (40.9) |
| Secondary school | 14 (33.3) | 3 (7.1) | 6 (14.3) | 19 (45.2) | 42 (31.8) |
| Tertiary | 1 (7.1) | 3 (21.4) | 1(7.1) | 9 (64.3) | 14 (10.6) |
| None | 2 (33.3) | 1 (16.7) | 0.0 | 3 (50.0) | 6 (4.5) |
| Total | 45 (34.1) | 18 (13.6) | 15 (11.4) | 54 (40.9) | 132 (100.0) |

*Figures in parenthesis are row percentages only.

^oJHS: Junior High School

4.3 Husbandry practices

4.3.1 Training of farmers in antimicrobial usage

Eighteen (13.6%) confirmed to have received their training from other experienced poultry farmers. All farms practiced intensive poultry farming where birds were housed

throughout the production period. Broilers were raised for between 6 to 7 weeks by 77.4% of the farms. Only 7 (6.1%) farms kept broilers beyond 8 weeks. All farms administered antimicrobial drugs to the birds for therapeutic and prophylactic purposes. Majority of the farms, 97 (86%) administered drugs to the birds for prophylactic purposes. Oral route medication was the method commonly employed by the farmers to medicate their birds, with 81 (70.5%) farms administering them in drinking water, whilst, 34 (29.6%) administered in both drinking water and feed.

Of 102 who kept records of activities on the farm only 9 (8.8%) kept medication charts (Table 4.5). Only 16 (12.1%) farmers administered the medication on prescription from the veterinary office before purchasing of drugs. Available records at the Dormaa veterinary office indicated that there were 15 private veterinary drug stores in the Dormaa township alone.

Table 4.5: Husbandry practices with regards to antimicrobial usage on poultry farms in Dormaa municipality, December 2010 – June 2011

| Husbandry practice | No (%) |
|--|------------|
| Record keeping (n=132) | |
| Yes | 102 (77.3) |
| No | 30 (22.7) |
| Type of records (n=102) | |
| Daily tally card | 39 (38.2) |
| Medication chart | 9 (8.8) |
| Production records | 21 (20.6) |
| *Unavailable records | 33 (32.4) |
| Source of drugs (n=132) | |
| Vet office | 4 (3.0) |
| Private vet shop | 107 (81.1) |
| Private shop and vet office | 21 (15.9) |
| Source of instruction (n=118) | |
| Other poultry farmers | 12 (11) |
| Shop attendants | 86 (72.9) |
| Vet staff | 19 (16.1) |
| Obtaining individual prescription before drugs purchase (n=132) | |
| Yes | 16 (12.1) |
| No | 84 (63.7) |
| Sometimes | 32 (24.2) |

*Unavailable: unavailable records for confirmation

4.3.2 Commonly used drugs

Drugs that were commonly used by the farms were antibacterial 110 (95.7%), concurrently with coccidiostats (antiprotozoals). Twenty farms (17.4%) had dewormed their birds. Among the antibacterials, tetracyclines, in various forms such as oxytetracycline and doxycycline were widely used (Table 4.6). Other antibacterials were sulfa-based drugs, enrofloxacin, tylosin and neomycin. These drugs were administered singly or in combination with other drugs.

Antiprotozoals commonly used were 25 (22.7%) Amprolium hydrochloride, 5 (4.3%) Pharmacox® (Totalzuril), 15 (10%) Prococ® (Amprolium hydrochloride and sulfaquinoxaline base), 7 (6.3%) Narcoc®, and others in smaller percentages.

Out of the 16 farms that had given dewormers, 6 (30%) gave kepromec® oral (ivermectin), 8 (40%) levamisole (water soluble), and 6 (30%) piperazine.

Table 4.6: Antimicrobial drugs used by farmers in the Dormaa municipality, December 2010 – June 2011

| Drug | n (%) (n=115) | 95% Conf Limits | Withdrawal period [∞] | Active ingredients |
|--------------------------------|------------------|--------------------|-----------------------------------|---|
| Oxyfuravit* | | | Not on label | Oxytetracycline, furaltadone and vitamins |
| Yes | 41(35.7) | 26.9 – 45.1 | | |
| No | 74 (64.3) | 54.9 - 73.1 | | |
| Doxin | | | 7 days | Doxycycline hyclate and tylosin tartrate |
| Yes | 31 (27.0) | 19.1 - 36.0 | | |
| No | 84 (73.0) | 64.0 - 80.9 | | |
| TCN | | | 21 days | Tetracycline, Chloramphenicol and Neomycin sulphate |
| Yes | 5 (4.3) | 1.4- 9.9 | | |
| No | 110 (95.7) | 90.1- 98.6 | | |
| Antibact* | | | Meat: 14 days Eggs: 4 days | Tylosin tartrate, Oxytetracycline HCL and Neomycin sulphate |
| Yes | 16 (13.9) | 8.2- 21.6 | | |
| No | 99 (86.1) | 78.4 - 91.8 | | |
| Enrocoli | | | 4 days | Enrofloxacin |
| Yes | 25 (21.7) | 14.6 - 30.4 | | |
| No | 90 (78.3) | 69.6 -85.4 | | |
| Tyo Dox* | | | 15 days | Tylosin and Doxycycline |
| Yes | 14 (12.2) | 6.8- 19.6 | | |
| No | 101 (87.8) | 80.4- 93.2 | | |
| Sulfa – based drugs | | | 7- 14 days | Sulfadimidine, sulfaquinoxaline |
| Yes | 7 (6.1) | 2.5 - 12.1 | | |
| No | 108 (93.9) | 87.9 - 97.5 | | |

*Commercially combined drugs.

∞Withdrawal period: for poultry only.

7-14 days of withdrawal period depending on the type of sulfa drugs.

