PREVALENCE OF CHLORAMPHENICOL RESIDUES IN COMMERCIAL CHICKEN EGGS IN THE FEDERAL CAPITAL TERRITORY, ABUJA, NIGERIA

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ZARIA – NIGERIA

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DEPARTMENT OF COMMUNITY MEDICINE,
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FEBRUARY, 2014

ATTESTATION

I attest that the work in this thesis entitled “Prevalence of Chloramphenicol Residues in Commercial Chicken eggs in the Federal Capital Territory, Abuja, Nigeria” was carried out by me in the Department of Community Medicine under the supervision of Dr. J. Kabir and Dr. E. C. Okolocha of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Mbodi, Felix Enson ______________ ______________

Name of Student Signature Date

CERTIFICATION

This thesis titled, “Prevalence of Chloramphenicol Residues in Commercial Chicken eggs in the Federal Capital Territory, Abuja, Nigeria” by Mbodi, Felix Enson meet the regulations governing the award of the Degree of Master of Public Health - Veterinary Epidemiology of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To my wife (Ladi Mbodi) and two daughters, Faith and Sagacity – for their emotional support and perseverance during the course of this studies.

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LIST OF ACRONYMS

ABJAC	Abaji Area Council
ADI	Acceptable Daily Intake
AMAC	Abuja Municipal Area Council
AMS	Agricultural Marketing Service
BWAC	Bwari Area Council
CAP	Chloramphenicol
EC	European Community
EEC	European Economic Community
ELISA	Enzyme linked immunosorbent assay
EPIA	Egg Product Inspection Act
FCT	Federal Capital Territory
FAO	Food and Agricultural Organization
FMIA	Federal Meat Inspection Act
FSIS	Food Safety and Inspection Services
GWAC	Gwagwalada Area Council
HRP	Horse radish peroxidase
IARC	International Agency for Research on Cancer
JECFA	Joint expert committee on food additives
KAC	Kuje Area Council
KWAC	Kwali Area Council
MRL	Maximum residue limit
NAFDAC	National Agency for food, drug administration and control
NFELTP	Nigeria Field Epidemiology & Laboratory Training Programme

PPIA	Poultry products inspection act
TMB	Tetramethylbenzidine
USDA	United States Department of Agriculture
USFDA	United States Food & Drug Administration
WHO	World Health Organization

SUMMARY

Drug residues in foods of animal origin are drugs and their metabolites which are found in edible tissues and milk of animals following their medication with specific drugs whose prescribed withdrawal period are not observed. Chloramphenicol (CAP) residues in the food chain are potential hazards to public health. Such hazards include: allergy, antibacterial resistance, carcinogenicity, genotoxicity, aplastic anemia and leukemia. Globally, aplastic anemia affects 1 in 10,000 to 50,000 patients receiving a typical course of CAP therapy and about 280,000 patients are susceptible to the development of aplastic anemia in Nigeria.[9,10] CAP has therefore been banned globally by FAO/WHO and considered a drug of zero-tolerance in food-producing animals. This study was carried out to determine the occurrence of CAP residues in chicken eggs in the FCT and to assess the usage and awareness of its ban amongst poultry farmers.

A cross-sectional survey using structured questionnaires was conducted on poultry farmers and a survey of commercial chicken eggs from poultry farms and government owned markets in FCT, Nigeria was also carried out using CAP ELISA kits to test for the presence of CAP in eggs. Frequencies and proportions were obtained by univariate analysis and odds ratios and Fischer's exact p-values at 95% confidence intervals were also obtained using OpenEpi and EpiInfo version 3.5.3 softwares.

Of the 57 questionnaire respondents, 30 (52.6%) were farm managers out of which 48 (84.2%) were males, and 27 (47.4%) were between ages 36-50 years. Pooled egg samples (10 eggs make a sample, n=288 i.e. 2880 eggs) were analyzed using CAP ELISA kits and 20 (7%) of the samples tested positive for CAP residues. Poultry farmers use both human (8.8%) and veterinary drug preparations containing CAP (43.9%) on their birds. Most poultry farmers (71.9%) were not aware that CAP is not recommended for use in food producing animals. There is a strong association (OR=14.8) between human CAP and its detection by ELISA test (Fischer's Exact P<0.05). Veterinarians are more likely (OR=1.4) to be aware of the CAP ban, while poultry attendants are less likely (OR=0.9; Fitscher's Exact P=0.4) to be aware.

There is an influx of CAP residue-containing eggs from within FCT and also from other States into FCT with 7% prevalence. Poultry farmers using both human and veterinary CAP preparations were not aware of its ban for use in food-producing animals. There is therefore the need for drug residue surveillance and education of poultry farmers on the prohibition of CAP in food animals and its hazards to public health.

Keywords: Chloramphenicol Residues, chicken eggs, ELISA, FCT-Nigeria

CHAPTER ONE – INTRODUCTION

1.1 BACKGROUND

Drug residues in foods of animal origin are drugs and their metabolites which are found in edible tissues and milk of animals following their medication with specific drugs whose prescribed withdrawal period are not observed. [1, 2] Drug residues eventually make their way to the food chain where they can potentially pose a risk to human health. Such health problems include development of allergic reactions, emergence of multiple resistant strains of pathogenic bacteria, development of cancer and mutations, aplastic anemia and leukemia in humans.[3] This has resulted in the global ban of the use of chloramphenicol in Veterinary Medicine to non-food use due to potential public health risks posed by its traces in edible tissues and was therefore considered a drug with an established zero-tolerance.[4] Chloramphenicol (CAP) was first isolated in 1947 from the soil bacterium *Streptomyces venezuelae*, and synthetic production began in 1949.[5] Due to its outstanding antibacterial properties, CAP is an often used antibiotic in the production of milk, meat and eggs. It is an inexpensive broad-spectrum antibiotic recommended for the treatment of salmonella infections and for the prevention of secondary infections associated with chronic respiratory disease and blue comb in poultry.[6] It is well absorbed by both oral and parenteral routes. CAP usually acts as a bacteriostatic, but at higher concentrations or against some very susceptible organisms it can be bactericidal. It is used in the treatment of human infection with *Salmonella typhi* and other forms of salmonellosis and other threatening infections of the central nervous system and respiratory tract.[7]

In Nigeria, many poultry farmers use CAP to control poultry diseases because of its claimed efficacy. [8] This situation could result from a state of very low and absolute lack of awareness of legislation guiding the use of veterinary drugs, particularly those with zero tolerance. For this reason there is a global recommendation for effective reporting of residues in foods destined for human consumption, especially in developing countries where poor perception of residues among farmers is being noticed. [8]

A number of agencies have reviewed CAP e.g United States Food and Drug administration (USDFA), 1985; International Agency for Research on Cancer (IARC), 1990; European Committee for Veterinary Medicinal products, 1994. Concerns have been expressed about the genotoxicity of CAP and its metabolites, its embryo and fetoxicity and its carcinogenic potentials in humans. The joint FAO/WHO expert committee on food additives (JECFA) concluded that CAP is genotoxic, which means it could cause genetic damages and possibly lead to cancer. Based on this advice, the Codex Alimentarius Commission, the International body on food standards, stated that because of the toxicity of CAP, an acceptable daily intake (ADI) has never been allocated and consequently a maximum residue limit (MRL) cannot be established. This has resulted in the global ban of the use of CAP in Veterinary Medicine to non-food use due to potential public health risks posed by its traces in edible tissues and was therefore considered a drug with an established zero-tolerance.[3]

Globally, aplastic anemia affects 1 in 10,000 to 50,000 patients receiving a typical course of CAP therapy.[9] About 280,000 patients are susceptible to the development of aplastic anemia in Nigeria.[10] Nigeria has no dedicated agency for the control and monitoring of veterinary drugs and there is non-enforcement of this ban.[11]

In humans, CAP is frequently found as a drug of choice for *salmonella* infections in the third world.[12] Due to its antimicrobial resistance and safety concerns, it is no longer a first-line agent for *Salmonella* infections in developed nations.[12] It is an inhibitor of protein synthesis and has shown dose-related reversible bone marrow depression and a severe aplastic anemia which is non-dose related and often irreversible in humans [13, 14]. Chloramphenicol is widely used in the treatment of human infectious diseases caused by susceptible bacteria, for instance, *Salmonella spp*. Its broad spectrum of activity previously contributed to its therapeutic usefulness being extended to the management of infectious bacterial diseases of food-producing and companion animals. However, major toxicological health problems have been reported in genetically predisposed humans as a result of direct (therapeutic administration) and indirect (residues) consumption [13].

Even low doses of administered CAP may result in residues in edible tissues from treated food-producing animals; therefore, consumers of milk, meat, aquaculture products, honey and eggs may be exposed to potentially harmful levels of drug residues. In Nigeria, veterinary drugs are freely available (not controlled) and livestock farmers are using them illicitly and indiscriminately without observing withdrawal period [15, 16].

1.2 STATEMENT OF THE PROBLEM

Due to concern over its potential public health hazards (Allergy, carcinogenicity, genotoxicity, fetotoxicity, antibacterial resistance, reversible dose-related and irreversible dose-related aplastic anaemia, leukemia e.t.c.) posed by its residues, CAP use in food producing animals has been banned globally by FAO/WHO joint expert committee on food additives (JECFA) and therefore considered as a drug with an established zero-tolerance.[3]

The Codex Alimentarius Commission, the International body on food standards, stated that because of the toxicity of CAP, an acceptable daily intake (ADI) has never been allocated and consequently a maximum residue limit (MRL) cannot be established. Nigeria has no dedicated agency for the control and monitoring of veterinary drugs and there is lack of enforcement of this ban.[11]

Furthermore, veterinary drugs are used indiscriminately by poultry farmers in Nigeria without observing withdrawal period (time interval after cessation of treatment before an animal or its product can be used for consumption), thereby exposing consumers to drug residues.[6,16,17] These residues in poultry products go undetected due to lack of routine veterinary drug residue surveillance and control programmes in Nigeria.

