



## Assessment of Enrofloxacin residues in table eggs obtained from open markets and supermarkets in Lusaka province of Zambia

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### ARTICLE INFO

#### Article history:

Received 10. 06. 2025

Received in revised form  
06. 09. 2025

Accepted 15. 09. 2025

#### Keywords:

Antimicrobial residues;  
Enrofloxacin;  
Laying hens;  
Table eggs;  
Zambia

### ABSTRACT

Globally, table eggs represent a vital dietary source. Unfortunately, antibiotic residue (AR) contamination in eggs remains a substantial food safety and public health concern. While the presence of antibiotic residues in poultry products is a known problem, there is still limited data, particularly with regard to the use of antibiotics that are prohibited in the production of poultry eggs. Herein, we used High-Performance Liquid Chromatography (HPLC) to determine the proportional distribution of branded and unbranded table eggs from supermarkets (regulated retail outlets) and open markets (traditional, informal markets) in Lusaka province that would contain enrofloxacin residues. Of the total samples tested, 31.7% (95% CI: 20.3-44.9%) contained enrofloxacin residues ranging from 0.16 to 1.52 µg/g. A higher proportion of the enrofloxacin residues were detected in open markets, 84.2% (95% CI: 60.4-96.6%), compared to supermarkets, 15.8% (95% CI: 3.4-39.6%). Similarly, the majority of enrofloxacin residues were detected in unbranded eggs, 89.5% (95% CI: 66.9-98.7%), compared to branded eggs, 10.5% (95% CI: 1.3-33.1%). Given the high risk of AR in Zambia and many other countries globally which is associated with extensive use of antibiotics in poultry, our data contributes to a fundamental knowledge gap and is relevant in generating hypotheses that will guide future research and developing targeted public health interventions to minimise human exposure to antibiotic residues.

**Citation:** Mutemwa VK, Silwamba I, Muma JB, Nchima G, Bumbangi F, Muzandu KM. **Assessment of Enrofloxacin residues in table eggs obtained from open markets and supermarkets in Lusaka province of Zambia.** J Food Safe & Hyg 2025; 11 (3): 256-267. <http://doi.org/10.18502/jfsh.v11i3.21340>

### 1. Introduction

Antibiotic residues (AR) in food describe the traces of antibiotics that are left in food products originating from livestock, such as meat, milk, and eggs, after being treated with these medications (1).

AR are present in food mainly due to improper antibiotic use, misuse without prescription, and disregard for withdrawal periods during food production processes (1-3). AR poses several food safety and health risks to humans, such as direct toxic

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effects, allergic reactions, and the development of antibiotic-resistant bacteria by exposing bacteria to sub-therapeutic antibiotic doses (1,2,4).

Resistant bacteria can spread from animals to humans and decrease the effectiveness of antibiotics used in human medicine; this connection presents a major public health issue (5,6). Consequently, to reduce consumer health risks and fight antimicrobial resistance (AMR), appropriate antibiotic usage and monitoring of antibiotic residues in food production remain crucial. Good agricultural practices, farmer education, adherence to withdrawal periods, and the development of antibiotic substitutes are some of the essential components towards control efforts.

In Zambia and other countries, poultry products such as eggs play a crucial role for livelihoods, economic development, nutrition, and food security (7,8). Poultry egg production requires appropriate management practices for laying hens, including feeding, lighting, and biosecurity for optimal egg production and poultry health (9). Table egg distribution and selling is performed through a variety of channels, such as farm gate sales, neighbourhood retail stores, wholesalers, open and closed marketplaces, all of which contribute to supply and demand balance (10). Global egg production has been increasing gradually, and the average annual consumption per person has increased by 14% over the last ten years (11,12). This increase reflects on the nutritional value of eggs and affordability. However, diseases pose a significant burden on poultry production, which often necessitates antibiotic use for both preventative and therapeutic purposes in order to manage diseases and maintain productivity. The use of antibiotics in laying hens