4.3.3 Frequency of drug use

Forty seven percent (63) of the farmers administered drugs to the birds on weekly basis to the broilers and 48 (36.4%) bi-weekly for to the layers. All veterinary antimicrobial drugs have withdrawal periods, and with this frequency of administration, farmers would not be able to adhere to them.

4.4 Extra-label use of drugs

The use of two antibiotics and a disinfectant that were not indicated for veterinary use was observed on some farms during the period of the study. They were tetracycline and chloramphenicol hydrochloride capsules for human use, and Omnicide®, a disinfectant (Table 4.7).

Table 4.7: Extra label use of 2 antibiotics and a disinfectant in the Dormaa municipality, December 2010 – June 2011

| Drugs | No of farms (n=115) | % | 95% Conf Limits |
|-------------------------------------|------------------------|------|-----------------|
| Tetra Capsules® | | | |
| Yes | 20 | 17.4 | 11.0 - 25.6 |
| No | 95 | 82.6 | 74.4 - 89.0 |
| Chloramphenicol Capsules | | | |
| Yes | 3 | 2.6 | 0.5 0- 7.4 |
| No | 112 | 97.4 | 92.6 - 99.5 |
| Omnicide® | | | |
| Yes | 51 | 44.3 | 35.1 - 53.9 |
| No | 64 | 55.7 | 46.1- 64.9 |

*Tetra: tetracycline hydrochloride capsules

*Omnicide: a disinfectant that contains gluteraldehyde, alkyl dimethyl and benzyl amomonium chloride.

4.4.1 Tetracycline capsules

In Table 4.7, it was found that 20 (17.4%) farms used tetracycline hydrochloride capsules (Tetra®) to water - medicate the birds. Tetracycline is a bacteriostatic antibiotic with a broad spectrum activity. The farmers said they used the capsules mainly to treat diarrheal diseases. This form of the drug is meant for human use and therefore did not have withdrawal periods indicated on the label. Emptied Tetra® capsules were found littered on the compound of some farms visited (Figure 4.1 and 4.2).



Emptied
Tetracycline
capsules

Figure 4.1: A photograph of tetracycline hydrochloride emptied capsules disposed off near a chicken pen, Dormaa, 2011



Spilled
tetracycline
from the
capsules

Figure 4.2 a closer look at the capsules

4.4.2 Chloramphenicol capsules

Three farms (2.6%) were also found to water-medicate their birds with Chloramphenicol capsules (Table 4.7; Figure 4.3, 4.4 and 4.5). Chloramphenicol is a broad spectrum antibiotic and the formulation (capsules) is meant for human use only.



Chloramphenicol capsules purchased from human drug store

Figure 4.3: Chloramphenicol capsules container on some farms, Dormaa municipality, 2011



Emptied Chloramphenicol capsules

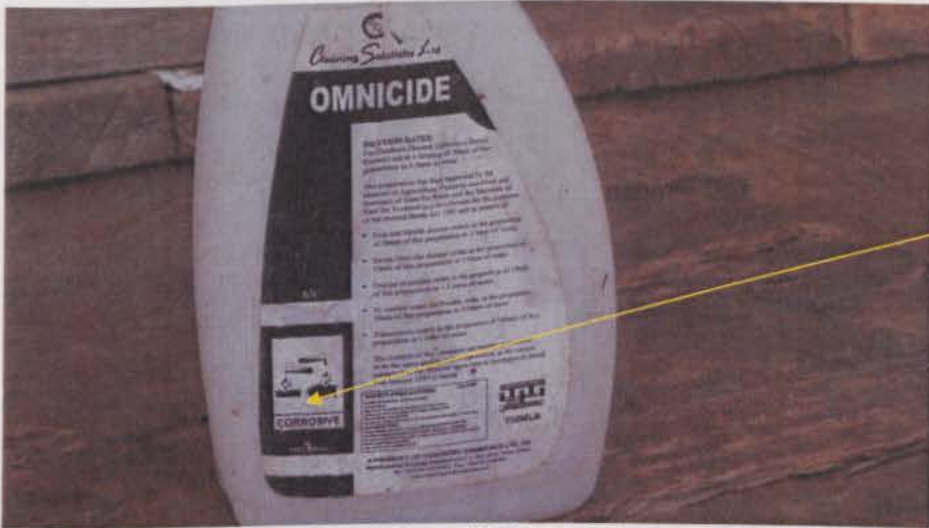
Figure 4.4: Emptied chloramphenicol capsules disposed off outside a poultry shed.



Figure 4.5: A closer look at the emptied chloramphenicol capsules.

4.4.3 Omnicide®

Table 4.7 again shows the use of Omnicide by 51 (44.3%) of the farms as an oral antimicrobial drug for the birds. Omnicide is a broad spectrum disinfectant concentrate, and it was used by the farmers to treat and prevent viral diseases such as Newcastle disease and Gumboro disease (Infectious Bursal Disease). Omnicides containers were found in some of the farms visited during the study period (4.6 and 4.7).



Corrosive
sign on
omnicide

Figure 4.6: A photograph of Omnicide, a disinfectant that some farmers administered to the birds, Dormaa municipality, 2011



Figure 4.7: Back cover of omnicide, where the product description and usage is indicated

4.5 Withdrawal Periods

Withdrawal period of all drugs used ranged from 1-21 days for meat and 1-14 days for eggs. Eighty one (61.4%) farmers did not know what withdrawal period was. Although 47 (35.6%) farmers said they knew, and gave different definitions, only 13 (28%) defined withdrawal period correctly as the interval from the time an animal is removed from medication until permitted time for slaughter. Other definitions of withdrawal period given by the farmers are summarized below:

"The number of days you withdraw the water before you give the drug"

"The time that the chicken will be safe for consumption"

"Time to prevent food poisoning"

"Problem that the user can get after using the product"

"You don't eat meat during that period"

The farmer's level of education did not seem to affect the knowledge of withdrawal period since 81 (61.4%) of the farmers who said they did know about withdrawal period had had some level of education and this was statistically significant ($p=0.04$). Most farmers (84.8%) said they would not wait to sell their broilers whilst they were on medication. The remaining 20 will wait as follows: 3 (2.3%) for a week; 3 (2.3%) for 3 days; 5 (3.8%) for 2 weeks, and 9 (6.8%) will wait till the end of the treatment. Majority of the farmers 123 (93.2%) would not wait to sell eggs when the laying birds were on medication.