Several reports document human fatalities resulting from CAP ophthalmic preparations with total exposure doses that could be achieved from food residues.[21] Due to few researches in this area in Nigeria, there is lack of knowledge about the level of exposure of the population to CAP residues, especially among small scale backyard poultry farmers who provide eggs strictly for home consumption.

1.3 JUSTIFICATION

Poultry eggs contribute to the palatability of many dishes by adding about the same amount of animal protein as pork and poultry meat. Poultry eggs have also attained industrial importance as a major ingredient in the baking of confectioneries. The egg powder is also a major input for beverage production, exclusive body soap, body shampoo, energy drink .[18]

Inadequate production and high demand for eggs makes the F.C.T. a hub for influx of eggs from neighboring States and beyond. These eggs that is high in demand if containing CAP residues are a major public health hazard.

Many families in Nigeria are keeping small scale backyard poultry for both home consumption and commercial purposes. They use veterinary drugs indiscriminately and illicitly without observing withdrawal periods. This leads to drug residues in eggs and therefore families are consistently exposed to residues tainted eggs which can constitute health problems associated with residues. Knowing the magnitude of these problems will provide baseline information for solving drug residue problems in F.C.T. and Nigeria at large.

As a result of reported occurrences in different parts of the world, coupled with low awareness reported in the developing world, the World Health Organization (W.H.O.) recommends effective reporting of drug residues in foods of animal origin meant for human consumption.[4] The challenge in residue surveillance is the availability of a rapid and sensitive method. Chloramphenicol ELISA test method is 99.9% sensitive with a short assay time. Public health agencies in many countries include surveillance for drug residues as part of routine food safety assurance in accordance with the recommendation of the World Health Organization. [4] A residue control programme in F.C.T. will serve as a prototype for other States in Nigeria. Drug residues control will ensure food safety from poultry products and provide quality nutrition for the public. It will also help reduce medical costs on families due to health hazards associated with drug residues.

1.4 RESEARCH QUESTIONS

- **i.** Are there chloramphenicol residues in commercial chicken eggs in F.C.T.?
- **ii.** Is chloramphenicol use for poultry production in the F.C.T.?
- **iii.** Are poultry farmers in F.C.T. aware of the ban on the use of chloramphenicol?

1.5 GENERAL AND SPECIFIC OBJECTIVES

1.5.1 GENERAL OBJECTIVE

The aim of this study is to determine the prevalence of CAP residues in commercial chicken eggs in F.C.T., Nigeria.

1.5.2 SPECIFIC OBJECTIVES

- **i.** To determine the occurrence of chloramphenicol and the proportion of egg-containing residues in commercial chicken eggs in the Federal Capital Territory.
- **ii.** To assess the usage of chloramphenicol and the production of residue containing eggs amongst poultry farmers in the Federal Capital Territory.
- **iii.** To determine the awareness of poultry farmers on the regulatory status of chloramphenicol.

1.6 Scope

This study will determine the occurrence of chloramphenicol in chicken eggs in registered poultry farms in F.C.T. and in eggs sold by major marketers (full time marketers who solely deal with eggs) in government owned markets in the Federal Capital Territory, Abuja, Nigeria. It will also assess the poultry farmers on the use and awareness of chloramphenicol ban.

CHAPTER TWO – LITERATURE REVIEW

2.1 Background Information

A number of agencies have reviewed CAP e.g United States Food and Drug administration (USDFA), 1985; International Agency for Research on Cancer (IARC), 1990; European Committee for Veterinary Medicinal products, 1994. Concerns have been expressed about the genotoxicity of CAP and its metabolites, its embryo and fetoxicity and its carcinogenic potentials in humans. The joint FAO/WHO expert committee on food additives (JECFA) concluded that CAP is genotoxic, which means it could cause genetic damages and possibly lead to cancer. Based on this advice, the Codex Alimentarius Commission, the International body on food standards, stated that because of the toxicity of CAP, an acceptable daily intake (ADI) has never been allocated and consequently a maximum residue limit (MRL) cannot be established. This has resulted in the global ban of the use of CAP in Veterinary Medicine to non-food use due to potential public health risks posed by its traces in edible tissues and was therefore considered a drug with an established zero-tolerance.[3]

Globally, aplastic anemia affects 1 in 10,000 to 50,000 patients receiving a typical course of CAP therapy.[9] About 280,000 patients are susceptible to the development of aplastic anemia in Nigeria.[10] Nigeria has no dedicated agency for the control and monitoring of veterinary drugs and there is non-enforcement of this ban.[11]

Chloramphenicol is produced naturally by the bacterium *Streptomyces venezuelae*. The crystallne antibiotic substance was isolated by Bartz in 1948. It may be produced by chemical synthesis followed by a step to isolate stereoisomers.[19] In Japan, production by a fermentation process has also been described. The process resulted from the discovery and isolation of a new strain of microbe and does not require separation of stereoisomers.[20]

Chloramphenicol is synthesized in Brazil, China, Czechoslovakia, the Federal Republic of Germany, Hungary, Italy, India, Israel, Japan, Mexico, Romania, South Africa, Spain and the USSR and has also been produced in France; Switzerland, the UK and the USA. Commercial production of chloramphenicol in the USA was first reported in 1948 [12, 20]

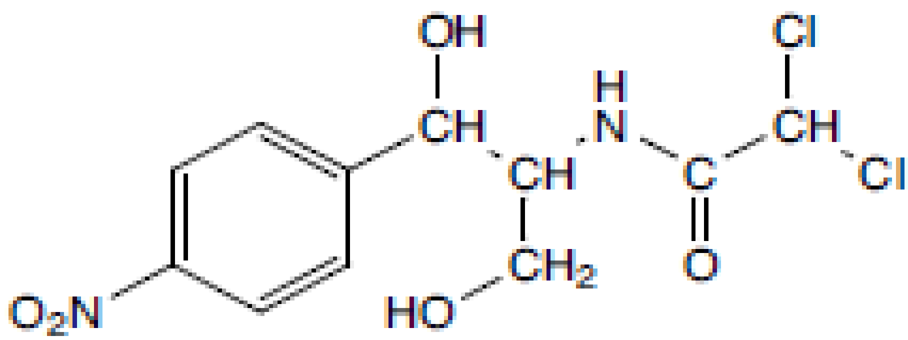
2.1.1 Physical and chemical properties of Chloramphenicol

Chloramphenicol exists as a white to grayish-white or yellowish-white fine crystalline powder, needles, or elongated plates, with a melting point of 150.5 to 151.5^{o}C. It sublimes in high vacuum and is sensitive to light.[19]

Solubility: 1:400 in water at 25°C; aqueous solutions are neutral; 1:6 in propylene glycol at 25 ^{0}C; very soluble in methanol, ethanol, butanol, ethyl acetate, acetone; fairly soluble in diethyl ether. It is fairly soluble in ether, but insoluble in benzene, petroleum ether, and vegetable oils.

Stability: Stable in the solid state as a bulk drug and when present in solid dosage form. Reasonable precautions taken to prevent excessive exposure to light or moisture are adequate to prevent significant decomposition over an extended period. ln solution, chloramphenicol undergoes a number of degradative changes related to pH, temperature, photolysis and microbiological effect. [12,19,20]

The chemical structure of chloramphenicol is illustrated below:



2,2-dichloro-N-[1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]acetamide

2.1.2 Pharmacokinetics of Chloramphenicol

Chloramphenicol is eliminated primarily following biotransformation. In humans, as much as 90% of administered chloramphenicol is eliminated in urine as the chloramphenicol glucuronide conjugate. In other species (e.g., dog and rat), urinary elimination is dominant, but larger amounts are eliminated in bile as aromatic amines.[21] In humans, as much as 10% of the administered dose may be eliminated unchanged in the bile.

Chloramphenicol is readily absorbed from the gastrointestinal tract after oral administration of a crystallne powder of the active drug itself or a palmitate ester; the latter is hydrolyzed in the small intestine to active chloramphenicol before absorption.[20] Esters of chloramphenicol, for example, the succinate-are converted to chloramphenicol in vivo. Peak levels of 10-20 µg/ml appear 2-3 h after administration of chloramphenicol orally at 15 mg/kg bw

Chloramphenicol is also well absorbed by infants and neonates after oral administration.[22] Serum (peak) concentrations of 20-24 µ/ml were noted after oral doses of 40 mg/kg bw to neonates. Infants given 26 mg/kg bw were found to have peak concentrations of 14 µg/ml.

Chloramphenicol is distributed extensively in humans regardless of its route of administration. The compound has been found in heart, lung, kidney, liver, spleen, pleural fluid, seminal fluid, ascitic fluid and saliva.[20] It penetrates the blood-brain barrier, and its concentrations in cerebrospinal fluid can reach about 60% of that in plasma. The concentrations in brain tissue equal or even exceed those in plasma. [23] Chloramphenicol easily crosses the placenta, and it is also excreted in breast milk.[20]

Chloramphenicol has a half-time ranging from 1.6 to 4.6 h; using different techniques and in different adult patients, apparent volumes of distribution ranging from 0.2 to 3.1 l/kg have been measured. The half-time is considerably longer in neonates: in one- to eight-day-old infants the half-life ranged from 10 to over 48 h, and in 11-day- to eight-week-old infants the range was 5-16 h[20, 22]

Six hours after an intravenous dose of 500 mg chloramphenicol succinate, the blood level was 4.5 µg/ml (2.8-6.9 µg/ml) in patients with chloramphenicol-induced bone-marrow depression, while in the control group the mean level was 1.2 µg/ml (0-2.3 µg/ml). Such findings suggest that patients susceptible to the effects of chloramphenicol on bone marrow may clear the drug from the blood more slowly than those who are not susceptible.