through their feed or water, combined with noncompliance with the withdrawal period, promotes the accumulation of antibiotic residues in eggs (13-15). While eggs represent a vital dietary source globally, several studies have reported the detection of antibiotic residues such as ampicillin, amoxicillin, tetracycline, and oxytetracycline in eggs from both large and small-scale poultry production systems, posing a substantial public health concern (2,13-19). To reduce these residues in eggs meant for human consumption, a regulatory framework is required, like that of the European Union which prohibits the use of certain antibiotics, such as Enrofloxacin, in laying hens (4,20,21). Enrofloxacin is a member of the fluoroquinolone group widely used in veterinary medicine and exhibits strong action against both Gram-positive and Gram-negative bacteria (22). Residues of enrofloxacin and its metabolite ciprofloxacin are known to accumulate in very high amounts in the egg white, egg shells, and yolk for several days after treatment is stopped, raising significant health and environmental issues (23,24). Consequently, enrofloxacin makes an interesting target to study, particularly in developing countries where antibiotic misuse is still a huge problem.

Although the presence of antibiotic residues in poultry products is a known problem, there is still limited data, particularly concerning the use of antibiotics that are prohibited in the production of poultry eggs. Furthermore, little is known about the distribution of these prohibited drug residues across various points in the poultry supply chain, especially between different market segments. In addition, data on the distribution of residues in branded versus unbranded eggs is often

limited or unavailable. This leaves a critical knowledge gap regarding the extent and patterns of prohibited drug usage in poultry production. These gaps make it difficult to determine where the risk to consumers is greatest and, consequently, where intervention efforts would be best targeted (25,26). In the Zambian context, there is a complete absence of data regarding the presence of prohibited fluoroquinolones, like enrofloxacin, in the table egg supply chain. This lack of local evidence hinders the development of targeted control efforts and consumer awareness campaigns.

In this study, we used High-Performance Liquid Chromatography (HPLC) to determine the proportional distribution of branded and unbranded table eggs from supermarkets (regulated retail outlets) and open markets (traditional, informal markets) in Lusaka province that contain enrofloxacin residues. Given the high risk of AR in Zambia and many other countries globally, as well as the extensive use of antibiotics in poultry, our data contributes to a fundamental knowledge gap and is relevant in control efforts to reduce antibiotic residues in poultry products and targeted public health interventions to address antimicrobial resistance resulting from the production of food animals.

## 2. Materials and Methods

### 2.1. Study area and design

This study was conducted in Lusaka province of Zambia, which included Lusaka and surrounding districts (Kafue, Chilanga, and Chongwe). We applied a cross-sectional descriptive study design to determine the proportion of table eggs from open and closed markets that contain Enrofloxacin residues. An open market was defined as an unrestricted market, not

housed in a building, where food products are often sold exposed, while a supermarket was defined as a restricted market, housed in a closed building with modernized facilities (27). Lusaka province was purposively selected due to its high population and demand for eggs in households, restaurants, and bakeries. Lusaka province has a proper balance of large-scale commercial farms and smallholder producers and represents 22% of the total number of layer chicken raising households and over 52% of the total number of laying hens raised in Zambia, making it an ideal study area (28).

### 2.2. Sample size estimation

The sample size was estimated based on a single-proportion estimation sample size calculator in Epitools (29). Inputs were the assumed or estimated value for the proportion 6.8-30.8%, with the actual assumed value (proportion) for the Enrofloxacin in table eggs at 7 % (14). The standard normal distribution corresponding to the desired confidence level was  $Z=1.96$  for 95% CI; and the desired precision (half desired CI width) was  $\pm 0.05$  (5%). The assumed number of farmers (egg brands) supplying eggs consistently on the market was 200.