Some of the drugs, such as Oxyfuravit did not have withdrawal period indicated on the label, which was contrary to what was expected from a pharmaceutically manufactured drug.

4.6 Knowledge of human health effect of poultry drugs

On the effect on health of antimicrobial drug administered to birds, 54 (40.9%) farmers said there was no effect on human health when the drugs are administered to birds. Thirty two (24%) did not know if there was any effect, however, 46 (34.8%) knew that it could have an effect on human health. Some of the effects given are shown below:

"Can cause coughing, example omnicide®"

"Can cause stomach pains"

"Can make us sick"

"Salivation"

"We may get diarrhea"

"Not sure of the kind of effects"

4.7 Laboratory investigation

4.7.1 Tissue standard concentrations

Tables 4.8 summarizes results of microbiological plate assay of tetracycline-spiked homogenates of eggs, kidney and liver standard concentrations. The limit of detection of tetracycline for tetracycline-spiked eggs, liver and kidney were 0.01µg/g. The correlation coefficient r calculated for the standard tissues were 0.89 (eggs), 0.97 (kidney) and 0.94

(liver) (Appendix 2), suggesting a positively strong linear relationship between the concentration of drug standards and the zones of inhibition.

4.7.2 Residue in samples

Table 4.8 show tetracycline residue levels that were found in eggs, kidney and liver. Twenty eight (24.3%) farms had detectable levels of tetracycline residue in eggs, 25 (21.7%) in kidneys and 14 (12.2%) in liver. The mean (Std dev; $\mu\text{g/g}$) for eggs, kidney and liver were 0.02 ± 0.003 , 0.02 ± 0.01 and 0.01 ± 0.008 respectively. In all the products, the concentration of tetracycline ranged from $0.01 - 0.03 \mu\text{g/g}$.

Table 4.8: No of farms in Dormaa municipality showing detectable levels of tetracycline in poultry products, 2011

| Type of poultry products | No of positive farms | Mean zone of inhibition (mm) | Mean Conc* ($\mu\text{g/g}$) | Range ($\mu\text{g/g}$) |
|--------------------------|----------------------|------------------------------|--------------------------------|---------------------------|
| Eggs | 28 (24.2%) | 10.2 ± 6.5 | 0.02 ± 0.003 | 0.01- 0.03 |
| Kidney | 25 (21.7%) | 9.7 ± 14.1 | 0.02 ± 0.01 | 0.01 – 0.03 |
| Liver | 14 (12.2%) | 6.0 ± 2.7 | 0.01 ± 0.008 | 0.01– 0.03 |

1*Concentration

4.7.3 Relationship between farm practices and residue in products

Table 4.8 describes the relationship between some farm practices and the presence of residues in their poultry products. Majority of the farms 26 (92.9%) that had sold eggs whilst the laying birds were on medication had detectable residue in the eggs.

Table 4.9: Influence of farm practices on the presence of tetracycline residue in poultry in Dormaa municipality, December 2010 – June 2011

| Farm practice | No of farms with residue by products (%) | | | | | |
|--------------------------------------|--|--------------|-----------------|--------------|----------------|--------------|
| | Kidney (n=25) | p values | Liver (n=14) | Pvalues | Eggs (n=28) | pvalues |
| Sale of eggs during treatment | | | | | | |
| Yes | | | | | 26 (92.9%) | 0.002 |
| No | | | | | 2 (7.1%) | |
| Age of birds at sale | | | | | | |
| 7-8 weeks | 17 (68%) | | 9 (64.3%) | | | |
| 8+ weeks | 5 (20%) | 0.004 | 2(14.3%) | 0.30 | | |
| 6 weeks | 3 (12%) | | 3 (21.4%) | | | |
| Tetra® Capsules use | | | | 0.001 | | 0.10 |
| Yes | 13 (52%) | 0.001 | 8 (57.1%) | | 7 (25%) | |
| No | 12 (48%) | | 6 (42.9%) | | 21 (75%) | |
| Oxyfuravit® Use | | | | | | |
| Yes | 15(60%) | 0.001 | 8 (57.1%) | 0.20 | 18 (64.3%) | 0.001 |
| No | 10 (40%) | | 6 (42.9%) | | 10 (35.7%) | |

∞ND: No detectable level *

Oxyfuravit® (Oxytetracycline, furaltadone and vitamins)

~Tetra (Tetracycline)

Over half (17; 68%) of the farms which showed the presence of tetracycline residue sold birds broilers between 7 and 8 weeks of age. (Table 4.9). It was also noted that 13 (52%) of the farms that had residue in the kidneys of the birds had administered Tetra® capsules to the birds. An association between Oxyfuravit® use and the presence of residues is also seen in Table 4.9 where farms had residues in kidneys 15 (50%; $p=0.001$), liver 8 (57.1%; $p=0.20$) and eggs 18 (64.3%; $p=0.001$).

CHAPTER FIVE

5.0 DISCUSSION

The present study was undertaken to determine the factors contributing to the occurrence of antibiotic drug residue in products of animal origin; assess poultry farmers' knowledge and practices of antimicrobial drugs usage with regards to adherence to withdrawal periods and to detect and quantify the type of antimicrobial agents in poultry products.

