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DETECTION OF TETRACYCLINE RESIDUE IN TABLE EGGS IN MAIDUGURI METROPOLIS, BORNO STATE, NIGERIA

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ABSTRACT

Tetracycline is the most commonly abused and misused antibiotic by layer poultry farmers in Maiduguri Metropolis. Lack of information on the residue of tetracycline prompted the need to ascertain the level of tetracycline residue in commercial eggs meant for human consumption.

Multistage sampling technique was used in selecting layer farms and retail outlets from 4 major districts within the city of Maiduguri Viz; Bolori, Gwange, Kyarimi Park and Shehuri North. Information on the sources of eggs were obtained from the commercial egg retailers. Tetracycline ELISA®Test Kits from Maxsignal® South Africa was used for the analysis according to manufacturer's instruction.

One hundred and eighty-six table egg samples were collected for the study, 68 were sourced from 35 randomly selected layer farms and 118 were from 36 randomly selected retail outlets. Forty-one (60.29%) and Eighteen (15.25%)had detectable tetracycline residue,with overall mean residue concentration of 3.0 µg/kg and 3.18 µg/kg in layer farms and retail outlets respectively. The tetracycline residue is far below the standard of 400 µg/kg maximum residue level set by Codex Alimentarius Commission.

Keywords: Tetracycline, Residue, ELISA, layer farm, Table egg, Retail outlets

Introduction

Tetracycline was discovered in the mid-1940s as the product of *Streptomyces aureofacians* (1). Because of the spectrum of activity, high therapeutic index and the low production cost, tetracycline has been widely used throughout the world in fighting infections in humans, animal

Vol. 2, No. 06; 2017

ISSN: 2456-8643

and plants (2). The first members of the tetracycline antibiotics to be described are chlortetracycline (CTC) and ox tetracycline (OTC) (3). The most common tetracycline with animal application is tetracycline, oxytetracycline and chlortetracycline. These drugs have been approved for beef cattle, calves, swine, sheep, chickens and turkeys (4).

The extensive use of tetracycline, both in clinics and in agriculture, imposed a massive selection pressure for resistant isolates (5). Given their long history of extensive use, resistance to tetracycline has become wide-spread resulting in reduced efficacy (2). In the 1950s about 98% of commensals and pathogenic bacteria were reported sensitive to tetracycline and recently 85 to 91% of food-borne pathogens were resistant to tetracycline (2, 3,and 6). Resistance to tetracycline has occurred dueto their indiscriminate use and abuse in medical, veterinary and agricultural practices (7, 8). The most common and practical method of administration of tetracyclines to poultry is via feed or water (9). Tetracycline is the most common antibiotic used in poultry farms throughout the world (10, 11, 12, 13, 14, 15, 16 and 17). In Nigeria, the wide use of tetracycline among layer farmershas been reported by several researchers (18, 19, 20, 21, 22 and 23). Tetracycline represents a greater percentage of antibiotic residues of animal products in Africa (24). In Maiduguri, the study area, antibiotics are freely marketed without veterinary prescription with tetracycline being the most commonly abused antibiotic by poultry farmers (25). Moreover, there is a paucity of information on the level of tetracycline residue of egg meant for human consumption in the study area.

Materials and methods

Study area

Maiduguri Metropolis, a major city in the North-eastern part of Nigeria, is located between latitudes 11˚04'N and 11˚44'N; and between longitudes 13˚04'E and 13˚44'E. It covers a total land area of 543 km^2, which makes it the largest city in the North-eastern region of Nigeria(26, 27). Maiduguri city now extends to four Local Government Areas: Maiduguri Metropolitan, Jere, Konduga and to a smaller extent part of Mafa Local Government Areas(28). The climate of Maiduguri is characterized by a long dry season with high evaporation rate from October to May and a short Wet season for the remaining part of the year (27). There are four identified seasons in the area which include the *Rainy Season*, (June to September)Harvest Season (September to November), Harmattan or Cool Season (December to February) and Hot Season(March to May)(29). It has a population estimated at 1.275 million people according to the 2006 census(30). With an annual growth rate of about 3.5% and a density of 1145 persons per square km which makes it the most densely populated city in Northeastern Nigeria (29, 27). Crop production and livestock farming are the predominant occupations of the people in the study area (31). Poultry layer production is a profitable business in Maiduguri Metropolis (31)

