 RESEARCH ARTICLE
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Immunologic determination of chloramphenicol residue in commercial birds at Nsukka, Enugu State, Southeast Nigeria

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Received: 01-02-2018, **Accepted:** 07-03-2018, **Published online:** 03-04-2018

doi: 10.14202/IJOH.2018.22-27 **How to cite this article:** Ezenduka EV, Okonkwo BC, Anyaoha CO, Nwanta JA, Anaga A. Immunologic determination of chloramphenicol residue in commercial birds at Nsukka, Enugu State, Southeast Nigeria. Int J One Health 2018;4:22-27.

Abstract

Aim: This study aimed to determine the presence and prevalence of chloramphenicol (CAP, a drug which was banned for use in food-producing animals due to many side effects) residue in commercial birds slaughtered at Ikpa abattoir and its awareness and usage in farms at Nsukka, Enugu State, Nigeria.

Materials and Methods: A cross-sectional survey was done with the use of a questionnaire on usage and awareness of CAP and screening for its presence in commercial poultry in the study area. The questionnaire was supplied to 35 commercial farms, and liver samples from 300 commercial broilers were analyzed using an enzyme-linked immunosorbent assay technique; the prevalence was then determined.

Result: Of the 35 farms evaluated, 33 (94%) responded. In the management practice, 57.6% of the farms use intensive deep litter, 18.2% intensive battery cage, and 24.2% extensive farming system. 19 (69.7%) farms rear only broilers, 12.1% layers, and 15.1% both. The feeding management showed that 21.1% of farmers produce their own feed with inclusion of antibiotics while 78.8% use commercial feed, of which 11.5% incorporate antibiotics. The findings also showed that 54.4% of the respondents use CAP and only 30.3% are aware of the consequences of antimicrobial residue in food and have knowledge of the legislation on the prudent use of antimicrobials in food animals. Of the 300 samples screened for CAP residue, 18.7% were positive with concentrations ranging from 0.5 to 6.2 parts per billion.

Conclusion: CAP is still very much in use in the study area, despite the ban, and it is present in the tissues of commercial birds meant for human consumption.

Keywords: chloramphenicol, drug residue, enzyme-linked immunosorbent assay, liver, poultry.

Introduction

Antimicrobials given to birds orally or parenterally may be found in tissues, particularly when birds are slaughtered without the observation of their withdrawal periods or when the eggs are harvested within the withdrawal period of the drug [1-3]. This is also likely to occur where the drug is fed continuously over a long period of time and when used as an extra-label dose or beyond manufacturers' recommendation [4]. Sometimes, the drugs are introduced accidentally through feed mill contamination [5] or recirculation through litter [3]. The presence of drug or antibiotic residues in food above the maximum level is recognized worldwide by various public health authorities as being illegal [6]. Their consumption could result in public health hazards including development of resistant strain of microorganisms. Antibiotics are used in poultry and livestock specifically for treatment, prophylaxis, and growth promotion in an attempt to increase the quantity and quality of output on focus. The overwhelming negative effects of most of these drugs in both humans and animals health when abused led to the setup of the regulations for the use of antibiotics in livestock [7] and the ban of some of these drugs particularly chloramphenicol (CAP) [8,9]. CAP, first isolated from *Streptomyces venezuelae,* is a bacteriostatic broad-spectrum antibiotic active against both aerobic and anaerobic Gram-positive, Gram-negative, and rickettsial organisms. It has a historical veterinary use in all major food-producing animals and with current uses in humans and companion animals.

in Nigeria, despite having an Act (Food and Drug act of Nigeria 1976) and food regulatory bodies such as NAFDAC that provide for residue avoidance as required by the WHO Codex Alimentarius Commission [10], the ban on CAP is yet to be enforced. The WHO has also since recommended the prohibition of use of CAP in all food-producing animals particularly lactating cows and laying birds [11,12]. The ban on CAP followed the concerns expressed about the genotoxicity of