5.1 Contributing factors to the occurrence of residue

5.1.1 Husbandry practices

The finding that out of 115 farms, 24.3% had detectable antimicrobial residue in eggs 21.7% in kidneys and 12.2% in liver, did not come as a surprise, although the tetracycline concentration in the products was not above the Maximum Residue level (MRL) recommended by Codex Alimentarius. All farms in the Dormaa municipality practiced intensive poultry farming and thus required the use of antimicrobial medication to keep the birds healthy. As many as 86% of the farms, administered antimicrobial agents to the birds for prophylactic purposes. This agrees with the assertion of Tollefson and Miller, (2000) that the increased density of livestock or poultry in intensive rearing practices required an aggressive approach to disease control, which could lead to heavy prophylactic and therapeutic antimicrobial use.

Another factor that could have led to the presence of drug residue in the poultry products was the non-adherence to withdrawal period. Although an appreciable number (35.6%) of farmers said they knew what withdrawal period was, 84.8% and 93.2% would sell broilers and eggs respectively, whilst birds were on medication. This finding supports those of previous studies which concluded that poultry producers did not always adhere

to withdrawal periods of the veterinary drugs used on their farms (Pavlov *et al*, 2008; Sharref *et al*, 2009 and Ezenduka *et al*, 2010).

The presence of residue in eggs from 23.4% farms in this study however, was lower than reported by Al-Ghamdi *et al*, (2000) in eastern province of Saudi Arabia. In that study 60.0% of layer farms had eggs contaminated with tetracycline and the amount detected exceeded the Maximum Residue Level in 14.4% of raw eggs tested.

The antimicrobial residue of 24.3% in this study is however higher than what Donkor *et al*, (2011) found in some parts of Ghana, namely Accra and Kumasi. They reported a prevalence rate of drug residues in eggs to be 6.8%. Reasons for the variance could be firstly, that samples were collected from various parts of the country. Secondly, in the current study, focus was on the locally produced poultry in Dormaa municipality only. Unlike in that study where eggs sampled may have come from various parts of Ghana and possibly from outside Ghana. In the current study, eggs were collected from the same farms, complemented by information from respondents.

A significant association ($p < 0.05$) between non-adherence to withdrawal period and the presence of residue in products from the farms was seen when 92% of the positive farms had not adhered to withdrawal periods. This finding is similar to what was found in Tanzania by Nonga *et al*, (2009) where 95% of the farmers slaughtered their birds without observing the withdrawal periods recommended for the antimicrobial drugs used.

Reasons given by the farmers in Dormaa for selling the products without observing withdrawal period was economic and availability of ready market for their produce. Eggs and broilers were sold for money to feed replacement birds. Turkson, (2008) and Donkor

et al, (2011) in separate studies in Ghana also found that farmers' non-adherence to withdrawal period was mainly due to economic reasons.

The non-adherence to withdrawal period by the farmers led to the presence of residue in the poultry products and this could have public health impact, especially because there is neither control from the poultry industry or the government regulatory agencies on the types of antimicrobial drugs used in layers. Further, there is no monitoring system for antimicrobial residues in poultry products. Oboegbulem and Fidelis, (1996) in a study to detect antimicrobial residues in poultry meat and slaughter cattle in Nigeria, concluded that there was no national program for routine monitoring of residues in food animals in most developing countries.

A statistically significant association between oxyfuravit® use and the presence of residues in products, was found in this study. where farms had residues in kidneys (60%), liver (57%) and eggs (64.3%). Oxyfuravit® is a commercially prepared combination drug that is used in the Dormaa municipality. About one third (35.7%) of the farms had administered it to the birds at the time of the study. It is a broad spectrum antibacterial agent composed chemically of oxytetracycline, furaltadone hydrochlorides and vitamins. In spite of the extensive use of this drug, withdrawal period was not indicated on the label. It was therefore not surprising that farmers were not adhering to a withdrawal period as there was none to which comply. In an FAO legal paper on Legislation for veterinary drugs control, Fingleton (2004), suggested that among the risks for a country with ineffective regulations for its drug supply are inadequate labeling of drugs. Users are therefore not properly informed on dosage, expiry date, dangers and precautions. A drug which was inadequately labeled was inappropriately used, the animal

was ineffectively treated and resistance would have developed in the animal and in humans consuming the animal's products (Fingleton, 2004). It is therefore a worrying situation that a drug being used for food animals did not show a withdrawal period.

5.2 Extra-label use of drugs

A disinfectant and two antibiotics formulated for human use were being administered to birds in the Dormaa municipality. They are omnicide, a disinfectant and, tetracycline and Chloramphenicol hydrochloride capsules.

5.2.1 Omnicide®

Nearly half, 44.3% of the farms sampled used Omnicide as water medication for the birds at the time of the study. Omnicide is a broad-spectrum disinfectant concentrate which contains Glutaraldehyde, cocobenzyl and Ammonium chloride. As a disinfectant, omnicide has no directions for oral use and as such did not indicate a withdrawal period. Omnicide has many applications such as cold sterilant and disinfectant in animal housing (CDC, 2010).

Glutaraldehyde, the main active ingredient in omnicide is a saturated, aliphatic dialdehyde that exists as a pungent oil at room temperature. It is most often used in a diluted form with solutions ranging from 0.1% to 50% glutaraldehyde in water. Trade names for glutaraldehyde-containing formulations include Cidex®, Sonacide®, Sporidicin®, Hospex®, Omnicide®, Metricide®, Rapicide® and Wavicide®.

In the health care industry, glutaraldehyde is most often used to disinfect equipment that cannot be heat sterilized such as dialysis instruments, surgical instruments, suction bottles, bronchoscopes, endoscopes, and ear, nose, and throat instruments.

