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Chloramphenicol Use and Prevalence of its Residues in Broiler Chickens and Eggs in Ibadan, Nigeria.

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SUMMARY

Antibiotic use in poultry could result in deposition of residues in edible products of which chloramphenicol is of particular concern because its toxicity and non-dose dependent fatal aplastic anemia in humans. This study assessed the use of antibiotic in poultry production, determined the prevalence and concentration of chloramphenicol residues in meat and eggs from poultry farms and commercial outlets in Ibadan. Semi-structured questionnaires were administered to 75 poultry farmers to assess the use of antibiotics in poultry and the presence of chloramphenicol in chicken eggs and meat randomly obtained from broiler farms and commercial egg outlets were screened for chloramphenicol residues using ELISA technique.

The results showed that 50% of the farmers reported administering chloramphenicol to their flocks. Also the survey of five veterinary drugs retail outlets revealed four brands of antibiotics sold for poultry use containing chloramphenicol as an active ingredient. The residue was detected in 51.1% of poultry organs and 25.4% of eggs with mean concentration ranged from 61.8 to 74.6 ng kg-1 in meat and 283.5 to 298.9 ng kg-1 in eggs. The prevalence of chloramphenicol residue was significantly higher in organs than in eggs, but the concentrations were significantly higher in eggs than in meat ($p<0.05$).

This study documented widespread sales and use of chloramphenicol in poultry in Ibadan with high concentrations of chloramphenicol residues posing public health risks and international trade barrier. We recommend prudent antibiotic use in food animals, alternative treatment regimens and enforcement of appropriate regulatory efforts to prohibit the use of chloramphenicol in food animals.

KEY WORDS: Antibiotics, Chloramphenicol Residues, Poultry.

INTRODUCTION

The Nigerian poultry industry constitutes an important agricultural enterprise to the country, contributing significantly to the national Gross Domestic Product (GDP) (Ambali et al., 2003). The population of poultry (both local and exotic chickens) in Nigeria has been estimated approximately 190 million (Orajaka, 2005). Poultry farming is a fast means of bridging the protein deficiency gap common in many developing countries of the world (Jibir and Usman, 2003) and efficient means of supplying the fast-growing human population with high-quality protein (Guéye, 2004). It also provides employment and income to the generally resource-poor small holder farmers to alleviate poverty (Guéye, 2004).

Antibiotics are among the major inputs of commercial poultry production in Nigeria. Veterinary drugs are usually imported from the developed nations and distributed to livestock and pet owners by about 150 private business merchants in Nigeria. (http://www.veterinaryproducts1.com/veterinary-suppliers/nigeria.html). The use of antibiotics in livestock production has become a global food safety concern due to the effect on human health via multiple pathways of directly or indirectly from food, water, air or manure.
Chloramphenicol (CAP) is a broad spectrum antibiotic that is active against both gram-positive and gram-negative bacteria (Sorensen et al, 2003). It has been used against salmonellosis and other bacterial diseases of poultry. However, due to the none-dose dependent toxicity in human, the use of chloramphenicol by humans has caused serious toxic side effects such as potentially fatal aplastic anaemia, agranulocytosis (Fabiansson et al, 1976) and leukemia (Dollery, 1999; Turton et al., 2002; JECFA, 2004). The use of chloramphenicol in food and feeds has therefore been restricted or prohibited in many developed nations.

However, in most developing countries including Nigeria, indiscriminate administration of antibiotics to food animals is a common practice due to unregulated distribution and access of farmers to veterinary drugs in the open markets or over the counter without veterinary prescription and supervision (Dipeolu, 2002). This misuse or abuse in food animals could be incriminated as a critical factor leading to antimicrobial resistance as well as posing health risks to consumers due to the presence of antimicrobial residues in animal products.

Preliminary studies suggested that chloramphenicol is among antibiotics frequently administered to poultry in the Nigeria (Kabir et al., 2004; Olatoye, 2011). Also in a recent study by Adewuyi et al. (2011), chloramphenicol residue was among detected in chicken from markets in Ibadan. Thus we hypothesised that the practice of antibiotic use in poultry results in high proportion of the chicken meat and eggs produced for human consumption in Nigeria with detectable chloramphenicol residues. In this study, we survey the use of antibiotics and assay for CAP residue in chicken eggs and broiler meat in Ibadan environs.