Chloramphenicol is excreted primarily in the urine (90%); up to 15% is excreted as the parent compound and the remainder as metabolites, inc1uding conjugated derivatives. Glomerular excretion is thought to be the major mechanism of excretion.

Approximately 48% of the chloramphenicol excreted in urine within 8 h of an oral dosing was the glucuronide conjugate; only 6% was excreted as the parent compound and 4% as the base derivative. The alcohol derivative has been detected in the urine of neonates.[22]

ln dogs, chloramphenicol was readily absorbed after oral administration of 50 mg/kg bw, giving plasma levels of 16.5 µg/ml 2 h after dosing. Similar findings were made in rabbits.[20, 21]

Five minutes after intravenous administration of chloramphenicol to newborn pigs at 0.52 mg/kg bw, most tissues had higher levels than the blood; however, levels of chloramphenicol in bone marrow did not reach those noted in serum.

Chloramphenicol and its metabolites were excreted in the urine of rats after oral dosing; up to 70% of an oral dose may be excreted in this way. About 0.4% of an intramuscuIar dose of 40 mg/kg to rats was detected in the bile within 4 h. ln newborn pigs, most of an intravenous dose of chloramphenicol was excreted in the urine. Following intravenous administration to goats, 69% of the dose was excreted in the urine within 12 h.

Chloramphenicol was detected in the milk of goats and cattle after parenteral administration; however, after oral administration (dose unspecified) to cattle, no chloramphenicol was detected in milk [19, 20, 21]

2.1.3 Toxicity and adverse effects of chloramphenicol

The primary routes of human exposure to chloramphenicol are oral and dermal, through its use as a drug. Exposure also may occur through inhalation, dermal contact, ingestion, or contact with contaminated water or soil [19, 20]

High oral doses of chloramphenicol of 500-2000 mg/kg to rats and mice and of 500 and 1000 mg/kg to rabbits produced high incidences of embryonic and fetal deaths and fetal growth retardation in all three species. Teratogenic effects- predominantly umbilical hernia-were observed only in rats. The pregnant animals showed no toxic sign, except that those given the highest dose gained significantly less weight than controls.[20, 21]

Groups of eight pregnant albino mice were given chloramphenicol orally at 25, 50, 100, or 20 mg/kg bw in 10 ml distilled water over the third stage of pregnancy for seven days. Animals were allowed to give birth, and the young were tested for conditioned avoidance response, electroshock seizure threshold and performance in open-field tests. Dose-related effects were seen in all three elements of the test: progeny of chloramphenicol-treated dams had reduced learning ability, higher brain seizure threshold and poorer performance in the open-field test.

Chloramphenicol was also investigated for its effects on avoidance learning in rats. Four groups of 15 pregnant Wistar rats each were treated as follows: chloramphenicol was given subcutaneously at 50 mg/kg bw on days 7-21 of gestation; chloramphenicol was given subcutaneously at 50 and 100 mg/kg bw to pups for the first three days after birth; and the fourth group served as controls. No adverse effect on pregnancy or postnatal weight gain was seen, but when the animals were 60 days old, they had significant impairment of avoidance learning [20]

The most important adverse effects of chloramphenicol involve the haematopoietic system[20, 21] Potentially fatal toxicity may develop in neonates exposed to excessive doses of chloramphenicol. This so-called 'grey baby syndrome' may also occur in older children and in adults receiving doses resulting in serum concentrations of 40-200 µg/ml[22] Other adverse effects include hypersensitivity reactions, gastrointestinal complaints and neurological complications after long-term treatment. [24] Chloramphenicol can also precipitate hemolytic anemia in subjects with glucose-6-phosphate dehydrogenase deficiency.[20]

Dose-dependent, reversible bone-marrow suppression affects primarily the erythroid series and occurs regularly when plasma concentrations of chloramphenicol are 25 µg/ml or higher. Another haematological side-effect is rare, unpredictable, non-dose-related aplastic anaemia, which often appears after the drug has been discontinued[25] A topical ophthalmologic concentration of CAP had been reported to cause bone marrow aplasia; this condition had been reported to cause the death of a rancher after feeding his cattle with chloramphenicol. [21]

There have been many case reports of the occurrence of aplastic anaemia following administration of chloramphenicol by various routes. [19] ln many of these cases, large doses had been taken repeatedly over periods of many years before the onset of symptoms of aplastic anaemia. [26] Case-control studies have also suggested an association between chloramphenicol use and aplastic anaemia. [20]

ln the Collaborative Perinatal Project, in which drug intake and pregnancy outcome were studied in a series of 50 282 women in 1959-65, 98 women had been exposed to choramphenicol during the first trimester of pregnancy. There were eight malformed children in the exposed group, giving a non-significant standardized relative risk (RR) of 1.17. A total of 348 women had had exposure at any time during pregnancy with no evidence of an increase in the incidence of congenital malformation. [20]

Numerous case reports have been published of leukaemia occurring following chloramphenicol-induced aplastic anaemia [27]. A case-control study of 309 childhood leukaemia cases (under 15 years) notified to a population-based cancer registry in Shanghai, China, during 1974-86, and 618 age and sex-matched population controls showed a significant dose-response relationship between chloramphenicol and risk of both acute lymphocytic leukemia and non-acute lymphocytic leukemia for more than 10 days being associated with risk of 11.0 and 12.0 respectively. Information was obtained from parents or guardians for lifetime use of selected drugs, including prescribed chloramphenicol and syntomycin (a racemic mixure of D- and L-chloramphenicol). The risk for all types of leukaemia combined showed a marked increase with accumulated use of chloramphenicol [27], yielding RR of 1.7 (95% confidence interval, 1.2-2.5), 2.8 (1.5-5.1) and 9.7 (3.9-24.1) for one to five days', six to ten days' and more than ten days' treatment, respectively [27]. The association was present in a subgroup in which first use had occurred more than five years prior to diagnosis and in one in which last use had been more than two years before diagnosis. Significant trends in risk with dose were observed both for acute lymphocytic leukaemia (56% of cases) and for acute nonlymphocytic leukaemia (30%). An association with leukaemia was also seen for use of syntomycin (RR, 1.9; 1.1-3.2). (The Working Group noted that interview was undertaken up to ten years after diagnosis, which adds to the possibility of differential recall between the parents of cases and controls. Little information was available with regard to use of other antibiotics, making it difficult to evaluate the possibility of bias.)

Due to resistance and safety concerns, it is no longer a first-line agent for any infection in developed nations, with the notable exception of topical treatment of bacterial conjunctivitis.[12,19] In low-income countries, chloramphenicol is still widely used because it is inexpensive and readily available [12]

The most serious adverse effect associated with chloramphenicol treatment is bone marrow toxicity, which may occur in two distinct forms: bone marrow suppression, which is a direct toxic effect of the drug and is usually reversible, and aplastic anemia, which is idiosyncratic (rare, unpredictable, and unrelated to dose) and generally fatal [21,26,28].

Chloramphenicol is released to the environment and may be found in various waste streams as a result of its use as a medicinal and research antimicrobial agent. [19, 20] Occupational exposure during the manufacture of chloramphenicol may occur through inhalation, dermal contact, or ingestion. Medical and veterinary personnel who administer drugs containing chloramphenicol also may be exposed. [19]

2.2 Prevalence of Chloramphenicol Residues in animal tissues

Drugs given indiscriminately to birds orally or parentally may be found in tissues, particularly when the birds are slaughtered without the observance of withdrawal period, or when eggs are harvested within the withdrawal period of the drug.[6,16] In Slovenia, between 1991 and 2000 a survey of 1,308 different animal tissue products including eggs, CAP residues were determined (using gas chromatography) in only one milk sample with a prevalence of 0.1%.[29] The results of the CAP residue monitoring are a consequence of the strict prohibition of this veterinary medicinal product for food producing animals, as well as a proper veterinary sanitary control of its residues in Slovenia.

In another study in Morogoro Municipality, Tanzania, agar well diffusion and Delvotest SP® assays was used to determine 10% prevalence of CAP in commercial chicken eggs.[30]

Thiamphenicol is a structural analogue of chloramphenicol with a broad spectrum and similar mechanism of action, which has been shown to be valuable for the treatment of bacterial Infections in both animals and humans.[31] Thiamphenicol has a greater *in vivo* activity against pathogenic bacteria than other structural analogues and it is also active against some bacteria that are resistant to chloramphenicol. However, in spite of their chemical similarity, the toxicity of thiamphenicol is lower (reversible dose-related bone marrow suppression).[31] In another study in Italy using high performance liquid chromatographic method (HPLC), thiamphenocol residues (>10 µg/kg) persisted longer in yolk than in albumin and were present in yolk for 10 days after administering a single dose and for 8 days after terminating the administering of multiple (five) doses. After multiple doses, the values of thiamphenicol concentrations were under the limit of detection of the method at the ninth day after the end of the treatment.[31]

In Nigeria, there is paucity of data concerning the prevalence of CAP in animal products. However, a similar study (using the same CAP ELISA technique) done in Ibadan, Oyo State, Nigeria in 2011 reported incidence of 51.1% CAP residue in broiler meat and 25.4% incidence of CAP residues in egg samples.[32] The prevalence in eggs from Ibadan was used to arrive at the sample size for eggs used in this thesis. Another study using specific thin layer chromatographic method (STLC) from Kaduna State, Nigeria, published (in 2012) a 0.7% prevalence of CAP residues in commercial eggs.[8,11]

2.3 Use of chloramphenicol by livestock farmers

The administration of drugs at levels in excess of those recommended or failure to observe withdrawal periods results in unacceptable level of residues in food products of animal origin such as meat, milk and eggs. It is common practice in Nigeria amongst livestock farmers to slaughter and sell milk from animals after treatment with therapeutic agents without observing the stipulated withdrawal time. [33] It is even more common in the poultry sector where treated birds and eggs produced by layers under chemotherapy are sold for human consumption. These result in consumption of meat, milk and eggs with high concentration of drug residues by humans. [33]

In animals, antibiotics are used for three primary reasons:

1. Therapeutically for curing existing diseases
2. Prophylactically at subtherapeutic levels for disease preventive measures
3. Growth promoters, for production enhancement from increased growth rate and efficiency of feed use.