Glutaraldehyde is known to be corrosive and irritating to the eyes, skin and respiratory tract and recognized as a cause of health problems in the exposed (CDC,2010; Tagikawa, 2006). It is also used as a cross-linking and tanning agent, a biocide in metalworking fluids and in oil and gas pipelines, a preservative in cosmetics, a slimicide in paper manufacturing, a tissue fixative in histology and pathology labs as well as hardening agent in the development of X-rays and in embalming solutions (CDC, 2010). The use of Omnicide® without any precautionary measures such as to protect facial mask could lead to respiratory problems (Tagikawa and Endo, 2006).

Workers can be exposed to gluteraldehyde through inhalation or skin contact and some of the adverse health effects include throat and lung irritation, asthma and difficulty breathing, burning eyes and conjunctivitis and contact and/or allergic dermatitis, and nasal irritation and sneezing.

The farmers in Dormaa administered Omnicide® to birds at a dose of 5mls per 5-10 liters of water (1ml/1-2liters of water) orally to birds for 3-5 consecutive days. They did this to treat and prevent viral diseases such as Newcastle disease and Gumboro.

It is interesting to note that among the health effects that the farmers mentioned, a respondent mentioned specifically that some of the drugs could cause coughing and gave as an example that of Omnicide®, “*Can cause coughing, example omnicide*” (See under section 4.6)

The above response suggests that the farmer may have experienced this effect as a result of omnicide use in poultry farming.

A study in Australia reported that the use of glutaraldehyde as a disinfectant in the animal housing industry has led to sporadic reports of adverse health effects, for example; an egg

collector in South Australia experienced facial and respiratory irritation after spraying eggs with 0.1-0.3% glutaraldehyde solution (Tkaczuk *et al*, 1993).

During the pre-testing of the research tool in Cape Coast, it was noted that the farmers were using Quincide[®], another disinfectant as water sanitizer. Quincide[®] is a non toxic and non corrosive disinfectant that has water sanitizing properties which is indicated on the label. It is made up of quaternary compound and glutaraldehyde solutions at 50%. However, Omnicide[®] does not have any indication on the label to show if it had water sanitizing properties.

5.2.2 Tetra[®] Capsules use.

Tetra[®] capsule is one of the trade names for tetracycline hydrochloride capsules. Tetra[®] capsule is a human formulation of tetracycline hydrochloride and so did not have withdrawal period on the label. There is an animal formulation of tetracycline which appears to be one of the most widely used antimicrobial around the world to promote animal health (Wilson *et al*, 2003). However, 17.1% of the farms was using Tetra capsules at the time of the study. Tetra capsules were emptied and dissolved in water to water-medicate the birds to treat diarrheal conditions. Ten to 15 of 250mg capsules of tetra were dissolved in 3-4 gallons (18 liters) of water for oral medication for 3-4 consecutive days. Farmers gave economic reasons for the use of tetra capsules in poultry. One kilogram of tetra capsules from the human drug store in Dormaa costs \$6.0 whilst the animal formulation of tetracycline from the veterinary shop cost more than twice that amount, (thus \$16.0).

This finding agrees with a report by Adam *et al*, (1995), that the high cost of drugs was the main reason why farmers resorted to unorthodox practices and medicaments to treat animal diseases. According to these workers, these practices included the use of antibiotic capsules, especially tetracycline and ampicillin, meant for human treatment to treat diarrhea and other ailments in animals,. Although the dosage frequency may be compromised, farmers appeared happy with the results obtained from the extra label use of the tetra capsules.

It was interesting to note that tetracycline hydrochloride capsules were normally not mentioned as part of the commonly used drugs on the farms, but the littering of empty capsules (Figure 4.1 and 4.2) confirmed it is used on the farms. It appeared the farmers who used the capsules were aware they were not doing the right thing and so were reluctant to mention the use.

According to FDA, (1998) Extra-label use of drugs by non-veterinarians in food-producing animals is a significant public health concern and a contributing factor to illegal residues in animal tissue. It therefore came not as a surprise to note that that 10 (62.5%) of the farms that had residue in the kidneys of the birds had administered tetra capsules to the birds. Salehzadeh *et al*, (2006) also found in a study that all farms they sampled showed oxytetracycline residue above the maximum residue level in the poultry products. This current study confirmed widespread abuse of oxytetracycline in the Dormaa municipality.

Tetracycline residues that exceed the tolerance level may be of toxicological concern in humans and animals. These include gastrointestinal disturbances, teratogenic risk to the

fetus, allergic reactions (Schenk and Collery, 1998) and development of resistant pathogens. (Bogaard and Stobberingh, 2000). Tetracyclines in meat may also potentially stain the teeth of young children (Walton *et al*, 1994). It is therefore worrisome to find that tetracycline is being used in an extra-label manner without any adherence to withdrawal period to.

A statistically significant association ($p=0.05$) was found between the use of oxyfuravit and the presence of tetracycline residue in poultry products. Fifty two percent of the farms that had residue in kidneys, had administered oxyfuravit to the birds.

5.2.3 Chloramphenicol hydrochloride capsules

Chloramphenicol is a broad spectrum antibiotic that is used to treat a wide range of microbial infections, including typhoid fever and other forms of salmonellosis, and central nervous system, anaerobic and ocular infections (Bartlett, 1982; Sande and Mandell, 1985).

Three farms (2.6%) were found to water-medicate the birds with Chloramphenicol hydrochloride capsules. Chloramphenicol was banned by the US Food and Drug Administration (FDA) in 1998 from use in food-producing animals because of potentially harmful effects on humans health (FDA, 1998). Countries that have banned its use include USA, Canada, Australia and EU member states (JECFA, 1994). In countries in which its veterinary use is permitted, food regulations require withdrawal periods so as to avoid residues in the final product (FAO/WHO Expert Committee on Food Additives, 1988).