MATERIALS AND METHODS

The Study Area

Ibadan, the capital city of Oyo State, is in the south western part of Nigeria, on grid reference latitude 07o22' North and longitude 03o58' East. It has a population of over 2.5 million people inhabiting a total of eleven Local Government Areas. Ibadan is a major poultry producing community in Nigeria, according to the Poultry Association of Nigeria (PAN), Oyo State Chapter there are 112 registered poultry farms in Oyo State as 2011; most of which are located at the Local Government Areas within and around the capital city. A lot of commercial poultry marketing activities such as sales of day old chicks, growing pullets, broilers and breeder chicken, veterinary drugs, vaccines and poultry equipment are operated within and around the city for distribution to many parts of Nigeria and neighboring countries. Large scale commercial chicken egg and broiler producers are also operating within the urban and peri-urban area of the city. Poultry markets of various structures ranging from day old chicks, retail eggs as well as live poultry slaughter and frozen chicken markets exist across the city.

Survey and sampling were done from three major broiler grow-out farms and egg retail markets from seven LGAs randomly selected from Ibadan Area (FIGURE 1) during a period of six weeks between April and October 2010 for oxytetracycline residue analysis.

Antibiotic Use Survey

Semi-structured questionnaires were administered to 75 randomly selected poultry farmers from the members of PAN (Oyo State Chapter) in different local governments of the study area. The questions were aimed at assessing the rationale and pattern of use of antibiotics in poultry production. Respondent farming practices, production data of the farms, poultry feeds and feeding, poultry health status, diseases treatment and prevention in poultry, drug use decisions and sources were assessed. Also, five veterinary drug retail shops in Ibadan metropolis were randomly selected for survey of poultry antibiotics commonly sold that contained chloramphenicol. The brand names, active ingredients, manufacturers' names, addresses and indication for use were recorded.

Sample collection

One hundred and sixty- eight poultry samples (84 eggs and 84 muscles, 84 liver and 84 kidney) were randomly obtained from 3 commercial broilers chicken slaughter farms and egg retail markets in 6 Local Governments Areas of Ibadan. The Organ samples were transported to the Food and Meat Hygiene laboratory, of Department of Veterinary Public Health and Preventive Medicine, University of Ibadan in ice cooled box and kept frozen until further analysis for chloramphenicol residues.

Chloramphenicol residue detection

Ridascreen® Chloramphenicol ELISA kits (R1501) R-BiopharmR, Germany was used to analyze by chloramphenicol residues in samples. Analysis was performed according to manufacturer's protocol which involved sample preparation and ELISA assay.

Sample preparation

Briefly, about 100g of each sample was homogenized with blender and stomacher machine after which about 3g each of the homogenates was weighed, 3ml of distilled water and 6ml of ethyl acetate were added. The suspension was vortexed for 10minutes after which it was centrifuged at 3000g for 10min at room temperature. Four microliters of the ethyl acetate supernatant (corresponding to 2g of sample) was evaporated to dryness at 60°C. The preparation of egg samples was similar to that of organ samples but 2g of the homogenized whole egg sample was weighed and vortexed with 12ml of ethyl acetate for 10minutes.The suspension was centrifuged at 3000g for 10min at room temperature. 6ml of ethyl acetate supernatant (corresponding to 1g of sample) was transferred into a new vial and evaporated to dryness.

The residue was re-dissolved in 1ml n- hexane to which 0.5ml of the chloramphenicol buffer was added to this solution and vortexed for approximately 1min. The solution was centrifuged at 3000g for 10min at room temperature. A 50µl of the aqueous (upper) layer was used per well of the microtitre plate for ELISA.

The ELISA Assay

Fifty microlitres (50µl) of the 6 standard solutions (these came with the ELISA kits) were added to duplicate ELISA wells as well as 50µl of diluted each sample was added duplicate wells and incubated for 2 hours at room temperature. Followed by washing with the washing buffer, 100µl of substrate/chromogen solution were added to each well and incubated for 30 min and the reaction was stopped with100 µl of stop reagent. The absorbance was measured at 450 nm by a Multiscan RC version 6.0 ELISA reader (Labsystems).