Vol. 2, No. 06; 2017

ISSN: 2456-8643

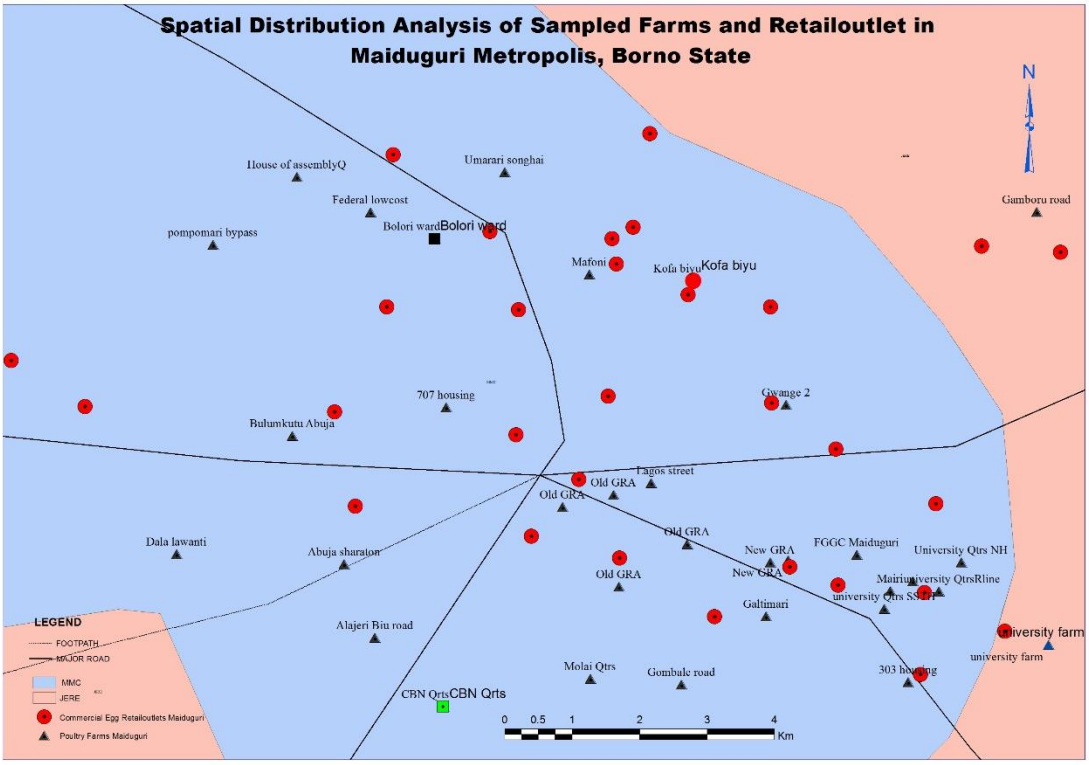

Figure 1: Map Showing Four Major Areas in Maiduguri Metropolis, Borno State, Nigeria.

Study design

Multi stage sampling method was used in selecting layer farms and commercial eggs retail outlets. Maiduguri metropolis is divided into 4 major districts by Borno state water board namely Bolori, Gwange, Kyarimi Park, and Shehuri North (32). (Refer to figure 1). In this study, these areas were taken as clusters or primary sampling units. In each area, 50% of sub areas were selected and sampled as secondary sampling units. Fifty percent of laying poultry farms and 10% of commercial egg retail outlets within each secondary sampling unit were randomly sampled and taken as tertiary sampling units.

Sample collection

One hundred and eighty-six commercial egg samples (68 from layer farm and 118 from retail outlets) were randomly collected from 35 layer farms and 36 retail outlets in Maiduguri Metropolis, Borno State. The table eggs collected were arranged in a clean crate, labelled and transported to the Department of Veterinary Medicine laboratory, Faculty of Veterinary Medicine, University of Maiduguri immediately for processing.

Vol. 2, No. 06; 2017

ISSN: 2456-8643

Tetracycline residue detection

Two ELISA®Test Kits (Cat NO: 1016 and 1017, LOT: T124 and T125), Maxsignal® South Africa was used to quantify tetracycline residue in table egg samples according to the manufacturer's instruction which includes sample preparation, reagent preparation and ELISA testing protocol.