Chloramphenicol is believed to have been widely used as a veterinary antibiotic, despite

legal controls in many countries, and there have been a few reports of residual amounts in various animal products (Allen, 1985). Recently, chloramphenicol residue was detected in imported products to EU including poultry, shrimps and honey causing a huge economical impact. (Tajick *et al*, 2010). It is still used in cats, dogs, and horses to treat both systemic and local infections (MVM, 1998).

Regardless of its route of administration to humans, chloramphenicol is readily absorbed and extensively distributed (HSDS, 1995). Medical and veterinary personnel who use drugs containing chloramphenicol may be exposed (MVM 1998; DFC, 2000). An allowed daily intake (ADI) has also not been set for chloramphenicol because of the dose-independence of chloramphenicol-induced aplastic anaemia (FAO/WHO Expert Committee on Food Additives, 1988).

In Ghana, there are veterinary antibiotic preparations containing chloramphenicol though these restricted on other countries (Turkson, 2008). This was evidenced in this study by the use of drugs such as TCN[®] (Tetracycline, Neomycin and Cloramphenicol) on the farms at Dormaa.

Environmental exposure to chloramphenicol may occur through inhalation, dermal contact, ingestion, or contact with contaminated water or soil (HSDB, 1995). Odeyemi, (1997) observed that, as liberalization of drugs importation in developing countries mostly took place before any effective control and enforcement of legislation was put in place, there was a potential for unbridled importation and distribution of all types of drugs and chemicals. Furthermore, poor handling of these drugs might ultimately result in residues in slaughtered animals, as well as contamination of the environment, with the emergence of microbial resistance problems in the future. Therefore, the littering of

emptied chloramphenicol capsules, (some with its contents) and containers on some farms as found in this study posed a serious health hazard for the farmers in the Dormaa municipality.

CHAPTER SIX

6.0 CONCLUSION

In this study, it was found that tetracyclines were the main antimicrobial drugs used by the farmers in the Dormaa municipality with little or no regards to withdrawal period. Detectable antimicrobial residue was found in eggs (24.3%), kidneys (21.7.9%) and liver (3.5%) of birds in poultry farms in the Dormaa municipality. However, the level of tetracycline residue detected was below the MRL set for tetracycline by Codex Alimentarius. There was a significant association between husbandry practice such as sale of eggs and broilers whilst birds were on medication and the occurrence of antimicrobial residue in the poultry products.

Poultry industry is dominated by young literate men with varying level of formal education. However, the association between level of education and knowledge of withdrawal period was not statistically significant. Almost three quarters of the farmers had not had any training in antimicrobial usage and they relied heavily on veterinary shop attendants as well as experienced poultry farmers for instruction on drug usage. Less than 15% of the farmers obtained prescription before purchase of drugs for their poultry. The level of education did not have influence on farm practices with regards to antimicrobial usage and withdrawal period.

Forty percent of the farmers did not know the effect on human health when the drugs are administered to birds. Without any monitoring measures in place for drug residue in poultry products, heavy responsibility is placed on the farmer to observe the period for a withdrawal of a drug prior to slaughter to ensure that illegal concentration of drug residue in chicken and egg do not occur.

Extra-label use of two antibiotics and a disinfectant to water medicate birds is a public health hazard that exposed the absence or weakness in the control of drugs sale and usage in poultry production. It has also exposed the improper disposal of drugs and containers on poultry farms, a practice that could cause drug resistance in the poultry industry.

6.1 Recommendations

Veterinary Service, Ministry of food and Agriculture

1. Conduct educational and awareness programs targeted at farmers and field workers on the correct and appropriate use of veterinary drugs in order to avoid residue from occurring in the animal products. Such campaigns must take into account the socio-economic status of the farmers and the level of veterinary advice available to them in the field.
2. Set up systems to monitor drug residues in poultry products, as is done for other meat products.

Food and Drugs Board

1. Develop simple and economic field test to identify drug residue in poultry products.
2. Conduct nationwide monitoring and periodic surveillance of microbial residue in animal products.

6.2 Scope for further work

Two further studies would be required as a follow up to this one:

- A study to determine the knowledge level of the veterinary drug store attendants. This will be useful in assessing the kind of instruction poultry farmers are receiving from them.
- Effects of omnicide[®] on the health of the farmer / administrators to the birds.

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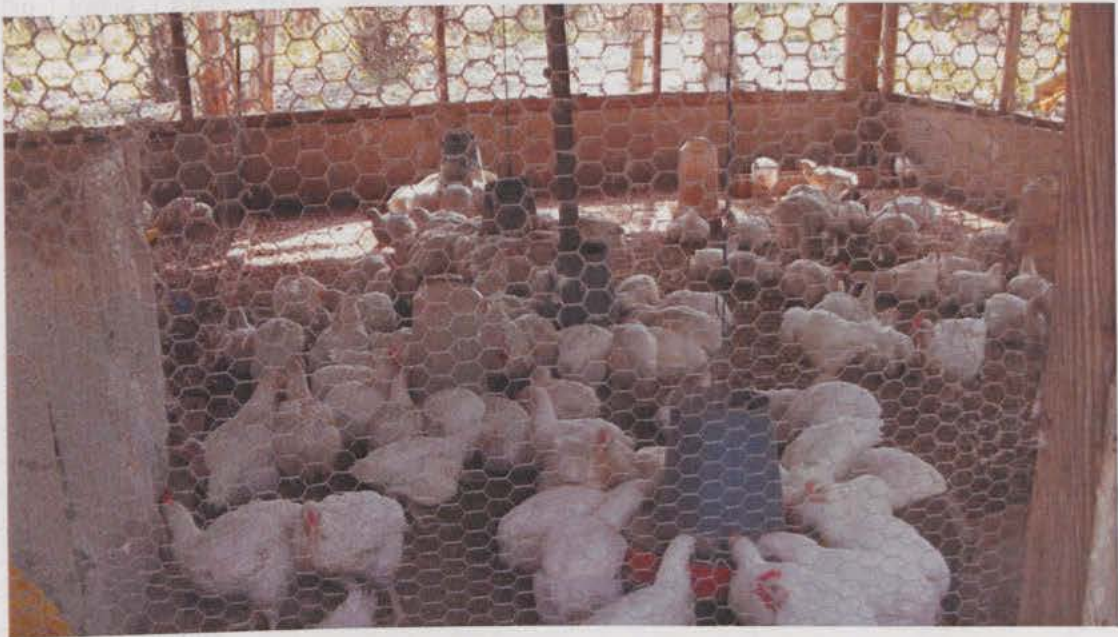
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APPENDICES