CAP and its metabolites, its embryo and fetotoxicity, and its carcinogenic potential in humans. Bone marrow depression and the lack of a dose-response relationship for aplastic anemia have also been associated with consumption of CAP residues in humans [13]. Two types of CAP-induced toxicity in humans have been widely discussed. The first is a frequently occurring, dose-related, bone-marrow depression that develops during treatment with CAP. The condition is seen as mild anemia, with decreased hemoglobin concentration and reticulocytopenia, with the bone marrow showing reduced erythroid precursors, increased myeloid: erythroid cell ratio, and vacuolation of erythroid cells. The patient returns to normal after drug withdrawal. Inhibition of protein synthesis in bone-marrow cells has been proposed as the mechanism of these effects [14]. The second type of bone-marrow toxicity has been clearly seen in man and is of concern wherever CAP or its residues may be ingested by human patients. In this condition, small amounts of CAP can cause irreversible bone-marrow depression leading to aplasia and fatal anemia. This is an unusual phenomenon, but the connection with CAP use (e.g., following treatment with ophthalmic ointment containing CAP) had led to the use of CAP in veterinary medicine in many countries to be restricted (or forbidden) in food-producing animals in view of the risk (albeit very slight) that trace residues may be linked with fatal aplastic anemia [15]. There is no withholding period recommended for CAP because its use in animals intended for food production is not allowed [16]. In general, residues of veterinary drugs in slaughtered chicken have been reported in Nigeria [17,18] and in commercial eggs [17,19]. Despite the ban, in some regions in Nigeria, the presence of CAP in foods of animal origin has been detected by Kabir *et al.* [20] and Mbodi *et al.* [10] in the North and by Olatoye *et al*. [21] in the West.

Detection of drug residues in foods of animal origin intended for human consumption is essential for the safety of consumers. Hence, this study aimed to determine the uses, awareness of ban, and presence of CAP residues in commercial birds in Nsukka, Southeast Nigeria.

Materials and Methods

Ethical approval

The study was conducted in accordance with the Ethics and Regulation Guiding the Use of Animals as approved by the University of Nigeria, Nsukka.

Study design

A cross-sectional survey was done using a questionnaire to determine awareness of the ban of CAP and their usage in poultry production among farmers and the detection of its residue using enzyme-linked immunosorbent assay (ELISA) immunoassay.

Study area

Ikpa slaughter was selected for this study, located at Ikpa market in Nsukka town, Enugu State, Southeastern geopolitical zone of Nigeria. Nsukka has a population of 309,633 (NPC 2006) making it the second biggest city in Enugu State. Poultry farming is a common practice since poultry is the most available source of meat in the Southeast. Ikpa slaughter is the major slaughterhouse serving Nsukka and environs.

Sample collection

Questionnaire survey

Thirty-five of the 45 listed farms in Nsukka and environs were randomly selected using simple random sampling technique, and questionnaire was distributed to them. Cross-sectional survey: Post-slaughter liver of chicken was used as sample matrix for the detection of CAP residue. Ikpa slaughter has daily slaughter capacity ranging from 200 to 300. Systematic random sampling was used to select 15 birds daily twice a week for 10 weeks. Liver samples were harvested from each selected bird after slaughter and sent for analysis. A total of 300 samples of liver from 300 chickens were sampled and tested for CAP residue during the course of the study.

Sample preparation/extraction

Following the ELISA kit's extraction instruction, 3±0.5 g of each liver sample was macerated and then homogenized with 3 ml of deionized water in a test tube. Ethyl acetate (6 ml) was added, shaken properly, and centrifuged at above 4000 rpm at room temperature (20°C-25°C) for 10 min. 2 ml of the supernatant (equivalent to 1 g sample) were taken from each test tube and let to evaporate to dryness. The dry residue from each sample was redissolved in 1 ml N-hexane and 1 ml of the diluted redissolved solution added and shaken vigorously for 30 s and centrifuged at above 4000 rpm at room temperature for 5 min. 50 µl of the lower part of the mixture were taken from each sample and stored for analysis.

CAP residue detection

CAP in the samples was detected using CAP ELISA kit (Shenzhen Lvshiyuan Biotechnology Co., Ltd). In preparation, the microtiter plates and the reagents were stabilized to room temperature before use. The test was based on competitive enzyme immunoassay, where CAP in the sample and the coupling antigen pre-coated on the microwell stripes compete for the anti-CAP antibody. After the addition of the enzyme conjugate, the 3, 3', 5, 5'-tetramethylbenzsidine substrate was added for coloration. The optical density of the sample has a negative correlation with the CAP in it. This value was compared to the standard curve and the CAP concentration is subsequently obtained. For each plate, a worksheet was prepared, and the standards and samples were run in duplicates.

