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High Concentrations of Quinolones Residues found in Eggs from Poultry Farms in Kaduna, Nigeria.

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ABSTRACT

Eggs are the major product generated from layer poultry production systems and are readily processed for consumption and sold to meet public demand. However, antimicrobial usage during production results in residues accumulating in poultry eggs. Thus, it is necessary to monitor and ensure that poultry eggs are safe for human consumption, with no antibiotic residues that can lead to allergic reactions/intoxication or development of antimicrobial resistance. Forty-five (45) farms around Kaduna metropolis, Nigeria were sampled for table eggs alongside a structured questionnaire to consider operation systems (cage or floor), stocking system (all-in-all-out or multiple batches), and location, along with egg producer sizes as either small-, medium-, or large-scale producers. A total of 450 fresh eggs were collated and analyzed using Enzyme-Linked Immunosorbent Assay. The result overall, showed a high prevalence (95.6%) of samples positive for quinolone residues with highest levels of 10,185.5 ng/ml and 5,519ng/ml from two farms (4.4%). These levels are higher than the Maximum Residue Limits (MRL) for edible tissue set by regulatory agencies, including the European Union and U.S. Department of Agriculture (100 ng/ml=100 mg/l). Thirty-three (33) samples from 33 farms (73.3%) overall had residue levels higher than the MRL, 10 farms (22.2%) had residue levels ranging between 2.5 ng/ml – 94.5 ng/ml, while only two farms had undetectable limits of less than 2.5ng/ml. No specific association was found for any of the assessed production factors. Quinolones are antibiotics used both in veterinary and human medicine, thus, their usage must comply with set safety criteria. Consequently, the presence of quinolones in poultry eggs may result in the transmission, to humans, of resistant bacteria and residues of fluoroquinolone active metabolites harmful to human health.

Keywords: Quinolones; Residues; Drug Resistance; Poultry Egg, Safety; One Health.

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INTRODUCTION

Poultry industry is a major part of livestock production throughout the world, and it is rapidly expanding in developing countries like Nigeria to fulfil the demands of safe and wholesome protein from animals as meat and eggs. Nigeria has the highest number of poultry farms in Africa is recorded to have 910% of the agricultural GDP contribution from the poultry industry [1]. Data from the Poultry Association of Nigeria (PAN) shows Nigeria produces about 1.25 million tons of egg yearly. A major threat to the safety of poultry eggs is the indiscriminate use of antimicrobials (antibiotics in particular) for empirical therapy, prophylaxis, and growth promotion without observance of prescribed withdrawal periods for these medications. Antibiotics such as tylosin, erythromycin, and tetracyclines, are commonly used at sub-therapeutic levels in the poultry industry as growth promoters. Administered through the feed, these antibiotics can stabilize the gut microbiome with reduced susceptibility to diseases [2]. On the other hand, quinolones (especially the fluoroquinolones) are used for therapy because they are very effective against chronic respiratory diseases in poultry [3]. The European Union and the U.S. Food and Drug Administration (FDA) permit fluoroquinolone use in broiler chickens with a set maximum residual limit (MRL: 100mg/kg equivalent to 100mg/l =100ng/ml) on the concentrations of residues that can be present in edible tissues [4]. However, these drugs are not allowed for use in laying hens due to the likelihood of transferring and accumulating the drugs in the eggs. Enrofloxacin, a commonly used quinolone, is de-ethylated to ciprofloxacin in the body. This active metabolite pharmaceutically is dispensed for use in human medicine because it is active against several pathogens that are known zoonoses, including *Salmonella*, *Campylobacter*, and *Shigella* [5], organisms that poultry are known to be colonized with frequently; thus, administration of quinolones to poultry has been shown to result in selection for resistant strains that jeopardize human treatment with ciprofloxacin [6]. Several studies have indicated high risks associated with use of Enrofloxacin in poultry and the US FDA has banned its use [7, 8]. Given the availability, large consumption, and production of eggs in Nigeria, the aim of this study was to determine the presence (prevalence) of quinolone residues in table eggs produced from commercial layer poultry chicken farms in Kaduna metropolis and its environs and quantification of any levels of quinolones residues detected in egg samples collected from these farms.