The use of CAP by unqualified farmers in this part of the world was observed to be due to the ineffective implementation of veterinary laws which guide and guard against incorrect veterinary usage. Such usage was seen to be a contravention of the global ban on CAP use in food-producing animals and may pose a public health risk to the susceptible consumers.[8]

For using chloramphenicol in food animals, an Iowa, Washington DC, large animal (veterinary) practitioner in 1991 was sentenced to 12 months imprisonment, the costs of imprisonment, a $15,000 fine, and two years' supervised release.[34] The conviction came after the trial, which was held in the United States District court for the Northern District of Iowa. It was established that the veterinarian acquired, processed, used, and dispensed chloramphenicol and other illegal animal drugs in his food animal practice. The trial also established that he had been aware of the prohibition against the use of chloramphenicol in food animals. If this kind of legal measures can be enforced in Nigeria, regulations concerning the use of chloramphenicol and other abused veterinary drugs will be adhered to. This will go a long a way in protecting public health.

In an article published in January 3, 1984, edition of the Wichita Eagle-Beacon, a rancher was described who 4 months after he started treating his cattle with chloramphenicol was diagnosed as having aplastic anemia.[21] A total of 5 months later, the rancher died of the condition. The information contained in the reports described here leads to the conclusion that aplastic anemia could occur in susceptible individuals who are exposed to concentrations of chloramphenicol that approach those that might remain as residues in edible tissues of chloramphenicol-treated, food-producing animals.[21] Based upon the information available to date, it is clearly impossible to establish a safe level of residues or a safe withdrawal period for animals treated with chloramphenicol.[3]

In Morogoro Municipality, Tanzania, a study published in 2008 reported that 10% of the poultry farmers in that survey use chloramphenicol on their birds.[30] In a study in Ibadan, Oyo State, Nigeria, 50% of the poultry farmers in that survey claimed the use of chloramphenicol for their poultry.[32] In a similar survey in Kaduna State, Nigeria, 20% admitted the use of CAP in forms veterinary and human preparations while 62.5% admitted the use of human CAP preparation, only.[8]

2.4 Awareness of chloramphenicol ban by livestock farmers

Due to concern over its potential public health hazards (Allergy, carcinogenicity, genotoxicity, fetotoxicity, antibacterial resistance, reversible dose-related and irreversible dose-related aplastic anaemia, leukemia e.t.c.) posed by its residues, CAP use in food producing animals has been banned globally and therefore considered as a drug with an established zero-tolerance.[3]

There is poor perception of the possible effects of antimicrobial residues on human health in Nigeria.[8] This has highlighted the low level of awareness of legislation that governs the application of drugs in poultry.[8] In Tanzania, similar observations were reported with 85% of farmers, particularly small poultry holders who were not aware of the effects of antimicrobial residues on human health.[30] In the survey of poultry farmers rearing commercial layers in Kaduna State, Nigeria, Only 26.7% of respondent farmers were aware that CAP was one of the drugs that is not recommended for use in food animals.[49]

2.5 Methods of Drug Residue Analysis

Chloramphenicol can be detected in blood serum, plasma, or cerebrospinal fluid by high-pressure liquid chromatography (HPLC). [5,7] HPLC or enzyme immunoassay may be used to determine chloramphenicol levels in blood.[33,36] Chloramphenicol can be measured in pharmaceutical preparations for humans and animals with microbiological, turbidimetric, and spectrophotometric assays.[33,36] Thin-layer chromatography and densitometry are used in the analysis of prescription drugs.[37] Chloramphenicol levels in meat, milk, and eggs have been determined with thin-layer HPLC and radioimmunoassay.[36, 37]

Immunoassays have been less frequently applied in residue analysis. They are relatively easy to perform and capable of detecting very low levels of residues even if the residues are covalently bound to proteins.[38,39] The enzyme linked immunoassay (ELISA) principle is based on the competitive enzyme immunoassay for the detection of chloramphenicol in the tissue (chicken, pork), honey, milk, fish, shrimp and egg. The assay operates on the basis of competition between the chloramphenicol in the sample and the anti-chloramphenicol antibody for the limited number of specific antigen binding sites.[40,41] After half hour incubation, the unbound reagents are removed in a washing step. The enzyme conjugate uses horseradish peroxidase (HRP) as a tracer. The amount of enzyme conjugate bound is measured by the addition of a chromogen substrate, tetramethylbenzidine (TMB). The colour intensity is inversely proportional to the chloramphenicol concentration in the calibrator or sample.[41]

Methods that include both microbiological and physicochemical procedures have been employed also. They include high voltage electrophoresis and thin layer chromatography with bio-autographic detection. In general these methods are not sufficiently sensitive but are particularly useful for screening purposes.[42]

Widely used methods in residue analysis are those based on chromatographic procedures. Thin-layer chromatography (TLC) is an efficient procedure for handling many samples simultaneously.[37] It should not be ignored as a powerful alternative to qualitative high performance liquid chromatography, since high resolution can be made possible, yet its precision cannot be compared with that of gas chromatography or high performance liquid chromatography.[37]

The gas liquid chromatography (GLC) provides higher resolution than TLC and most high performance liquid chromatography methods. In combination with a mass spectrometer, GLC is the most powerful, confirmatory tool available for residue identification.[37] However, non-volatile antimicrobial agents cannot be analyzed by GLC unless they are made volatile by derivatization. For highly polar or thermally unstable compounds, HPLC is the method of preference. [37] HPLC offers many advantages in residue analysis over other analytical methods. It is a very useful technique for the separation of compounds which are not usually separable by other means. [5,7] However, by its very nature, liquid chromatography is a slow process as low flow rates give the best separation efficiency. This is due in great part to the very slow diffusion rate of a solute in the solution being chromatographed. This diffusion rate is more than 50 times slower in a liquid than in a gas. Though modern HPLC instruments have overcome this difficulty by the development of columns containing very small particles. This increases the rate of diffusion dramatically and permits separation nearly as quickly as with gas chromatography

The monitoring of antibiotic residues has received much attention because of their widespread use in animal production. Two methods used in drug monitoring are the AH- Test and RIV-Test.

The AH-Test uses *Bacillus subtilis*, which is highly sensitive to a variety of inhibitory substances. It was introduced in Germany due to widespread availability of antibiotics on the 'black market.' A 10mm diameter sample of tissue is placed on an agar plate inoculated with *Bacillus subtilis* and incubated for 18 hours at 30^0c. A zone of inhibition in excess of 1mm is considered to be positive. However the suitability of this test for declaring animal tissues unfit for human consumption has been criticized.[37]

Unlike the AH-Test which detects many kinds of inhibitory substances in different tissue samples, the RIV-Test using *Sarcina cutea,* ATCC 9341, is specifically designed for the detection of unacceptable antibiotic residue in kidney sample, only. [37] The level of sensitivity has been adjusted so that the test organism is not affected by low level, acceptable residue of certain antibiotics. Thus it employs the principle of tolerances so that only cases of gross misuse are detected and penalized by rejection of the animal carcass.

The colorimetric/spectrophotometric methods can be used only to detect chemical substances that could be detected under ultra-violet (UV) ray or substances that could absorb UV light within a range of wavelength from which the highest absorbance is used to calculate the concentration.[42]

Trace analysis of chloramphenicol has been analysed using Radioimmunoassay (RIA) in eggs, milk and meat with a sensitivity of 1 ppb for milk and 5 ppb for meat.[42]

2.6 Drug Residue Legislation

A number of agencies have reviewed CAP e.g United States Food and Drug administration (USDFA), 1985; International Agency for Research on Cancer (IARC), 1990; European Committee for Veterinary Medicinal products, 1994. Concerns have been expressed about the genotoxicity of CAP and its metabolites, its embryo and fetoxicity and its carcinogenic potentials in humans. The joint FAO/WHO expert committee on food additives (JECFA) concluded that CAP is genotoxic, which means it could cause genetic damages and possibly lead to cancer. [3,4] Based on this advice, the Codex Alimentarius Commission, the International body on food standards, stated that because of the toxicity of CAP, an acceptable daily intake (ADI) has never been allocated and consequently a maximum residue limit (MRL) cannot be established.[3,4] This has resulted in the global ban of the use of CAP in Veterinary Medicine to non-food use due to potential public health risks posed by its traces in edible tissues and was therefore considered a drug with an established zero-tolerance.[3]

As a result of reported occurrences in different parts of the world, coupled with low awareness reported in the developing world, the World Health Organization encourages effective reporting of drug residues in foods of animal origin destined for human consumption, especially in developing nations where poor perception of residues among farmers is being noticed.[4]

Public health agencies in many countries include surveillance for drug residues as part of routine food safety assurance in accordance with the recommendation of the World Health Organization.[4]

Due to the serious impact that drug residue could have on the public health if not prevented or controlled, various countries have laws governing the use of veterinary drugs and regulate drug residues in food. For instance, the Northern Ireland's meat shipping regulations (1964) require veterinary officers to give an opinion on the suitability of any animal tissue for human consumption. In general, these regulations are interpreted to mean freedom from detectable antimicrobial, synthetic hormone and other residues.