Sample preparation

Preparation of 1X OXYTET extraction buffer was performed by diluting 1 volume of 5X by diluting OXYTET extractions Buffer into 4 volumes of distilled water. Preparation of 1X TET sample Diluent was performed by mixing a Volume of 10X TET sample diluents with 9 volumes of distilled water. Preparation of 1X TET balanced buffer formed by mixing 300µl of TET balanced buffer concentrate with 29.7ml of 1X TET sample Diluent. Preparation of 1X feed extraction buffer was performed by mixing 1 volume of 30X Feed extraction with 29 volumes of distilled water.

Whole egg preparation: To one gram of sample 3ml of 1X OXYTET Extraction buffer was added and vortex for 10 minutes using multi tube vortexer and was centrifuged for 10 minutes at 4000 x g. Two hundred microliter of the supernatant was transferred to a new tube containing 300µl of 1X TET sample buffer and were vortex for 1 minute. Seventy-five microliter per well was used in the assay.

Reagent preparation

Preparation of 1X wash solution was performed by adding 1 volume of the 20X wash solution with 19 volumes of distilled water. Preparation of 1X HRP-Conjugated Antibody #2 was performed by mixing 1 volume of the 100X HRP-Conjugated Antibody #2 with 99 volumes of Ab #2 Diluent.

ELISA testing protocol

Tetracycline standard 75µl each (Negative control, 0.05, 0.15, 0.4, 0.8, 1.6ppb) was added in duplicate into different wells. Seventy-five microlitre of each sample in duplicate was added into different wells. Hundred microlitres of Antibody #1 was added to the plate then mixed well by gently rocking the plate manually for 1minute and the plate was incubated at room temperature for55 minutes. The plate was washed three times with 250µl of 1X wash solution, after the last wash the plate was then inverted and gently tapped on paper towels to dry. Hundred and fifty microliters of 1X Antibody #2 solution was added and incubated for 25minutes at room temperature (20-25^{0}c). The plate was washed 3 times with 250 µl of 1X wash solution. After the

Vol. 2, No. 06; 2017

ISSN: 2456-8643

last wash, the plate was inverted and gently tapped on paper towels to dry. Hundreds of TMB substrate was added to each well and the plate was incubated for 15minutes at room temperature and 100µl of stop buffer was added to stop enzyme reaction. Immediately after the addition of stop buffer solution, the plate was read with a plate reader (450 nm primary filter).

Tetracycline (TET) concentration calculations

The standard curve was constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/g on a logarithmic curve.

$$\text{Relative absorbance}(\%) = \frac{\text{absorbance standard (or sample)x100}}{\text{absorbance zero standard}}$$

The mean relative absorbance values for each sample were used to determine the corresponding concentration of the tested drug in ng/g from the standard curve. According to the tetracycline, ELISA kits 1.6 ηg/g is the detection limit, so any sample with a concentration below the detection limit was considered negative and the concentration was converted to appropriate µg/kg.

Data analyses

The data was compiled and analysed with Statistical Package (SPSS statistical package version 21). ANOVA and independent student T test were used to determine the association between variables at significant levels of $P< 0.05$.

Results

The 68 table egg samples collected from 35 randomly selected layer farms were subjected to tetracycline ELISA, out of which 41 (60.29%) table eggs were positive for tetracycline residue. The positive egg samples include 14 (63.64%) out of 22 samples from Bolori, 14 (58.33%) out of the 24 positive eggs from Gwange, 13 (61.90%) out of 21 positive eggs from Kyarimi Park. The M± SD residue concentrations were 3.11±1.27 µg/kg, 2.93±1.42µg/kg and 2.62±0.86µg/kg for Bolori, Gwange and Kyarimi Park respectively. There was no significant difference ($p=0.780$) in mean residue concentration among the clusters (Table 1).