METHODOLOGY

Experimental Design and Sampling

A cross-sectional study of commercial egg laying poultry chicken farms in Kaduna metropolis, the capital of Kaduna State, northwestern Nigeria, was conducted in September 2017. Kaduna metropolis consists of four local government areas (LGAs), namely Kaduna North, Igabi, Kaduna South, and Chikun LGAs. All four LGAs have a significant number of poultry chicken farms located therein. For this study, sampling spread across all the four LGAs. This was achieved using simple random sampling technique with the aid of a random numbers table and a numbered sample frame of all commercial poultry chicken farms obtained from the State Ministry of Agriculture, Department of Livestock and Pest Control (DLPC). The sample frame was authenticated by the Poultry Farmers Association of the State. At the time of this study, there were a total of 194 poultry egg producers in Kaduna metropolis; 128 of this number were in Kaduna North and Igabi combined while 66 were in Kaduna South and Chikun LGAs. Data regarding each producer that was sampled included the size of the producer in terms of egg production (small scale, ≤500 crates of eggs per day; medium scale, 500 to 1,000 crates of eggs per day; or large scale, ≥1,000 crates of eggs per day), the type of production system (caged or floor laying system), stocking system (all-in-all-out or multiple batches) and location within the metropolis.

Sample Collection and Processing

In total, 45 farms were sampled, with ten eggs from each farm collected in a one-stage single sampling, making a total of 450 eggs collected and analyzed (Table 1). All the egg samples were fresh unprocessed and collected on the day of lay. They were identified with coded labelling using a permanent marker on each egg specimen, and safely transported unbroken to the Microbiology Laboratory at the College of Agriculture and Animal Science Mando, Kaduna, of the Division of Agricultural Colleges, Ahmadu Bello University, Zaria.

Eggs albumen and yolk from each farm (10 eggs) were deposited and gently beaten in a clean beaker until homogenized into a single homogenate. From the homogenized sample representing the farm, 2 g was weighed and added into a 15-ml centrifuge tube. Distilled water (5 ml) was added to the egg homogenate along with another 5 ml of 70% methanol, the mixture was well shaken to mix for 1minute in a shaker incubator. This mix was centrifuged at 4000 g for 5min (Xiangtian 800-1 Bucket Centrifuge, Jiangsu,

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China), and 50 µl of the supernatant was taken for use in the assay [10].

Assay Preparation and Testing Procedures

Each of the sample obtained was run using the Quicking Quinolone ELISA Kit (W81126 Quicking Biotech, Shanghai China) following the manufacturer's guide. The kit is a direct enzyme-linked immunosorbent assay for the quantitative detection and presence of quinolone antibiotics in samples of meat and egg (detection limits: meat=5 ppb and egg=2.5 ppb). Briefly, all reagents were brought to room temperature on laboratory bench (air-conditioned lab temperature, 22^{o}C). The standards and samples quantity of 50 µl was placed in each standard well and sample wells in duplicate. This was followed by the addition of 50 µl of quinolone antibody into each test well and the wells were gently rocked for adequate mixing and incubated for 30 minutes at room temperature. The contents of all the test wells were then poured out completely after initial incubation and prepared wash buffer was used to fill each test well and poured out almost immediately. This wash step was repeated 4 times, after which absorbent paper was used to tap the micro-wells held upside down to help absorb and ensure complete liquid removal. This was followed with the addition of 100 µl of enzyme-linked conjugate into each well and the wells were incubated for 30 min at room temperature. The wash step was repeated as described earlier at the end of the incubation period. This was then followed with addition of 50 µl of substrate A and substrate B, ensuring thorough mixing with subsequent incubation for 15 min at room temperature. Lastly, 50 µl of stop solution was then added to each well and rocked gently to mix and absorbance was read at 450 nm filter using a microplate reader (Absorbance Microplate Reader KC-100, Caretium Medical Instruments, China) with 5 min of stopping reaction. Any sample with detectable quinolone residues considered to have greater than the MRL 0.0 ng/ml established by the U.S regulatory body FDA and the European counterpart EFSA.

Data Analysis

Prevalence data for quinolone residues were sorted by size of the producer in terms of egg production, the type of production system, stocking system and location within the metropolis.

Data were analyzed using the Fisher's exact test with SPSS version 20.0 (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