Appendix I: A photograph ready-for-sale birds (broilers) sampled in the Dormaa Municipality, Jan 2011

Appendix II: Tetracycline concentrations ($\mu\text{g/g}$) in poultry products from sampled farms in the Dormaa municipality, December 2010 – June 2011

| No | Farm ID | Eggs Drug concent ($\mu\text{g/g}$) (n=3 eggs / farm) | Kidney Drug concent ($\mu\text{g/g}$) (n=3 birds / farm) | Liver Drug concent ($\mu\text{g/g}$) (n=3 farms / farm) |
|----|---------|---|--|---|
| 1 | Q1 | 0.03 | | |
| 2 | Q2 | 0.03 | 0.01 | |
| 3 | Q4 | 0.03 | 0.01 | |
| 4 | Q5 | 0.03 | 0.01 | 0.03 |
| 5 | Q17 | | 0.03 | 0.01 |
| 6 | Q19 | | 0.01 | |
| 7 | Q20 | 0.03 | 0.01 | |
| 8 | Q30 | | 0.03 | 0.01 |
| 9 | Q33 | | 0.03 | 0.01 |
| 10 | Q34 | 0.03 | 0.03 | 0.01 |
| 11 | Q35 | 0.03 | 0.01 | |
| 12 | Q50 | 0.01 | | |
| 13 | Q52 | 0.03 | | |
| 14 | Q53 | 0.03 | | |
| 16 | Q56 | | 0.03 | |
| 17 | Q57 | 0.03 | | |
| 18 | Q58 | 0.03 | | |
| 19 | Q61 | 0.03 | | |
| 20 | Q64 | | 0.01 | |
| 21 | Q65 | | 0.03 | 0.01 |
| 22 | Q73 | 0.03 | | |
| 24 | Q81 | 0.03 | | |
| 25 | Q82 | 0.03 | 0.01 | 0.01 |
| 26 | Q84 | 0.03 | 0.03 | 0.03 |
| 27 | Q89 | 0.03 | | |
| 28 | Q90 | 0.03 | | |
| 29 | Q94 | 0.03 | | |
| 30 | Q95 | 0.03 | | |
| 31 | Q101 | | 0.03 | 0.01 |
| 32 | Q102 | 0.03 | 0.03 | 0.01 |
| 33 | Q105 | 0.03 | 0.01 | |
| 34 | Q106 | | 0.03 | 0.03 |
| 35 | Q108 | 0.03 | | |
| 36 | Q112 | | 0.03 | 0.01 |
| 37 | Q113 | 0.03 | 0.01 | |
| 38 | Q115 | 0.03 | 0.01 | |
| 39 | Q117 | | 0.01 | |
| 40 | Q124 | 0.03 | 0.03 | 0.01 |
| 41 | Q131 | 0.03 | 0.03 | 0.01 |

Appendix III, IV and V depicts a response of the microbiological plate assay to the standard concentrations of tetracycline - spiked egg, kidney and liver homogenates. A regression analysis of zones of inhibition and tetracycline concentration gave a correlation coefficient r of 0.87 ($r^2 = 0.77$) for eggs, 0.97 ($r^2=0.9486$) for kidney, and 0.94 ($r^2=0.9483$) for liver respectively. A strong and positively linear relationship between the concentration of tetracycline in tissues and zone of inhibition was established.

Appendix III: Response of microbial plate to tetracycline – spiked eggs homogenate standards concentration

| Standard concentration ($\mu\text{g/g}$) | Zones of Inhibition for six trials (mm) | | | | | Mean Zone of inhibition (mm \pm SD) |
|--|---|----|----|----|----|---------------------------------------|
| 0.0078 | ND | ND | ND | ND | ND | - |
| 0.0156 | 4 | 5 | 4 | 4 | 5 | 4.4 \pm 0.5 |
| 0.0312 | 4 | 4 | 5 | 5 | 6 | 4.8 \pm 0.8 |
| 0.0625 | 28 | 30 | 28 | 28 | 29 | 28.6 \pm 0.8 |
| 0.125 | 30 | 29 | 29 | 30 | 30 | 29.6 \pm 0.5 |
| 0.25 | 31 | 32 | 32 | 30 | 31 | 31.2 \pm 0.8 |
| 0.5 | 33 | 32 | 33 | 32 | 32 | 32.4 \pm 0.5 |

Appendix IV: Response of microbial plate to tetracycline – spiked kidney homogenate standards concentration

| Standard concentration ($\mu\text{g/g}$) | Zones of Inhibition for six trials (mm) | | | | | Mean Zone of inhibition (mm \pm SD) |
|--|---|----|----|----|----|---------------------------------------|
| | | | | | | |
| 0.0078 | ND | ND | ND | ND | ND | - |
| 0.0156 | 8 | 7 | 7 | 6 | 8 | 7.2 \pm 0.8 |
| 0.0312 | 10 | 11 | 12 | 10 | 11 | 10.8 \pm 0.8 |
| 0.0625 | 12 | 11 | 11 | 10 | 12 | 11.2 \pm 0.8 |
| 0.125 | 16 | 16 | 15 | 16 | 15 | 15.6 \pm 0.5 |
| 0.25 | 19 | 20 | 21 | 19 | 20 | 19.8 \pm 0.8 |
| 0.5 | 21 | 20 | 21 | 21 | 20 | 20.8 \pm 0.8 |