In the United Kingdom, the principal Acts concerned with food for human consumption are the food Act of 1984 and the food safety Act of 1990. Section of the safety Act enables the ministers to make regulations implementing a wide range of food safety and consumer protection measures. This may include regulations on food composition and the presence of residues in food sources such as live animals.[44]

Because of its tendency to cause blood abnormalities in humans, the U.S. Food and Drug Administration in 1997 banned the use of chloramphenicol in food-producing animals.[19] Several reports document human fatalities resulting from ophthalmic preparations containing chloramphenicol with total exposure doses that could be achieved from food residues.[21] Consequently, several Veterinarians have been fined or imprisoned for distributing or misbranding chloramphenicol for use in food animals.[26] A case in point was that of an Iowa (Washington) large animal Vet. practitioner who was sentenced (May 9, 1991) to 12 months imprisonment, the cost of imprisonment, a $15,000 fine for using chloramphenicol in food producing animals. [34]

Generally the intent of these laws in protecting Public Health does not vary from country to country, but there are differences in disease patterns and animal husbandry practices that affect the choice of drugs in use, the level of pattern of use, the maximum residue levels that are permitted to remain in food and the enforcement residue limits and other control. [9]

In the U.S.A., the Following agencies are responsible for the work of protecting the public from residue and microbial hazards: The U.S. Department of Agriculture (USDA), the Food Safety and Inspection Sservices (FSIS), the Agricultural Marketing Service (AMS), the Food and Drug Administration (FDA), the (EPA) U.S. Environmental Protection Agency.[43] USDA is charged with enforcing the Federal Meat Inspection Act (FMIA), the Poultry Products Inspection Act (PPIA) and the (EPIA) Egg Product Inspection Act. [45] Within USDA, FSIS is responsible for the wholesomeness and safety of fresh meat, poultry and processed meat and poultry products intended for human consumption .[11] Inspection and analysis are intended to ensure among other things that meat and poultry do not contain residues of drugs, pesticides or pathogens that cause them to be adulterated as defined in FMIA or PPIA. When residue violations are detected, FSIS notifies FDA, as FDA is authorized to take legal action against violators.[11] Studies on the occurrence of chemical residues (for which no safe limits have been established) such as trace metals, industrial chemicals and mycotoxins, may be conducted by FSIS with input from FDA, to establish information on the frequency and concentrations at which such residues occur. The results are then given to appropriate agencies such as EPA or the FDA which have the responsibility of establishing tolerance for contaminants in food under the Federal Food Drug and Cosmetic Act. [11]

Illegal residues in animals are investigated by the FDA; however, the dynamics of tissue residue program are such that no single agency can accomplish the goal of residue reduction and subsequent enhancement of consumer confidence .[36] Therefore, joint FDA and State cooperative work-sharing programs have been formed to perform residue follow-up investigations on first time residue violator. In case of environmental pesticides residues, the information is channeled to EPA in conjunction with FDA and FSIS to correct the problem. In case of drug or pesticide residue in meat, FSIS and FDA join forces to correct the problem.

European Economic Community (EEC) came up with legislations that gave power to European Community (EC) countries to come up with ways aimed at harmonizing legislations including those of trade of food animals and animal products. Some of these legislations cover national markets of member states, intra-community markets and importations from other countries. In general, EC regulations allow no residues in meat. [36]

2.7 Drug Residue Regulations in Nigeria

Food and Drugs Decree of 1974 and the draft of Veterinary Public Health meat hygiene Decree of 1992 are the two basic regulatory laws guiding the use of veterinary drugs and there residues in animal tissues in Nigeria. The National Agency for Food and Drug Administration and Control (NAFDAC) was established under sections 1 and 2 of NAFDAC law of 1993.[46] NAFDAC has the power to monitor and carry out surveillance through inspection, sampling and analysis of samples. However, there are no clear regulations for residues of veterinary drugs in animal products nor a provision of a link under the Decree between NAFDAC and the Federal Department of Livestock and Pest Control Services of the Federal Ministry of Agriculture and Natural Resources as practiced by other countries e.g. U.S.A. [36] However, in April 2012 the Federal Government of Nigeria approved the creation of the Directorate of Veterinary medicine and Allied Products in NAFDAC. When backed by law, the Veterinary Directorate will be able to monitor and control drug residues in foods of animal origin destined for human consumption.

CHAPTER THREE - METHODOLOGY

3.1 STUDY AREA

Federal Capital Territory, Abuja, was officially created as Nigeria's Capital city on December 12, 1991. It is located in the centre of Nigeria and has a land area of 800 Km^2 with a human population of over 1.4 million people and 3.8 million poultry [4,487] with a population of 100 Register poultry farmers and a large population of unregistered poultry farmers. The human population in Abuja is cosmopolitan in nature due to the influx of different people from other parts of the Nigeria and other foreign nationals.

Most veterinary services for the Territory are provided by the Animal Health Divisions of the Federal Capital Territory Administration and the Area Councils. Services are provided to all categories of animals e.g. poultry, large animals, small animals, dogs and cats and other pets. Private veterinary practitioners, though few, also provide some significant veterinary services in the territory. Administratively, districts constitute the Six Area Councils in the Territory, viz: Abaji, Kwali, Kuje, Gwagwalada, Municipal and Bwari Area Councils.

Abuja city centre consist of five government owned markets, this include: Garki International market, Utako Ultra-modern market, Gudu market, Wuse market and Garki modern market.

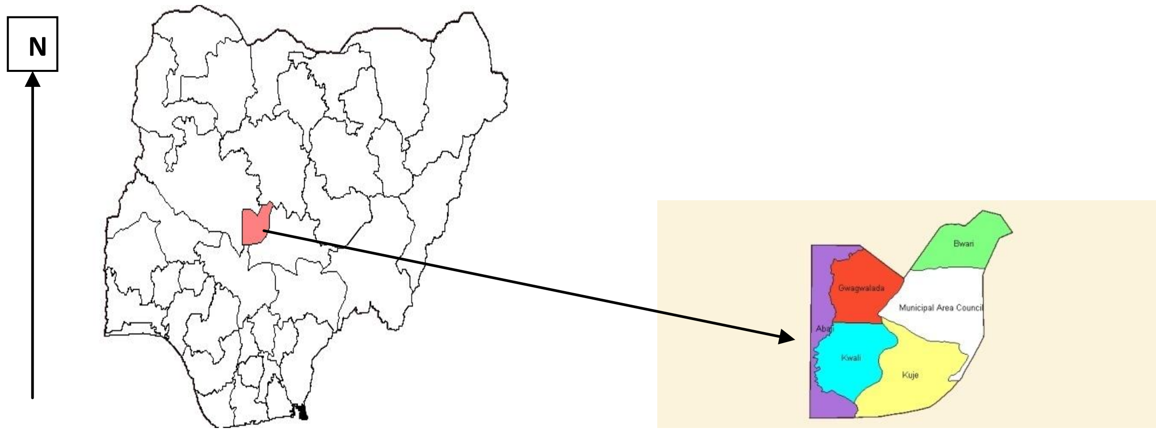


Figure 1. Map of Nigeria highlighting the F.C.T. and the six Area Councils

3.2 STUDY DESIGN

A cross-sectional survey using structured questionnaires was conducted on poultry farmers to assess the usage of CAP and the awareness of its ban. A survey of commercial chicken eggs from poultry farms and government owned markets in FCT was also carried out.

3.3 STUDY POPULATION

The study populations were registered poultry farmers in F.C.T. and chicken egg samples from the farms and the markets.

3.3.1 INCLUSION CRITERIA

- Intact commercial chicken eggs sold by consenting major marketers (full time egg sellers) in government owned markets.

- Questionnaires were administered to only register poultry farmers in F.C.T.

3.3.2 EXCLUSION CRITERIA

- Egg using food processors such as tea joints

- Supermarkets and other egg retailers

3.4 SAMPLE SIZE DETERMINATION

1. For the questionnaire respondents, total population of 100 register poultry farmers were used, out of which only 57 functional farmers were available for the study.

2. The minimum sample size for eggs was determined using:

$n = z^2pq/d^2$ [53]

Where n = Sample size for eggs

z = Standard normal deviate (1.96 at 95% confidence interval)

d = Level of precision (0.05)

p = Proportion having the characteristic (0.254).[32]

q = proportion that does not have the characteristic (1-p=0.746)

Therefore, $n = (1.96)^2(0.254)(0.746)/(0.05)^2$

$= (3.8)(0.189484)/0.0025$

= 288 samples (10 pooled eggs makes a sample).[8]

3.5 SAMPLING TECHNIQUE

1. Questionnaires were administered to all the 57 operational register poultry farmers. Ten (10 freshly laid) pooled eggs were sampled (first 10 eggs makes a sample) from each farm, making a total of 57 egg samples. Egg samples from each farm were properly labeled for identification purpose. Each farm was visited only once.

2. Egg samples were also sourced from the markets. Stratified sampling of markets was first employed. Federal Capital Territory was divided into two strata: government owned markets in Abuja city center (50% proportionate to size) versus government own markets in satellite Area council's headquarters (50% proportionate to size).

Sampling of the markets was done using (OpenEpi software) computer generated table of random numbers. Of the five government owned markets in Abuja City Centre (Garki International market, Utako Ultra-modern market, Gudu market, Wuse market and Garki modern market), three markets (Utako Ultra-modern market, Wuse market and Garki modern market) were randomly selected. Of the five government owned markets in the satellite Area Council's headquarters (Abaji, Kwali, Kuje, Gwagwalada and Bwari) three markets (Kwali market, Kuje market and Gwagwalada market) were randomly selected.