Sample size was calculated using the formula:  $n = (Z^2 \times P \times (1 - P)) / e^2$

Where:

- $Z$  = value from standard normal distribution corresponding to desired confidence level ( $Z=1.96$  for 95% CI)
- $P$  is the expected true proportion
- $e$  is the desired precision (half the desired CI width)

Since the number of farmers supplying eggs on the market consistently was considered to be about 200, the

estimated sample size ( $n$ ) was adjusted so that  $n(\text{adj}) = (N \times n) / (N + n)$ . (Thrusfield M, 2005.). Based on these assumptions, we calculated the number of samples to be collected to be 68 (farms/egg brands). This sample size was distributed between open markets and closed markets. A sample consisted of one egg tray (30 eggs) of a particular brand or coming from a specific farmer.

### 2.3. Sampling

The primary sampling units were farms (egg brands) that supplied eggs to both open markets and supermarkets. We used a tray of 30 eggs as the sample to represent a farm (egg brand), and egg trays were bought from open markets and supermarkets across the Lusaka and the surrounding districts. At the time of the study, information collected from the respective local authorities (District council of office) revealed that there were 47 supermarkets and 33 open markets in Lusaka province. This formed the sampling frame from which a study population was drawn. Samples were purchased from the selected markets in November and December 2018. During this study, samples were collected from seven (7) open markets and eight (8) supermarkets in Lusaka district; one (1) open market and two (2) supermarkets in Kafue district, two (2) open markets and one (1) Supermarket in Chilanga district, and one (1) open market and no supermarket in Chongwe district. A total of 45 samples were collected from open markets and 15 from the supermarkets. An additional egg tray was bought from the hatchery, bringing the total to 61, but the hatchery sample was used as a blank in this analysis, leaving a total of 60 samples analysed. Information obtained from the hatchery indicated that the birds were not on any antibiotic treatment, which made it a suitable

sample for the blanking. The number of collected samples was eight (8) in Chilanga; thirteen (13) in Chongwe; twenty-four (24) in Lusaka, and fifteen (15) in Kafue. In Chongwe, Kafue, and Chilanga, three visits were made on different days and each time targeting different farms.

### 2.4. Laboratory sample analysis

From each sample of 30 eggs, the eggs were thoroughly mixed in a mixing bowl, and from it, 2g was weighed and placed in a 50mL centrifuge tube to which 4 mL HPLC grade water was added. The mixture was further homogenized by vortex agitation for 1 min, and 10 mL of 1% acetic acid in acetonitrile solution was added. This mixture was further subjected to vortex agitation for 1 min. Extraction salts, sodium sulphate and sodium chloride (4g  $\text{Na}_2\text{SO}_4$ , 1g  $\text{NaCl}$ ) were added to the centrifuge tube containing the mixture above, and then it was shaken vigorously for 1 min, after which centrifugation at 5000 rpm for 5 min was done. The tubes were allowed to stand for 30 min. Six millilitres (6 mL) of the supernatant was transferred into an empty 15 mL centrifuge tube, to which 15 mg of Super clean ENVI-carb SPE was added and subjected to vortex agitation for 1 min. The mixture was centrifuged at 5000 rpm for 5 min, and 4 mL of the supernatant was transferred into another clean 15 mL centrifuge tube. The tubes were dried using nitrogen compressed gas flow at 40°C, after which HPLC water and acetonitrile were added to make 1 mL in the ratio of 2:8 acetonitrile: water, and centrifuged the samples at 10,000 rpm for 10 min. The supernatant was transferred into vials for HPLC analysis. The instrument conditions were programmed as follows: Column: Agilent ZORBAX solvent saver HD Eclipse plus C18, 3.0 x 100mm, 1.8m.

Flow rate: 0.5 mL/Min. Column temperature: 30°C. Injection volume: 5µL. Mobile phase: A H<sub>2</sub>O 0.1% Formic acid, B: ACN. Gradient 1-9. Time (min 0.0 and max. 15 min); %A (min 5% and max. 90%); and %B (min 10% and max. 95%).