Detection and calculation of CAP concentration using absorbance values

The standard curve for CAP is given in Figure-1. The concentrations of the standards (0, 0.5, 0.15, 0.45, 1.35, 4.05, and 10) in ng/ml were plotted against their

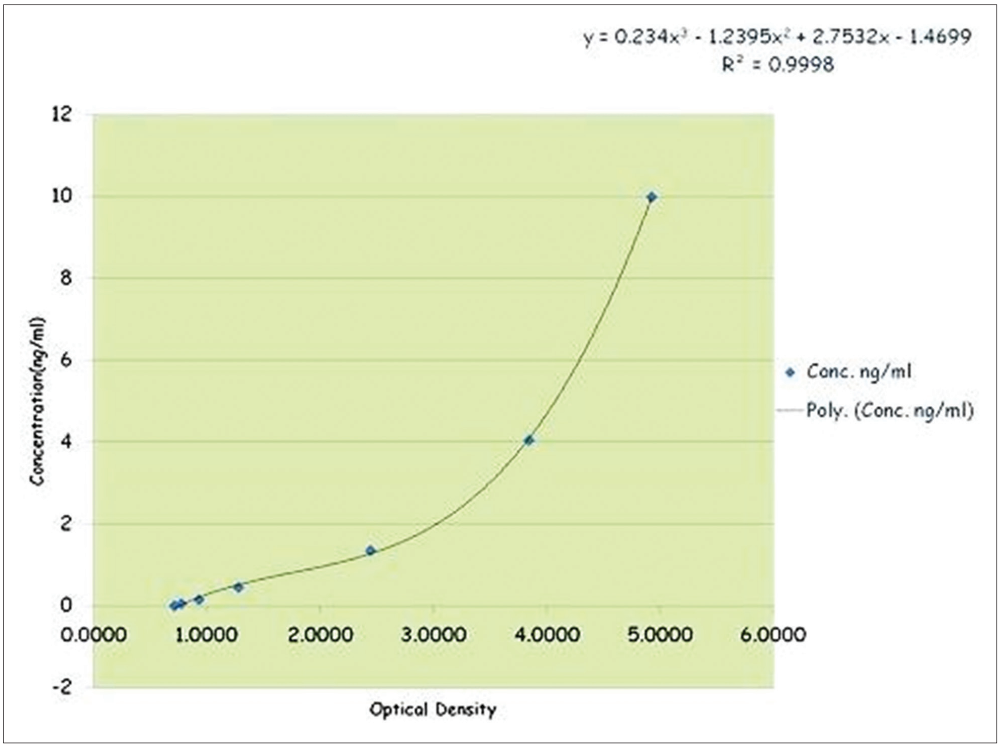

Figure-1: Standard curve of chloramphenicol concentrations (ng/ml).

absorbance. The calibration curve was used to extrapolate the concentration of the samples using Microsoft Excel software supplied by Shenzhen Lvshiyuan Biotechnology Co., Ltd. The procedure for calculating the concentration of the samples is as follows:

- Since in competitive ELISA, the concentration of the samples is inversely proportional to the absorbance, and the reciprocal of the absorbance measurement (1/absorbance) was calculated to have a direct proportion.
- The standard curve was plotted, with absorbance directly proportional to the concentration; a polynomial trend line is used to give a perfect curve.
- Equation $y=0.234x^3-1.2395x^2+2.7532x-1.469$ for calculating the concentration was formed and displayed from the polynomial curve with a correlation ($R^2=0.9998$) (Figure-1).

Statistical analysis

Data from the study are presented in tables with mean±standard error of mean, frequencies, and proportions and analyzed in GraphPad Prism Statistical Software Version 5.02 (www.graphpad.com). Fisher's exact test (p value) was used for statistical significance and inference, while odds ratio was used to test strength of association. The alpha value of significance was set at the probability level of <0.05.