In each market, List of all the major egg marketers (full time egg sellers) in the sampled markets were obtained and served as the sampling frame for eggs. Pooled sample of ten (10) eggs per marketer per origin were collected and recorded. Sampled markets were visited sequentially until the required sample size (288 including egg samples from farms) was obtained. Each pooled sample (10 eggs) shells were then cleaned with 75% ethanol, broken at the taper ends to drain the albumen and 10ml of the homogenized egg yolk were collected into capped test-tubes, properly labeled and frozen, ready for laboratory analysis.

3.6 STUDY INSTRUMENTS

The instruments used in carrying out this study are enumerated below:

A. Questionnaires

The questionnaires are structured and contain the following sections:

- i. **Section I**: consist of questionnaire ID, name of farm, farm address and Area council.
- ii. **Section II**: Socio-demographic information, comprising of respondent's occupation in the farm, age sex.
- iii. **Section III**: Type of farm management, birds reared and approximate egg production per day
- iv. **Section IV**: Chloramphenicol usage and other antibiotics.
- v. **Section V**: Awareness on Chloramphenicol ban.

B. CAP ELISA Kits (4 No.) sourced from Antibodies-online Inc., Atlanta, U.S.A. A Kit component for 96 analyses include:

1. Micro-well strips: 12 strips with 8 removable wells each
2. 6x standard solution (1 mL each):

   0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb and 4.05 ppb,
3. Enzyme conjugate (12 mL) red Cap;
4. Concentrated antibody working solution (1 mL) blue cap;
5. Substrate A solution (7 mL) white cap;
6. Substrate B solution (7 mL) black cap;
7. Stop solution (7 mL) yellow cap;
8. 20x Concentrated washing buffer (40 mL) white cap;

9. 2x concentrated redissolving solution (50mL) transparent cap.

Additional materials not part of the kit set:

1. **Equipment**: microplate reader, homogenizer, nitrogen-drying device, vortex, centrifuge, measuring pipets, and balance (a sensibility reciprocal of 0.01 g).
2. **Micropipettors**: single-channel 20-200 µL, 100-1000 µL, and multi-channel 250 µL.
3. **Reagents**: Ethyl acetate, Acetonitrile (CH_3CN), N-hexane, $Na_2Fe(CN)_5.NO.2H_2O$, $ZnSO_4.7H_2O$,

3.7 DATA COLLECTION METHODS

1. Data were collected using interviewer-administered structured questionnaires. Two interviewers who are Livestock Superintendents, working with the Avian Influenza Unit, Animal Health Department, Federal Capital Territory Administration, were recruited and trained for a day. Questionnaires were then pretested and validated. The pretested questionnaires were administered on ten (10) backyard poultry farmers who were not part of the target population.

2. Chloramphenicol residue ELISA test kits were used for the laboratory analysis. This test principle is based on the competitive enzyme immunoassay for the detection of chloramphenicol in the tissue (chicken, pork), honey, milk, fish, shrimp and egg. The assay operates on the basis of competition between the chloramphenicol in the sample and the anti-chloramphenicol antibody for the limited number of specific antigen binding sites. After half hour incubation, the unbound reagents are removed in a washing step. The enzyme conjugate uses horse radish peroxidase (HRP) as a tracer. The amount of enzyme conjugate bound is measured by the addition of a chromogen substrate, tetramethylbenzidine (TMB). The colour intensity is inversely proportional to the chloramphenicol concentration in the calibrator or sample.

The following protocol by Antibodies-online Inc. was employed for analyses of the egg samples:

A.

i. Egg yolk from a pooled sample was homogenized.

ii. Three grams (3.0g) of the homogenized sample was weighed and 9 mL of the acetonitrile-H2O solution ($V_{acetonitrile}$:V_{H2O} = 84:16) was added, shaken for 5 minutes, and centrifuged at 4000 r/minute for 10 minutes.

iii. Three (3 mL) milliliters of the upper layer was transferred into a centrifuge tube, and 3 mL of deionized water added, mixed properly, then 4.5 mL ethyl acetate added, mixed properly for 5 minutes and centrifuged at
4000 r/minutes for 10 minutes.

iv. The organic phase (upper layer) was transferred into a new centrifuge tube and blew to dry with nitrogen at 50 ^{o}C.

v. The dry residues were dissolved in 1 mL N-hexane, and 2 mL of the diluted re-dissolving solution was added, mixed properly for 30 seconds, and centrifuged to remove N-hexane.

vi. Fifty (50) µL was then taken for final analysis.

Fold of dilution of the sample: 2

Detection limit: 0.1 ppb

Quantitative limit: 0.3 ppb

B.

i. All the necessary reagents from the kit were taken out and placed at room temperature for 30 minutes, shaken to mix evenly before use.

ii. Fourty (40) mL of the concentrated washing buffer (20 x concentrated) was diluted with distilled water at 1:19 to 800 mL for use.

iii. The micro-wells were numbered according to samples and standard solution.

iv. The concentrated antibody working solution was diluted with the redissolving solution at 1:10

v. Fifty (50) µL of the sample or standard solution were added to separate wells; 50 µL of the diluted antibody working solution was added to each well, mixed gently by shaking the plate manually. The microplate was then sealed with the cover membrane and incubated at 25 ^{0}C for 30 minutes.

vi. The liquid was poured off the microplate and washed with buffer at 250 µL/well 4-5 times for 15-30 seconds and flapped to dry with absorbent paper.

vii. One hundred (100) µL enzyme conjugate was added into every well, sealed with cover membrane and incubated at 25 ^{0}C for 30 minutes (and repeat step 5 above).

viii. Fifty (50) µL of substrate A solution and 50 µL of substrate B solution were added into each well, mixed gently by shaking the plate manually and incubated at 25 ^{0}C for 15 minutes at dark for coloration. The colour intensity is inversely proportional to the chloramphenicol concentration in the sample.

3.8 DATA MANAGEMENT

The two data sets from:

i. Questionnaires and egg samples from poultry farmers

ii. Egg samples from markets

were validated and cleaned for consistencies and analyzed using Microsoft Office Excel 2007 and Epi Info version 3.5.3 softwares.

3.8.1 MEASUREMENT OF VARIABLES

Using the structured questionnaires, data obtained on the following variables were analyzed:

- Sociodemographics of respondents

- Relationship with the farm
- Age
- Sex

- Chloramphenicol usage

- Awareness on chloramphenicol ban

3.8.2 STATISTICAL ANALYSES

Univariate analysis was carried out to obtain frequencies and proportions for data summary. Bivariate analysis was conducted using 2 X 2 tables to examine the strength of associations from the odds ratios. Fischer's exact (P) values were used for statistical significance and inferences.

3.9 ETHICAL CONSIDERATIONS

Informed consent (verbal and written) of questionnaire respondents was obtained. Confidentiality of respondents was maintained. Ethical clearance and approval was obtained from the Ahmadu Bello University Teaching Hospital health research and ethics committee.

3.10 LIMITATIONS

a) Study design bias, in that egg using food processors (e.g tea joints) and egg retailers (e.g. supermarkets) who are not full time egg marketers were excluded from the survey.

b) Underestimation of CAP residue exposure in the population due to exclusion of small scale poultry producers that provide eggs strictly for home consumption and those derived from unregistered farms.

c) Possible deterioration of CAP residues in eggs under protracted period of storage at room temperature in the markets. This might have reduced chances of detecting the CAP residues (if present)

d) Recall bias by questionnaire respondents.

CHAPTER FOUR – RESULTS

A total of 57 questionnaires were administered and all the study participants (poultry farmers) responded appropriately giving a response rate of 100%. A total of 288 egg pooled samples (10 eggs make a sample) obtained from the poultry farms and markets were analyzed using CAP ELISA kits. Results of the data analysis (univariate and bivariate) in line with the specific objectives of the study are presented in the following tables:

4.1 Baseline socio-demographic data

Table 1. Socio-demographic characteristics of Respondents from Poultry Farms in FCT.

Characteristic	Frequency (n=57)	Proportion (%)
Relationship with farm		
Farm owner	12	21.1
Farm manager	30	52.6
Farm Veterinarian	3	5.3
Others		
Poultry attendant	9	15.7
Farm supervisor	3	5.3
Age		
<20 years	3	5.3
21-35 years	27	47.4
36-50 years	21	36.8
>50 years	6	10.5
Sex		
Female	9	15.8
Male	48	84.2

Out of the 100 poultry farms registered by the Avian Influenza unit of the Animal Health Department in FCT, only 57 (57%) were operational and all responded to the questionnaires.

Of the 57 respondents, 84.2% were males, 47.4% were between ages 36-50 years, 52.6% were farm managers.

4.2 Other tables and figures based on specific objectives

Table 2. ELISA Test result by egg sample (source) sold in FCT Markets, April, 2013

State of origin	**No. of (*pooled) samples: n=288**	**ELISA Result**		
		Positive	**Negative**	**Prevalence (%)**
Zamfara	1	0	1	0
Kaduna	3	0	3	0
Nassarawa	6	0	6	0
Kogi	1	0	1	0
Anambra	1	0	1	0
Ekiti	1	0	1	0
Ondo	10	0	10	0
Osun	4	0	4	0
Kwara	5	0	5	0
Ogun	2	0	2	0
Lagos	4	0	4	0
FCT	128	**4**	284	1.4
Niger	4	**1**	287	0.4
Oyo	53	**10**	278	**3.5**
Plateau	65	**5**	283	1.7
Total	**288**	**20**	**268**	**7.0**

*10 eggs (pooled) make a sample; therefore, 2880 eggs make 288 (pooled) samples.

A pooled sample is obtained from the same source, the same marketer and the same market or farm.