From this study, we found that 96% of the farms where eggs were collected contained quinolone residues. This implied that the hens were administered antibiotics and these eggs were collected within days of the antibiotic administration since residues in eggs can only be detected for days after antibiotics are withdrawn. When enrofloxacin is given to laying poultry chicken by intramuscular injection, residues can and have been detected in eggs within 2 days post administration and even persisting in the yolk and albumen for 9 days after withdrawal [9,10]. However, if drug is administered orally through drinking water, residue concentrations are higher in the egg than when it is delivered intramuscularly [10,11,12]. Furthermore, the secondary metabolite ciprofloxacin persists for 7 to 10 days, relative to the route of administration and in all the farms studied, the drug is administered orally through drinking water. Eggs obtained from Igabi and Kaduna North LGAs had the highest percentage of samples positive for quinolone residues (100%), while 89% and 90% of eggs from Kaduna South and Chikun LGAs were positive, respectively (Table 2). However, there was no difference statistically in prevalence between samples from all these areas. Furthermore, the concentration of residues was averagely higher in eggs collected from Igabi (144.9 ng/ml) and Chikun (158.5 ng/ml) than in eggs from the other two municipalities. A study conducted in South Korea reported 2.5% of eggs collected from several cities being positive for enrofloxacin [13], which is a lower prevalence than in our study. This difference in prevalence may be due to stringent enforcement of legislative regulations for quinolone use in laying hens in South Korea [14] compared to what is obtainable in Nigeria. The samples obtained from small-scale farms were 91% of total farms sampled and as expected had a high prevalence of quinolone residue (95%). The three-medium scale (7%) and single largescale (2%) farms sampled had a prevalence of 100% for quinolone residues, thus explaining the absence of difference between the different production sizes/production scales statistically. However, bad farm practices by small-scale farmers that includes self-medication may explain why residues are present in such high rates [15]. This is in addition to lack of adherence to drug withdrawal periods for both large and medium scale farms as well as the small-scale

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farms. Also, small-scale farmers have less economic resources, leading to more deficient infrastructures, and decreased biosecurity, resulting in more infections with the resultant need for antibiotic therapy [10,15]. In the eggs analyzed from egg-laying hens raised on the floor, quinolone residues were detected in 96% of eggs, while 95% of eggs from hens raised in cages contained residues, with no statistically significant difference. Egg-laying hens reared on deep-litter have greater contact with pathogens, which exposes them to more frequent diseases and parasites than hens raised in battery cages [16]. Thus, egg producers would likely increase the use of antibiotics to prevent and control diseases [17]. Additionally, pecking behavior commonly observed in hens raised on deep litter can indirectly contribute to the increased usage of antibiotics and consumption (litter pecking) of antibiotic residues that can be transferred to the eggs. Antibiotic usage in floor-rearing systems has been demonstrated to result in residue accumulation in the litter [18]. On the contrary, the need for antibiotic usage in battery cage systems are usually lower, thus the high prevalence here could be explained as empirical usage by producers and poor observance of withdrawal period protocols.

Also, there was no statistically significant difference in the prevalence between farms with multiple batches system and all-in-all-out systems. This could not be explained in this study as the farms with all-in-all-out systems even had a relatively higher prevalence (96%) and higher mean concentration of the quinolone residues (133.49ng/ml) compared to the multiple batches stocked farms (94%) and (121.8ng/ml). Thus, other explanations might include as earlier mentioned, empirical usage by producers and the lack of observance of withdrawal period.

Quinolone residues are heat stable and can persist long after treatment, thus, the concern for presence of residues in significant concentrations. Studies previously showed that concentrations of ciprofloxacin and norfloxacin in milk were reduced by only 12% when heated to 120^{o}C for 20 min [19,20]. However, in poultry meat, it was shown that concentrations did not change due to boiling or microwaving, even while residues were displaced from the meat into the water medium [21]. However, the same study demonstrated an increase in the concentration of enrofloxacin following roasting or grilling, attributed to moisture removal from the meat during the cooking process.

CONCLUSION

Relative stability of quinolones, the consumption of eggs by many, and the significant prevalence of contaminated eggs by residues point to the fact that quinolone usage in laying poultry chickens presents a risk to public health.

Table 1: Factor Distribution in Each Municipality and Number of Farms Separated by Housing System, Size/Scale of Farm, And Stocking System from Which Commercial Eggs were Obtained in the Study Area

Location	***Scale/Size of Farm***		***Housing System***		***Stocking System***	
Local Gov't Area (LGA)	**≤10000birds**	**≥10000birds**	**Litter**	**Cage**	**All-In-All-Out Batches**	
Kaduna North	6	1	5	2	5	2
Igabi	14	5	8	11	10	9
Kaduna South	9	0	7	2	5	4
Chikun	8	2	4	6	7	3
Total	37	8	24	21	27	18
	82.2%	17.8%	53.3%	46.7%	60.0%	40.0%

Table 2: Percentage of Eggs Testing Positive for Quinolone Residues Obtained from 45 Farms in Four Localities of Kaduna Metropolis Sampled

	Quinolone Residues	
Factors	*Percentage Positive (%)*	*Mean Concentration (ng/ml)*
Localities		
Kaduna North	100	134.1
Igabi	100	144.9
Kaduna South	88.9	121.3
Chikun	90.0	158.5
Housing System		
Deep Litter	95.8	135.6
Battery Cage	95.2	136.0
Size/Scale		
≤10000 birds	94.5	142.0
≥10000 birds	100	201.5
Stocking System		
All-In-All-Out	96.3	133.5
Multiple Batches	94.4	125.8

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