### 2.5. Data analysis

A calibration curve with the formula  $Y = 0.0141X + 0.0581$  was obtained using a standard negative sample. The retention time for Enrofloxacin was 6.91 min, and the recovery rate was between 79% and 120%. The coefficient of correlation for the calibration curve was 0.99908. Each sample produced a unique chromatogram, and using the retention time and the area under the peak produced at that time, were used to calculate the Enrofloxacin concentration for each sample. The data were summarized and analysed using STATA 12. The important parameters that were considered were the market segment, branding, and enrofloxacin concentration.

The method's limit of detection (LOD) and limit of quantification (LOQ) were determined to be 0.001 µg/g and 0.003 µg/g, respectively, confirming the method's sensitivity for detecting enrofloxacin at the levels relevant for food safety monitoring.

### 3. Results

From the sixty (60) egg samples (trays) that were collected, 31.7% (95% CI: 20.3-44.9%) tested positive for Enrofloxacin residues. Chongwe had the highest proportion of the antibiotic residues at 61.5% (95% CI: 31.6-86.1%), followed by Kafue at 33.3% (95% CI: 11.8-61.6%) and Lusaka districts 25.0% (95% CI: 3.2-65.1%). Similarly, Chongwe had the highest concentration of antibiotic residues at 0.579 µg/g, followed by Kafue at 0.332 µg/g (Table 1).

Fig. 1 shows a chromatogram from a sample which was collected from Chilanga district. This sample gave the lowest reading of all the positive samples.

A higher proportion of the enrofloxacin residues among the positive samples was detected in open markets, 84.2% (95% CI: 60.4-96.6%), compared to supermarkets, 15.8% (95% CI: 3.4-39.6%). Similarly, the majority of enrofloxacin residues were detected in unbranded eggs, 89.5% (95% CI: 66.9-98.7%), compared to the branded eggs, 10.5% (95% CI: 1.3-33.1%) (Table 2).

The district proportional distribution of total samples in each market and branding segment is displayed in Tables 3 and 4. In supermarkets, the predominant proportion of positive samples, 27.3% (95% CI: 6.0-60.9%), were detected in Lusaka district, while Kafue, Chilanga, and Chongwe districts had no positive samples recorded. In open markets, a high number of positive samples were detected in Chongwe district, 61.5% (95% CI: 31.6-86.1%), followed by Kafue, 41.7% (95% CI: 15.2-72.3%). Lusaka district had the lowest proportion, 7.7% (95% CI: 0.19-36.0%), of positive samples from the open markets. Of the total branded samples, only the Lusaka district recorded positives with a proportion of 50.0% (95% CI: 6.7-93.2%) while the other three districts had none. Among the unbranded samples, Chongwe district 61.5% (95% CI: 31.6-86.1%) recorded the highest proportion followed by Kafue 33.3% (95% CI: 11.8-61.6%) and Chilanga districts 25.0% (95% CI: 3.2-65.1%) while Lusaka recorded the lowest proportion of positives at 10.0% (95% CI: 1.2-31.7%).