Appendix V: Response of microbial plate to tetracycline – spiked liver homogenate standards concentration

| Standard concentration ($\mu\text{g/g}$) | Zones of Inhibition for six trials (mm) | | | | | Mean Zone of inhibition (mm \pm SD) |
|--|---|----|----|----|----|---------------------------------------|
| | | | | | | |
| 0.0078 | 0 | 0 | 0 | 0 | 0 | - |
| 0.0156 | 10 | 9 | 9 | 10 | 9 | 9.4 \pm 0.5 |
| 0.0312 | 15 | 14 | 15 | 14 | 16 | 14.8 \pm 0.8 |
| 0.0625 | 25 | 23 | 23 | 24 | 24 | 23.4 \pm 0.8 |
| 0.125 | 26 | 25 | 25 | 25 | 26 | 25.4 \pm 0.5 |
| 0.25 | 32 | 30 | 31 | 32 | 31 | 31.2 \pm 0.8 |
| 0.5 | 33 | 32 | 32 | 33 | 33 | 32.6 \pm 0.5 |

Appendix VI
QUESTIONNAIRE

Department of Epidemiology and Disease Control, School of Public Health University of Ghana, Legon

Antimicrobial Residue in Poultry Products in the Dormaa municipality of the Brong Ahafo Region

ID Number.....

Date of interview:.....

Name of Farm.....

Location.....

Sub-district.....

District.....

GPS Reading: Longitude.....

Latitude.....

SECTION A: Respondent's Socio-demographics

A1: Name of Respondent.....

A2: Position on Farm: 1. Caretaker 2. Manager 3. Owner
4. Others

A3: Sex Male Female

A4: Age.....

A5: Educational Background Primary JHS Secondary Tertiary
None

A6: How long have you been in the poultry business?.....

SECTION B: Farm Characteristics

B1: Type of Farm:

B2: Type of Birds: Layers only Broilers only Mixed

B3: Farm Capacity-----

B4: Current Bird Population (By types if applicable).....

B5: Total Stock.....

B6: Date of acquisition of stock of Birds
(DD/MM/YYYY):.....

B7: Type of feed Self Prepared Commercial

If commercial, indicate Source.....

B8: Type of Husbandry: Intensive Semi-intensive Extensive

B9: Point of Sale: Within Farm Outside of Farm If outside,
where?.....

SECTION C: Current Antimicrobial Drug Use

C1: Do you give drugs to your birds? Yes / No

If No, go to **Section D**

C2: What type of drugs do you give to your birds? (See drug container(s) for names if available)

| Name of Drug | A. Ingredients | Expiry date | withdrawal Period |
|--------------|----------------|-------------|-------------------|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

Note to interviewer: List as many as are given to you. You do have to list all the vitamins on the container, the name of the vitamin, example "Supervit" should be enough.

C3: Who administers the drugs to the birds?.....

C4: Why do you give them drugs?.....

C5: When do you give them?.....

C6: How do you administer the drugs to your birds?

Through their Drinking Water Feed others (specify).....

C7: How often do you administer drugs to your birds?.....

C8: What prompt the administration of drugs to your birds?

C9: Do you keep record of drugs you give to the birds? Yes /No

C10: What type of records do you keep? *Ask to see records for confirmation.*

C11: Where do you get drugs from for your poultry? Vet office Vet Shop
Agrochemical shop others (specify).....

C11: Do you take prescription from the vet before purchase? Yes /No

C12: Explain your answer

.....
.....
.....

C13: Do you receive any instruction on how to use the drug? Yes /No Don't emember

C14: If yes, from where?.....

C15: Have you ever had any training on the use of drugs in poultry? Yes /No Don't remember

C16: If yes, source of training?

.....
.....

C17: If no, do you think you need training? Yes / No

C18: Have you heard of drug "withdrawal period" before? Yes / No

Please show them the withdrawal period on the containers brought to you and have them explain what it means to you if they answer yes.

C19: What does it mean to you?

.....
.....

SECTION D: For Layer birds only

D1: Do you give drugs to your layers? Yes / No

D4: What kind of drugs do you give them?

.....

D5: Have you ever refused to sell eggs to any buyer? Yes / No Not sure

D6: when birds begin to lay, do you at some point decide not to sell the eggs? Yes/ No

D7: If yes, give reasons?

.....

D8: If No, give

reasons.....

D9: How long do you wait to sell your eggs when the layers are on drugs?

.....

SECTION E: Broiler Birds Only

E1: Do you give drugs to your broiler? Yes / No

E4: What kind of drugs do you give them?

.....

E5: How long do you keep your broilers before you sell them?

.....

E6: Why?

.....

E7: How long do you wait to sell your birds when they are on drugs?

.....

E8: What do you do when someone wants to buy birds that are taking drugs?

.....

SECTION F: For both Layer and Broiler Farms

F1: Do you think drugs administered to birds have any effects on human health? Yes /No

F2 If yes, what kind of effects can you think of.....
.....
.....

SECTION G: SAMPLES FOR LABORATORY TEST

G1: Egg samples taken from this farm? Yes / No

G2: If Yes, How many?

G3: If No, reason
.....
.....

G4: Tissue sample taken from this farm? Yes / No

G5(a): If Yes, How many kidney tissues?

G6(b): If Yes, How many liver tissues?

G7: If No, reason
.....
.....

Note to the interviewer: REMEMBER TO LABEL EGG AND TISSUE SAMPLES TO TALLY WITH YOUR INTRVIEWER ID AND THE QUESTIONNAIRE NUMBER BEFORE YOU LEAVE THE FARM.