Statistical Analysis

Data of prevalence were expressed by descriptive statistic (i.e. percentage, range, mean and standard deviation). The amounts of chloramphenicol residue in the samples were obtained from the percentage absorbance calculated from the linear regression equation of the standard curve. The prevalence and mean residues levels in meat and eggs were compared using Student T-test while the mean residues levels in all the samples were compared by ANOVA and Tukey's multiple comparison test at 95% confidence level using GraphPad Prism 4.0 (GraphPad software Inc., CA)

RESULTS

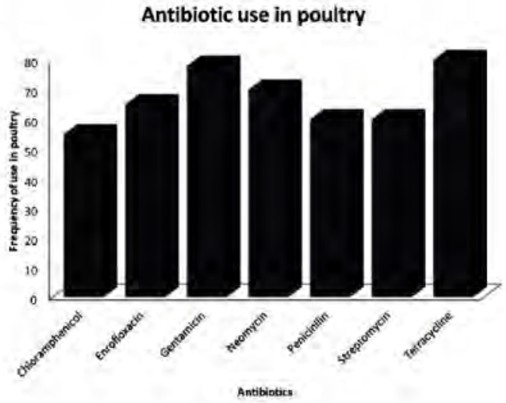
Questionnaire Results

Respondent's Background Information

Out of the 75 poultry farmers respondents, 93.3% practiced intensive management system of production requiring the use of drugs but the remaining 7.2% practiced semi intensive system and flock population ranged from 250 to 15000 chicken. Their academic qualifications ranged from non-formal to university education (FIGURE 2) while 64% of the respondents had above 10 years of poultry farming experience. Egg production was engaged by 32% of the respondents while 13.3% reared only broiler chicken and majority (64.7%) reared both commercial meat and egg chickens. The result also showed that all the respondents engaged in self milling of feed their poultry from different ingredients including drugs as feed additives. About 87.7% of the farmers routinely included antibiotics as routinely added feed additives aimed at disease prevention and to improved productivity.

Drug use in poultry

Majority of the farmers (67.9%) engaged in self diagnosed and drug prescription for their poultry diseases (based on experience of clinical signs and mortality pattern) without engaging the services of veterinarians or laboratory confirmation. They also claimed to source the drugs from retail shops, sales vendors and hawkers. Most of respondents claimed to administer the drugs following manufacturer's instructions on drug labels while the remaining 10% claimed that they needed to increase the dosage or use combinations drugs.



All the respondents (100%) administered different antibiotics (including chloramphenicol) to their poultry flocks for disease prevention, treatment and productivity performance (as egg booster and growth promoter) in different frequencies as shown in FIGURE 2. Oxytetracyline was the most frequently administered antibiotics to the flocks surveyed. Also, majority of the farmers reported that most of the antibiotics are not effective at the recommended dosages but usually combined multiple antibiotics or increase the dosage.

TABLE I: Brands of antibiotics containing chloramphenicol on shelves of veterinary drug stores in Ibadan

Brand name	Composition	Country of Manufacturing	Indication for use
NCO mix WSP	Neomycin Sulphate Chloramphenicol Oxytetracycline HCl	Holland	Infection s of the alimentary tract caused by G+ve and Gneg bacteria such as Actinomyces, Pasteurella, Mycoplasma infections
Tetracure plus	Neomycin Sulphate Chloramphenicol Oxytetracycline HCl	United Kingdom	Chronic respiratory disease, Enteritis, Stress.
Neocloxyl WSP	Neomycin Sulphate Chloramphenicol Oxytetracycline HCl	United Kingdom	Staphylococcal, Streptococcal, Corynebacteria, Typhua and Parathyphus infections
Neofuraseryl plus	Furazolidone, Erythromycine, Oxytetracycline, Neomycine sulphate, Streptomycine, Chloramphenicol	Sweden	Salmonellosis, Pasteurellosis, E coli infections.

The survey of veterinary drug retail shops identified four different brands of antibiotics containing chloramphenicol as part of active ingredients identified from all the retail shelves of the drug marketers were presented in TABLE I.

Prevalence of CAP Residue

The overall prevalence of CAP residue in the poultry tissues is 51.1% while the prevalence in eggs is 25.4%. The distributions across the surveyed farms and markets are shown in FIGURES 3a and 3b. The overall residue prevalence was highest in the muscle (71.4%) and from farm 1 while eggs from Ido LGA had the highest residue prevalence (40%) and none of the egg samples from Oluyole LGA was positive for CAP residue. The prevalence of CAP residue in chicken meat was significantly higher than in eggs (P<0.05).