The 118 commercial egg samples collected from 36 randomly selected retail outlets were subjected to tetracycline ELISA and 18 (15.25%) egg samples had detectable tetracycline residue. Jos had 12 (15.00%) samples with detectable tetracycline residue whilst Ibadan had 6 (15.278%) egg samples positive for tetracycline residue. Mean residue level is 3.86 µg/kg and

Vol. 2, No. 06; 2017

ISSN: 2456-8643

3.1 µg/kg from Ibadan and Jos respectively. There was no significant difference in Tetracycline levels between the sources (P=0.106) (Table 2).

Table 1: Concentration of tetracycline residues in table eggs from layer farms in Maiduguri Metropolis, Borno State Nigeria

Cluster	No. of eggs	Samples with detectable tetracycline	Mean ± SD (µg/kg)	Samples exceeding MRL
Bolori	22	14 (63.64%)	3.11 ±1.27	None
Gwange	24	14 (58.33%)	2.93± 1.42	None
Kyarimi Park	21	13 (61.90%)	2.62±0.86	None
Shehuri North	01	Nil (0 %)	Nil	None
Total	68	41 (60.29%)	2.89±1.24	None

(ANOVA P=0.780 at 95 % confidence interval)

Table 2: Concentration of tetracycline residues in table Eggs from retail outlets in Maiduguri Metropolis, Borno State Nigeria

Sources of eggs	No. of eggs	Samples with detectable tetracycline	Mean ± SD (µg/kg)
Ibadan	38	6 (15.78%)	3.86±0.06
Jos	80	12 (15.00%)	3.1 ± 1.89
Total (%)	118	18 (15.25%)	3.48± 1.61

(Independent student test P= 0.106 at 95 % confidence interval)

Vol. 2, No. 06; 2017

ISSN: 2456-8643

Discussion

The study on tetracycline residues in table eggs in Maiduguri revealed that there was a very low concentration of the residues in both egg samples collected from farms and retail outlets with overall mean residue concentration of 3 µg/kg and 3.48 µg/kg respectively. This is far below the standard of 400 µg/kg maximum residue level set by Codex Alimentarius Commission. The low concentration of tetracycline residue found in this study is in reminiscence to the finding of Fagbamila et al., (19) which reported very low concentration of tetracycline contamination in table eggs in Jos, Plateau State and disagrees with the finding of Omeiza and Nafarnda, (33) which reported 479 µg/kg concentration of tetracycline residue in table eggs in Ibadan. Nonga et al., (12) also reported the high concentration of tetracycline contamination of table eggs in Tanzania. The reason for this low concentration of tetracycline residue might be partly due to the use of substandard drugs or partly due to elapsing of withdrawal periods of tetracycline as reported by Saba et al., (34). The findings of this research also indicated that there is small flock layer production farming in Maiduguri Metropolis, this might not be unconnected with the unfavourable weather and financial constraint that characterized the farming sector in Maiduguri Metropolis, the same finding reported by Tijjani et al., (31).

Conclusion and Recommendation

There are small flock size (back yard) poultry layer farms in Maiduguri Metropolis, The major sources of commercial eggs for human consumption in Maiduguri Metropolis is Jos, Plateau state and Ibadan, Oyo state. The overall mean concentration of tetracycline recorded is 3.0µg/kg and 3.18µg/kg from layer farms and retail outlets respectively and is below the Maximum Residue Level of 400 µg/kg set by Codex Alimentarius Commission.

Recommendation

Based on the findings of the study the following recommendations were made:
Farmer education on the use of antibiotics and its public health implication. Antibiotics being a prescription drug should not be freely sold to farmers over the counter. The established Veterinary Directorate in National Agency for Food and Drug Administration and Control should ensure the control of importation and subsequent use of antibiotics prohibited for use in food animals. More research using sensitive techniques should be carried out to quantify the residue levels of individual tetracyclines e.g. Doxycycline and other antibiotics in table eggs in the study area. Legislation regarding the maximum residue level of approved antibiotics and the use prohibited antibiotics on food animals by National Agency for Food and Drug Administration and Control.

Authors Agreement

Vol. 2, No. 06; 2017

ISSN: 2456-8643

I affirm that all the authors have seen and agreed to the submission of paper and their inclusion of name as co-author.

Conflict of interest: There is no conflict of interest

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Vol. 2, No. 06; 2017

ISSN: 2456-8643

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Vol. 2, No. 06; 2017

ISSN: 2456-8643

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