Results

Questionnaire survey

Of the 35 farms evaluated, 33 (94%) responded while 2 (6%) did not. In the management practice (Table-1), 57.6% of the farms use intensive deep litter, 18.2% intensive battery cage, and 24.2% extensive farming system. 19 (69.7%) farms rear only broilers, 12.1% layers, and 15.1% both. The feeding management showed that 21.1% of farmers produce their own feed with inclusion of antibiotics while 78.8% use commercial feed, of which 11.5% incorporate antibiotics. Table-2 shows that 54.5% of respondents use CAP in form of N.C.O mix®, 45.6% Tyfurchlor®, 33.3% Typhoid care®, 21.2% Neocloxin®, 9.1% Oxytet Phenicol 20/20®, and 0% as human formulation.

In Table-3, 54.5% of the respondents are aware of the consequences of antimicrobial residues in foods, and 30.3% know that there is legislation on the prudent use of antimicrobials in food animals and the ban on the use of CAP in livestock and poultry production.

Detection of CAP residues in commercial birds

The CAP ELISA kit has a detection limit of 0.5 parts per billion (ppb). Samples ≥0.5 ppb are regarded as positive; samples above 0.00 ppb but below 0.5 are below the detection limit of the kit, therefore regarded as negative. Results of the analysis as indicated in Table-4 show that of the 300 samples analyzed, 56 (18.7%) tested positive for CAP residue with a mean value of 1.88 ppb, ranging from 0.54 ppb to 6.25 ppb, while 244 (81.3%) samples of liver tested negative. 24 negative samples were detected but below the detection limit of the kit at 0.5 ppb.

Discussion

Commercial birds reared in Nsukka and environs are sold and slaughtered at Ikpa abattoir. Majority of the farms in Nsukka are involved in deep

Table-1: Farm management and feeding system of farms in Nsukka, South East Nigeria.

Housing	Extensive	Intensive deep litter	Intensive battery cage	
Birds reared	8 (24.2%)	19 (57.6%)	6 (18.2%)	
	Broilers only	Layers only	Breeders	*Combination
	23 (69.7%)	4 (12.1%)	1 (0.0%)	5 (15.1%)
Source of feed	Compound		Commercial	
Incorporate	7 (21.2%)		26 (78.8%)	
Antibiotics	7 (100%)		3 (11.5%)	

*Combination of both deep litter and intensive battery cage

Table-2: CAP drug usage by farms in Nsukka, South East Nigeria.

Drug	Frequency (n=33)	Proportion (%)
N.C.O. Mix®	18	54.54
Tyfurchlor®	15	45.45
Typhoid care®	11	33.33
Neocloxin®	7	21.21
Oxytet Phenicol 20/20®	3	9.10
CAP human formulation	0	0

N.C.O. Mix®=Neomycin, chloramphenicol, and oxytetracycline, Tyfurchlor®=Tylosin, furazolidone, and chloramphenicol, Typhoid care®=Chloramphenicol, amoxicillin, ampicillin, and neomycin, Neocloxin®=Neomycin, chloramphenicol, and oxytetracycline, Oxytet Phenicol 20/20®=Oxytetracycline and chloramphenicol, CAP=Chloramphenicol

Table-3: Awareness of the prudent use and ban of certain antimicrobials in livestock and poultry production in Nsukka and environs, Southeast Nigeria.

Awareness	Yes (%)	No (%)	p value	Odd ratio
To antimicrobial residues	18 (54.5)	15 (45.5)	0.78	1.356
To legislation on the prudent use of antimicrobials	10 (30.3)	23 (69.7)	1.00	1.00
To banned drugs (furazolidone and CAP)	10 (30.3)	23 (69.7)	1.00	1.00

CAP=Chloramphenicol

Table-4: CAP residue in liver samples of commercial birds.