Influxes of eggs into FCT were from 15 States (including FCT) of the federation.

Of the 288 samples, 20 (7%) tested positive for chloramphenicol, with the highest number of positive samples 10 (3.5%) from Oyo State.

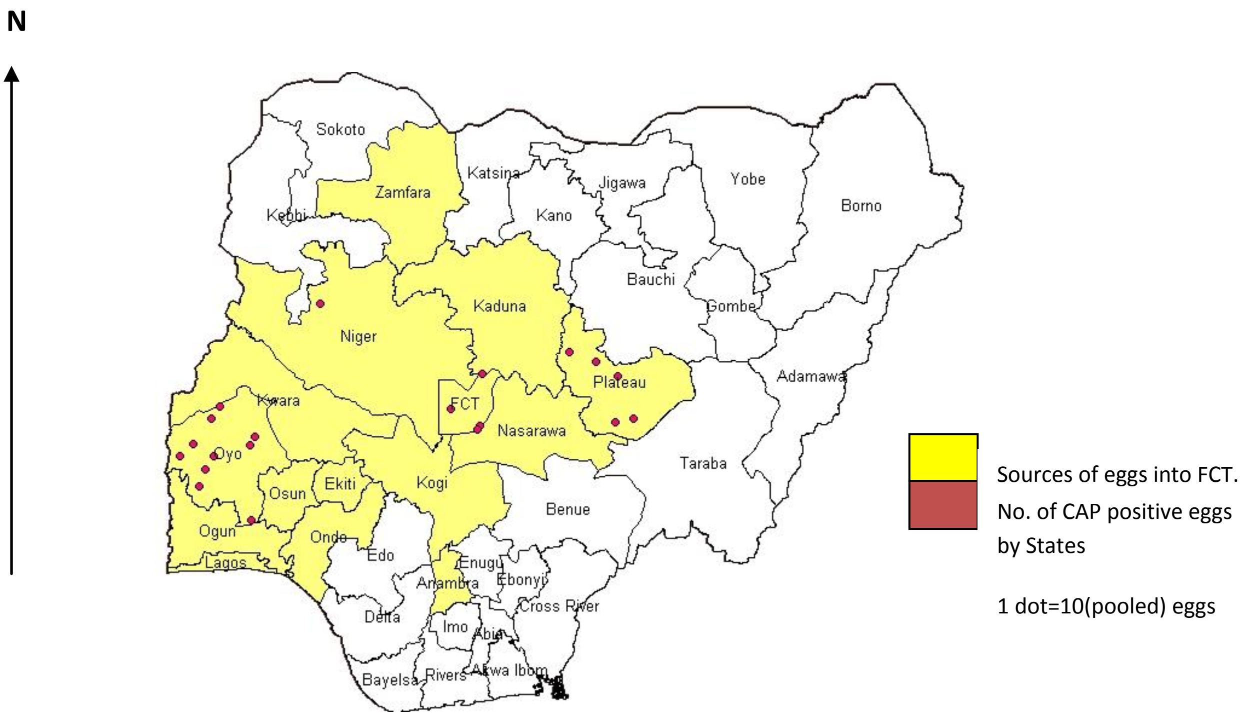


Figure 2. Distribution of sources of CAP positive eggs in FCT., April, 2013

Aside from eggs produced within FCT, there is a high influx of eggs into FCT from the neighboring States and beyond.

Of the eggs that tested positive for chloramphenicol, 50% originated from Oyo State.

Table 3. ELISA test results of egg samples from farms and markets in FCT, April, 2013

Sample source	Frequency of (pooled) samples (%): n=288	ELISA Result	
		Positive	Negative
Farms			
***GWAC Farms**	6 (2.1)	0	6
*ABJAC Farms	1 (0.3)	0	1
*AMAC Farms	4 (1.4)	0	4
*BWAC Farms	3 (1.0)	0	3
*KAC Farms	10 (3.5)	**4**	7
*KWAC Farms	7 (2.4)	0	6
Sub Total	**31 (10.7)**	**4**	27
Markets			
Garki modern market	55 (19.1)	**3**	52
Wuse market	43 (15.0)	**6**	37
Utako market	57 (19.8)	**5**	52
Kwali market	34 (11.8)	**1**	33
Gwagwalada market	36 (12.5)	**1**	35
Kuje market	32 (11.1)	0	32
Sub Total	**257 (89.3)**	**16**	241
Grand Total	**288 (100)**	20	**268**

*These are poultry farms in Gwagwalada, Abaji, Abuja Municipal, Bwari, Kuje and Kwali Area Councils, respectively.

Farms that tested positive for chloramphenicol (4[13%]) were all from Kuje Area Council.

Amongst the markets, Wuse market has the the highest No. of CAP positive eggs = 6 (2.3%)

Table 4. Usage and awareness of CAP amongst poultry farmers in FCT, April, 2013

Variable	Frequency (n=57)	Proportion (%)
Usage		
Neocloxin®	15	26.3
N.C.O. Mix®	25	43.9
Tyfurchlor®	3	5.3
Use Human CAP?		
Yes	5	8.8
No	52	91.2
Aware of CAP ban?		
Yes	15	26.3
No	41	71.9

Poultry farmers in FCT were not aware that CAP is not recommended for use in food producing animals (71.9%). They use Both Human (8.8%) and vet. drug (N.C.O. Mix® 43.9%) preparations containing CAP on their birds.

Table 5. Relationship between CAP usage and residue containing eggs in FCT Poultry Farms

Use of CAP	ELISA Test (Farms)		OR	P-value (CI)
(n=57)	Positive	Negative		
Neocloxin®	2	10	8.3	0.05 (0.59-264.5)
N.C.O. Mix®	4	21	5.7	0.06 (0.67-150.4)
Tyfurchlor®	1	2	7.8	0.1 (0.22-132.0)
Human Cap	2	3	**14.8**	**0.01 (1.2-188.3)**

There is an association between CAP use and the eggs from poultry farms that tested positive for CAP residues.

The strength of the association (OR) is 14.8 times more likely for human CAP use to be detected in eggs by ELISA test and it is statistically significant ($P<0.05$).

Table 6. Awareness of CAP ban for use in food producing animals amongst Respondents in FCT Poultry Farms, April, 2013

Variable (n=57)	Awareness Yes	No	OR	Fischer's Exact P-value (CI)
Farm Owner	3	9	1.03	0.9 (0.15-5.19)
Farm Manager	8	22	1.0	0.9 (0.27-4.05)
Farm Veterinarian	1	2	**1.4**	0.3 (0.02-29.27)
*Others	3	9	**0.9**	0.4 (0.14-4.56)

Farm Veterinarians are 1.4 times more likely to be aware of the ban on CAP use in food producing animals.

* Poultry attendants and farm supervisors are 0.9 times less likely to be aware of the ban on CAP use in food producing animals.

CHAPTER FIVE - DISCUSSION

Following the ban on the use of CAP in food animals and the evidence of its indiscriminate use by poultry farmers in different parts of the world, coupled with low awareness reported in the developing world, the FAO/WHO joint committee on foods and additives (JECFA) recommends effective monitoring and reporting of drug residues in foods of animal origin meant for human consumption.[4] Very few researches have been done in Nigeria in this area in a bid to find a solution to this problem and this form the basis for this research.

The outcome of this study revealed that CAP residues are present in commercial chicken eggs in poultry farms and markets in FCT with 7% prevalence. This implies the contravention of the ban on the use of CAP in food animals. There is a high influx of commercial chicken eggs (56%) from other States into FCT and these also were found to contain CAP residues (5.6% prevalence). This implies that CAP residues in chicken eggs have a nationwide spread. The high influx of eggs into FCT suggests an inadequate egg production by FCT poultry farmers for her residents. This is also a good economic indicator for those interested in egg production in the FCT.

Similar studies in Ibadan, Oyo State [32] and Kaduna State[49] have also confirmed occurrences of CAP in chicken eggs with 25% and 0.7% prevalence respectively. Of the eggs that tested positive for CAP in this study, 50% of them originated from Oyo State. This confirms the findings of Olatoye in 2012[32], that there is high prevalence of CAP residues in poultry tissues in Ibadan, Oyo State. The low prevalence recorded in Kaduna State by a similar study in 2012[49], may be due to the less sensitive STLC test used. A more sensitive ELISA test (used in this study) would have probably detected a higher prevalence. This nationwide occurrence of CAP residues poses an enormous health risk to Nigeria's teeming human population and therefore calls for a national drug residue surveillance and control program.

In most countries the total ban of CAP is in place with very few reports of CAP residue occurrence. In Slovenia, between 1991 and 2000 a survey of 1,308 different animal tissue products including eggs, CAP residues were determined (using gas chromatography) in only one milk sample with a prevalence of 0.1%.[29] This low prevalence results from the CAP residue monitoring and a consequence of the strict prohibition of this veterinary drug for food producing animals, as well as a proper veterinary sanitary control of its residues in Slovenia. Findings from another study in Morogoro Municipality, Tanzania, showed 10% prevalence of CAP in commercial chicken eggs.[30] This does not vary widely with the prevalence in this research even though the detection methods were different. This goes to show that Nigeria and Tanzania may have similar problems of poor regulation and enforcement of the ban on the use of CAP in food animals.

The frequency of occurrence of CAP residues showed that Kuje Area Council amongst the six Area Councils in the FCT recorded the highest. In fact it is only farms from Kuje Area Council that tested positive for CAP. This is not surprising because Kuje Area Council has the highest poultry (population) farms in FCT. High density of farms increases the risk of spread of diseases between farms, leading to using antimicrobials more readily.[50]

Residues of antibiotics degrade in tissues after long term storage. It is not clear whether CAP exhibit same properties in eggs which if so could underestimate the level of occurrence of this problem in Nigeria. Since most of the eggs come from outside FCT, a lot of the eggs are no longer fresh due to long period of haulage and most are not even sold immediately on arrival, until days later.