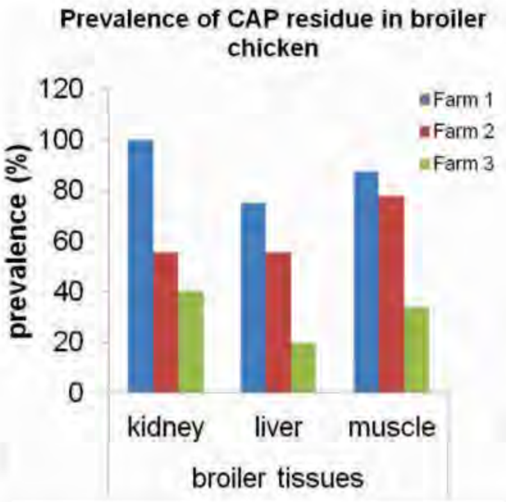


Fig. 3a: Prevalence of chloramphenicol residue in chicken tissues from surveyed farms in Ibadan

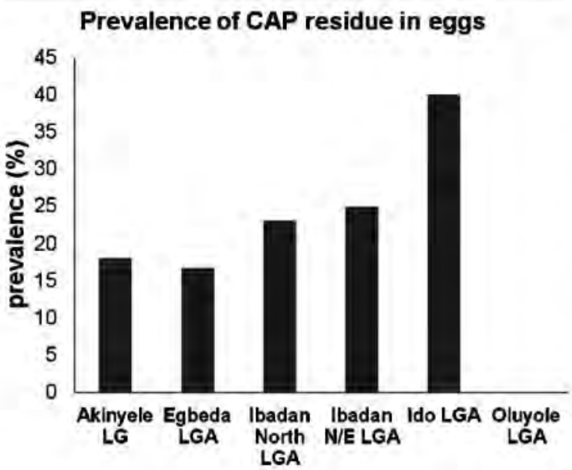


Fig. 3b: Prevalence of chloramphenicol residue in chicken eggs from surveyed markets in Ibadan

CAP Residue Concentrations in chicken tissues and eggs

The standard curve was plotted using linear regression with linear equation formula obtained y= 90.7 – 0.26x, where y = Absorbance (%) and x = concentration (ppt) correlation coefficient (r^2) of 0.93 ($p<0.001$) (FIGURE 4). The limit of detection of the test was 25ng/kg or ppt), samples with values above this concentration were considered as positive, while the coefficient of variation (CV) was 19.89% (25ng/kg or ppt), n=5). The detection limit of the assay was 6.25ppt for tissue samples and 25ppt for egg samples. The overall mean concentrations of CAP residue in the chicken tissues was 68.73 (±7.19 SEM)

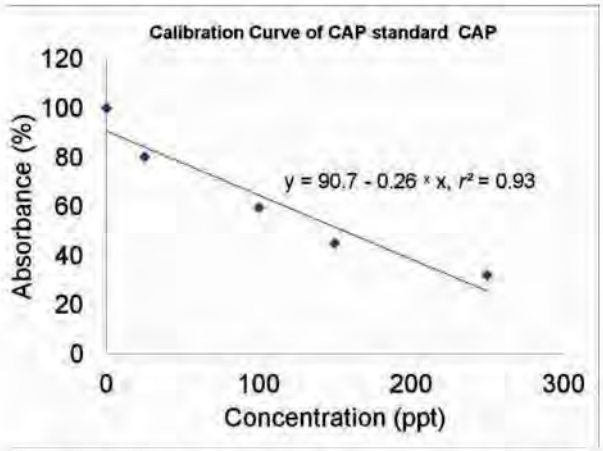


Fig 4: Calibration curve of chloramphenicol standard solution of the ELISA

ng/kg (ppt) ranging from 61.8 to 74.6 ng/kg (ppt) while the overall mean concentrations of CAP residue in the chicken eggs was 239.70 (±11.16 SEM) ng/kg (ppt) with a range of 0.0 to 298.9 ng/kg (ppt). The mean concentrations of CAP residues in eggs and tissues from different locations in the metropolis are shown in TABLES IIa and IIb. The overall mean concentrations of CAP residue in egg samples were significantly higher (P<0.05) than in the tissues, but there was no significant difference in the mean residue levels in the different tissues (kidney, liver and muscle) and eggs from different locations.