Concentration (ng/ml)	n (%)	Decision	Mean concentration (ppb)±SEM	Range (ppb)
≥0.5	56 (18.7)	Positive	1.89±0.37	0.5-6.2
<0.5	220 (73.3)	Negative	-	-
0.00	24 (8)	Negative	-	-
Total	300 (100)	-	0.44±0.09	0.00-6.2

CAP=Chloramphenicol, SEM=Standard error of mean

litter intensive farming, and since there is very high possibility of cross infections among birds/pens, it suggests that the use of antimicrobials to prevent and treat infections is inevitable. Almost all the farms have used CAP in one way or the other, either by incorporating it into their feed or simply used for treatment. This is buttressed by the fact that all those that compound their feed incorporate antibiotics. Although majority of the farms use commercially prepared feed, however, some of the farmers believe that some feeds contain antibiotics though not indicated by the manufacturer. Despite the non-usage of human and single formulation of CAP in the study area, it is still very much available in the market but in combination with other drugs. More than half of the farms surveyed use one CAP combination or the other. The use of CAP in this case is somewhat inevitable since most of these drugs contain mixtures of another very important antibiotic normally used in poultry production; they are also locally manufactured and easily available. It is therefore not surprising from the outcome of this study that CAP residue was present in commercial chicken tissues slaughtered in Nsukka Municipality of Enugu State with 18.4% prevalence.

Similar studies in Ibadan, Oyo State [21]; Federal Capital Territory (FCT), Abuja [10]; and Kaduna State [22] have also confirmed occurrences of CAP in chicken eggs with 25%, 7%, and 0.7% prevalence, respectively. Findings from another study in Morogoro Municipality, Tanzania, showed 10% prevalence of CAP in commercial chicken eggs [23]. This implies the contravention of the joint Food and Agriculture Organization (FAO)/WHO ban on the use of CAP in food animals in developing countries. The continuous existence of the use of CAP in food animals could be attributed to ignorance (lack of awareness) as pointed out by some farm management in this study where only 30% of respondent farmers were aware that CAP was banned and not recommended for use in food animals. This is also in agreement with the works of Omeiza *et al*. [22] and Mbodi *et al*. [10], where 26.7% and 26.3% of commercial bird farmers in Kaduna State and FCT Abuja, respectively, are aware of the ban. Interestingly, only those who are aware of the perils of antimicrobial residues are also aware of the ban. The low level of awareness of drug residues among the developing nations was also reported by the FAO/WHO [24] as having great public health significance. Other potential causes could be a lack of routine monitoring of antimicrobial residues in foods of animal origin, regulation, and enforcement of the ban on the use of CAP in food animals in developing countries. In Slovenia, a survey of CAP residues was determined in 1308 different animal tissues and their products such as eggs and milk, and CAP residue was seen in only one milk sample with a prevalence of 0.1% [25]. This low prevalence was attributed to routine monitoring, strict prohibition of this veterinary drug for food-producing animals, as well as a proper veterinary sanitary control of its residues in Slovenia as is also obtainable in the most developed countries. In 1991, a large animal (veterinary) practitioner was sentenced to 12 months' imprisonment for using CAP in food animals in Iowa, Washington DC, despite the ban [26]. It was established that the veterinarian acquired, processed, used, and dispensed CAP and other illegal animal drugs in his food animal practice. If this kind of legal measures can be enforced in Nigeria and other developing countries, regulations concerning the use of CAP and other abused veterinary drugs will be adhered to. This will go a long way in protecting the public from exposure to drug residues.

Conclusion

There is a presence and high prevalence of CAP in commercial chickens destined for human consumption in Nsukka Municipality of Enugu State, Nigeria, with limited awareness of the ban of CAP in use in livestock and poultry production by farmers. Chicken consumers in Nsukka (and other parts of the country) are therefore exposed to health hazards associated with CAP residues and the presence of CAP can also jeopardize international chicken and their products trade from Nigeria.

Further studies should aim at the determining the effect of different cooking methods on the levels of CAP in poultry meat.

Authors' Contributions

EVE and AA got the concept of the work and designed the study. BCO collected samples and did the laboratory analysis with EVE. COA and JAN designed the questionnaire and carried out the field work. EVE and AA wrote and edited the work. All authors read and approved the final manuscript.

Acknowledgments

The authors appreciate the efforts of the Enugu State Director of Veterinary Services for providing a complete list of Veterinary farms and drug sale outlets for the questionnaire survey and also the technologists at the Veterinary Public Health and Preventive Medicine Laboratory for their assistance. The authors funded this study.

Computing Interests

The authors declare that they have no competing interests.

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