This study has shown that 43.9% of poultry farmers in FCT use veterinary preparations of CAP for their birds which is almost close to the finding in Ibadan, Nigeria (50%)[32] but vary from the findings in Tanzania (10%).[30] and Kaduna, Nigeria (20%).[49] However, findings on the use of human CAP in Kaduna, Nigeria (62.2%)[49] differ from the findings on the use of human CAP in this research (8.8%). Due to lack of understanding by poultry farmers of the pharmacokinetics of CAP in chickens, who erroneously believe that human CAP is as good or even better than veterinary CAP preparation, leads to their abuse and illicit use of these drugs. This implies a bilateral lack of effective control of both human and veterinary drugs.

There are indications in this study that use of human CAP in place of veterinary CAP preparation does promote the occurrence of its residues in eggs (OR=14.8; Fischer's Exact P=0.01). Two (2) out of five (5) farms tested positive to human CAP in this research contrary to the findings in Kaduna, Nigeria[49] , where all the farms that use human CAP tested positive. Use of human CAP therefore lends credence to the suggestion that using human drug preparations is likely to cause overdosing and residue occurrence and thus can be considered as an unsafe practice due to varied pharmacokinetics in the species mentioned by *Anadon, et al*.[51] The strong association (OR=14.8) of detecting human CAP in this study may also be due to the pure single preparation of human CAP used by poultry farmers unlike the veterinary CAP preparations that comes in combination with other drugs which may reduce its chances of detection. There is paucity of information on the pharmacokinetics of human CAP preparation in chickens, thus no certainty of appropriate dosage and withdrawal period even in those drugs that are recommended for use in poultry.

For using chloramphenicol in food animals, an Iowa, Washington DC, large animal (veterinary) practitioner in 1991 was sentenced to 12 months imprisonment, the costs of imprisonment, a $15,000 fine, and two years' supervised release.[34] The conviction came after the trial, which was held in the United States District court for the Northern District of Iowa. It was established that the veterinarian acquired, processed, used, and dispensed chloramphenicol and other illegal animal drugs in his food animal practice. The trial also established that he had been aware of the prohibition against the use of chloramphenicol in food animals. If this kind of legal measures can be enforced in Nigeria, regulations concerning the use of chloramphenicol and other abused veterinary drugs will be adhered to. This will go a long a way in protecting public health.

In the survey of poultry farmers rearing commercial layers in Kaduna State, Nigeria,[49] Only 26.7% of respondent farmers were aware that CAP was one of the drugs that is not recommended for use in food animals. This agrees with the findings in this research whereby only 26.3% of the poultry farmers were aware that CAP is not recommended for use in food animals. It was also found in this study that poultry attendants specifically are less likely (OR=0.9) to be aware of the CAP ban for use in food producing animals. There is poor perception of the possible effects of antimicrobial residues on human health in Nigeria.[49] This has highlighted the low level of awareness of legislation that governs the application of drugs (especially CAP) in poultry.[49] This lack of awareness amongst poultry farmers on the ban of CAP for use in food animals is of great public health concern and importance. Earlier reports made by FAO/WHO [52] over the low level of awareness of drug residues amongst the developing nations also emphasized this great public health significance.

CHAPTER SIX – CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From the study we can conclude that chloramphenicol residues of 7% prevalence are present in commercial chicken eggs destined for human consumption in the Federal capital Territory, Abuja, Nigeria. These residue-containing eggs were both from poultry farms within F.C.T. and other geo-political regions of the country and most poultry farmers in F.C.T. using both human (8.8%) and veterinary CAP preparations (43.9%) were unaware of the FAO/WHO global ban on its use in food-producing animals. These results indicate that egg consumers in F.C.T. (and other parts of the country) are exposed to health hazards associated with CAP residues and this can jeopardize international egg trade from Nigeria.

6.2 Recommendations

1. The NAFDAC should enforce the food and drug decree (1974) that provides for residue avoidance in accordance with the recommendations of FAO/WHO Codex Alimentarius Commission towards implementing total ban of CAP use in food animals in Nigeria.

2. The FCT Animal Health Department should establish integrated drug residue surveillance and control program in order to protect the public from health hazards associated with drug residues. This will serve as a prototype for other States to emulate.

3. Poultry farmers in FCT should be educated on best practices for the use of veterinary drugs. Veterinarians should promote alternative management options such as vaccinations, drug withdrawal period adherence, environmental sanitation and disease containment, which would decrease the use of antibiotics that could reduce the frequency of antimicrobial drug usage in poultry, occurrence of drug residues and spread of drug resistant bacteria.

4. There is a need for continuous education of Veterinarians and other Poultry Professionals by the Veterinary Council of Nigeria, on chemical contamination of drug residues in foods of animal origin.

5. Due to possible underestimation of CAP residue exposure in the population in this study, there is the need for further research about CAP residues amongst small scale poultry producers that provide eggs strictly for home consumption in FCT and other States of the federation.

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APPENDICES

APPENDIX I

DEPARTMENT OF COMMUNITY MEDICINE

FACULTY OF MEDICINE

AHMADU BELLO UNIVERSITY, ZARIA

Dear Respondent,

QUESTIONNAIRE ON CHLORAMPHENICOL RESIDUES IN CHICKEN EGGS

I am a student from the Nigeria Field Epidemiology & Laboratory Training Program. This questionnaire is designed as part of a scientific study of chloramphenicol use and its residues in chicken eggs. Your identity will not be required. The information you will provide will be strictly treated as confidential and used solely for academic purposes.

Please, tick your preferable answer.

Thanks for your cooperation.

Dr. F.E. Mbodi

Tel.: 08065491626

Questionnaire No.:………………..

Name of farm………

Farm address………

Area Council………

1. Relationship with the farm
   a) Farm owner
   b) Farm manager

c) Farm Veterinarian
d) Animal health practitioner
e) Others (please, specify)……………………………………….

2. Age
   a) Less than 20 years
   b) 21 - 35 years
   c) 36 – 50 years
   d) Above 50 years
3. Sex: ☐ Male ☐ Female
4. Type of farm management system
   a) Extensive
   b) Intensive deep litter
   c) Intensive battery cage
5. Type of birds reared on the farm
   a) Broilers only
   b) Layers only
   c) Cockerels only
   d) Breeders only
   e) Combination of any of the above (please, specify)……………………………….
6. If the flock consists of layers, what is the approximate egg (crate) production per day?
   a) Less than 10
   b) 10 – 59
   c) 60 – 99
   d) 100 and above
7. With respect to commercial outlet, where do you sell your eggs?
   a) Sell by self in the market
   b) Sell to retailers in the local market
   c) Sell to wholesalers in F.C.T.
   d) Sell to wholesalers in other parts of the country
   e) Others (please, specify)……………………………………………….
8. Which of the following brand of poultry antibiotics do you administer to your birds? **(If applicable choose more than one option)**
   a) Neocloxin®
   b) N.C.O. mix WSP®
   c) Tyfurchlor®
   d) Others (please specify)……………………………………………………
9. Do you use chloramphenicol (human preparation) for your birds? □ **Yes** □ **No**

10. If you use any of the antibiotics mentioned in (8) or (9) above, how will you describe the frequency of usage in your farm?
    a) Always
    b) Sometimes
11. Why do use the antibiotics mentioned in (8) and (9) above?
    a) Very effective
    b) Cheap
    c) Others (please, specify)………………………………………………………………………………………
12. Who recommends your use of the antibiotics mentioned in (8) and (9) above?
    a) Veterinary Doctor
    b) Vet. Assistant/Animal health/Livestock superintendent
    c) Poultry attendant
    d) Others (please, specify)……………………………………………
13. Do you have any knowledge about legislation guiding the use of chloramphenicol for veterinary use? □ **Yes** □ **No**
14. What is your feeling towards consumption of eggs containing chloramphenicol residues?
    □ Concerned □ Not concerned
15. If concerned, which of the following describes the reason for your concern?
    a) Treat sick birds to prevent death and improve production
    b) The eggs may be containing some of the chloramphenicol administered
    c) The chloramphenicol residues in eggs may have effect on the health of consumers
16. Do you know that chloramphenicol is not recommended for use in food producing animals?
    □ I know □ I don't know
17. What exactly would describe your judgement over eggs containing chloramphenicol residues?
    a) Eggs could be sold to the public
    b) Eggs could be consumed in my household
    c) Eggs should be discarded

APPENDIX II

Ahmadu Bello University Teaching Hospital

P.M.B. 06, Shika - Zaria, Kaduna State, Nigeria. 069-876305

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Chairman of Board:

Chief Medical Director: **DR. LAWAL KHALID,** ***MBBS, FMCS, FWACS, FRCS(ED) mni***

Chairman, Medical Advisory Committee: **DR. ABDULLAHI MOHAMMED,** ***MBBS, FWACP FICS***

Director of Administration: **BARR. ISHAK BELLO,** ***LL.B, BL, LL.M, PGDM, AHAN, FCAI***

Our Ref: ABUTH/HREC/TRG/36

7th March, 2013

Dr. Mbodi Felix Enson,
Comm. Medicine Department,
A.B.U. Zaria.

ETHICAL CLEARANCE.

Your application for ethical clearance on the research proposal titled: - **'Determination of Chloramphenicol residues in commercial chicken eggs in the Federal Capital Territory (FCT)."** refers.

This is to convey ethical approval for you to commence the study. The ABUTH Scientific and Health Research Committee require an annual update from the Principal Investigator.

Thank you.

PROF. J.U OKPAPI
CHAIRMAN, ABUTH HREC