TABLE IIa: Mean chloramphenicol residue in broiler edible tissues from surveyed farms in Ibadan

Location	Samples	Mean (±SEM) ppt
Farm 1	kidney 1	62.7 (3.9)
	liver 1	61.8 (2.8)
	muscle 1	62.6 (5.3)
Farm 2	kidney 2	74.9 (3.9)
	liver 2	73.9 (0.7)
	muscle 2	73.7 (2.4)
Farm 3	kidney 3	73.7 (1.4
	liver 3	72.3 (2.9)
	muscle 3	73.0 (3.5)

TABLE IIb: Mean chloramphenicol residue in eggs from surveyed markets in Ibadan

Source	Mean (±SEM) ppt
Bodija market (Ibadan North LG)	283.6 (12.1)
Ojoo market (Akiyele LG)	283.5 (19.9)
Oje market (Ibadan North-East LG)	288.5 (11.3)
New Garage market (Oluyole LG)	0 (0.0)
Apata market (Ido LG)	283.7 (17.2)
Iwo road market (Egbeda LG)	298.9 (6.6)

DISCUSSION

Chloramphenicol induced bone marrow aplasia which is not dose dependent. Extremely low doses of the drug are known to cause this aplastic anemia in human. This, together with other toxic and carcinogenic effects of chloramphenicol, has caused particular concern for the public health and have led to prohibition of its usage in food animals (Papich and Riviere, 2001; Wareham and Wilson, 2002). This study showed that several imported antibiotics (feed-grade, water-soluble) containing chloramphenicol and Nitrofuran both of which are "forbidden" for use in food producing animals were being sold in veterinary shops located in the study area. All the antibiotics were indiscriminately used for production of chicken meat and eggs in the study area where most of the farmers do not rely on professional diagnosis and prescription.

The farmers either lacked food-safety knowledge or refused to follow recommended practices. This could be an indication of the level of effectiveness of regulatory control of veterinary drugs and food safety monitoring system in the country. The wide-spread and uncontrolled use of antibiotics is cause for concern from both a veterinary and public health point of view. This practice has been reported as enhancing the development and spread of multiple drug resistance and deposition of violative levels of residues in meat and eggs. There are overlapping functions of control agencies such as National Food and Drug Administration Control (NAFDAC) and Veterinary Council of Nigeria (VCN) that could also create loopholes in veterinary drug control.

Chloramphenicol residue was detected from the chicken tissues and majority of eggs screened except in eggs from Oluyole LGA market. The results of the study agreed with Adewuyi et al., (2011) who also obtained CAP residue in four out of twenty (20%) broilers chicken obtained from the same city by chloramphenicol residue by high performance liquid chromatography. However, the prevalence of CAP obtained in our study was higher than those of Adewuyi et al., (2011), this could result from the fact that most of the samples used in this study were obtained directly from the producers and the ELISA method used was also validated to be accurate and precise. The results of this study is also comparable with the findings of Mehdizadeh et al., (2010) who reported 54.8% of broiler chicken samples from Iran with detectable concentrations of chloramphenicol residue.

The higher concentrations of CAP residue obtained in eggs than in the meat could be due to more frequent use of antibiotics in laying chicken as egg boosters by farmers and also as a result of sequestration of drug in egg without further metabolism (Donoghue, 2001). Also, the mean for CAP residues are in chicken tissues from the different farms were not significantly different, but the prevalence is much lower in farms 2 and farm 3. This could be as a result of similar patterns of use of antimicrobials in the poultry across the different farms for chemotherapeutic, prophylactic and growth promotion purposes.

Since there is no maximum residue limit (MRL) set for CAP residue i.e. zero tolerance in food (JECFA 2004; FAO-WHO, 2004), we consider the CAP residues identified in this study to be of significant public health concern demanding compelling need for effective regulatory compliance (and monitoring) for drug residue and antibiotic resistance in Nigeria.

CONCLUSION

The use of CAP in food producing animals has been prohibited in several countries, but in Nigeria, the drug is still on sales and used in poultry production. This study revealed the public health threat of the residues resulting from the use of chloramphenicol among other antibiotics in poultry production. This study confirmed indiscriminate use of many antibiotics including CAP in poultry without food safety consideration by the farmers and with indications of weak regulatory control on the use of veterinary drugs in Nigeria. The food safety (residue) hazard associated with such practice was also confirmed by the high prevalence of chloramphenicol concentrations residue detected from chicken eggs and meat in this study. This is also important as could also cause international trade barrier for livestock products in Nigeria.

We recommend prudent antibiotic use in food animals and consumer protection, alternative treatment regimens and enforcement of appropriate regulatory efforts to prohibit the use of chloramphenicol and other dangerous drugs in Nigerian food animal production.

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