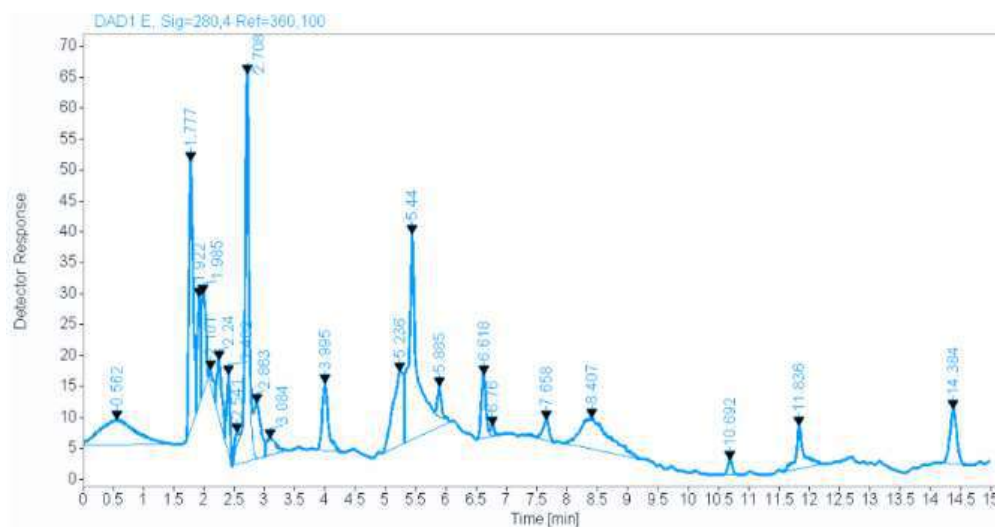


Figure 1. Chromatogram for a sample collected from Chilanga (CHL7)

Table 1. Proportion and concentration of HPLC positive samples

Provincial districts and overall	Total no. of samples (n = 60)	Total no. HPLC positive	% positive (CI)	Mean conc. (µg/g)	Range: Min-max range (µg/g)
Chilanga	8	2	25.0 (3.2-65.1)	0.245	0.16-0.33
Chongwe	13	8	61.5 (31.6-86.1)	0.579	0.23-1.52
Lusaka	24	4	16.7 (4.7-37.4)	0.303	0.25-0.37
Kafue	15	5	33.3 (11.8-61.6)	0.332	0.28-0.42
Overall total	60	19	31.7 (20.3-44.9)	0.365	0.16-1.52

Table 2. Distribution of positive samples according to brand and market segment

Provincial districts and overall	Total no. HPLC positive (n = 19)	Branded		Unbranded		Open markets		Supermarkets	
		Total positive	% Positive (CI)	Total positive	% Positive (CI)	Total positive	% Positive (CI)	Total positive	% Positive (CI)
Lusaka	4	2	50.0 (6.7-93.2)	2	50.0 (6.7-93.2)	1	25 (0.6-80.6)	3	75 (19.4-99.4)
Kafue	5	0	0.0 (0.0-0.0)	5	100.0	5	100.0	0	0.0 (0.0-0.0)
Chongwe	8	0	0.0 (0.0-0.0)	8	100.0	8	100.0	0	0.0 (0.0-0.0)
Chilanga	2	0	0.0 (0.0-0.0)	2	100.0	2	100.0	0	0.0 (0.0-0.0)
Overall total	19	2	10.5 (1.3-33.1)	17	89.5 (66.7-98.7)	16	84.2 (60.4-96.6)	3	15.8 (3.4-39.6)

Table 3. Distribution of total samples by market segment

Provincial districts and overall	Total no. of supermarket samples (n = 15)	Total no. positive	% Positive (CI)	Total no. of open market samples (n = 45)	Total no. positive	% Positive (CI)
Lusaka	11	3	27.3 (6.0-60.9)	13	1	7.7 (0.19-36.0)
Kafue	3	0	0.0 (0.0-0.0)	12	5	41.7 (15.2-72.3)
Chongwe	0	0	0.0 (0.0-0.0)	13	8	61.5 (31.6-86.1)
Chilanga	1	0	0.0 (0.0-0.0)	7	2	28.6 (3.7-70.9)
Overall total	15	3	20.0 (4.3-48.1)	45	16	35.6 (21.9-51.2)

Table 4. Distribution of total samples by branding status

Provincial districts and overall	Total no. of branded samples		% Positive (CI)	Total no. of unbranded samples		Total no. positive	% Positive (CI)
	(n = 4)	Total no. positive		(n = 56)	Total no.		
Lusaka	4	2	50.0 (6.7-93.2)	20	2	10.0 (1.2-31.7)	
Kafue	0	0	0.0 (0.0-0.0)	15	5	33.3 (11.8-61.6)	
Chongwe	0	0	0.0 (0.0-0.0)	13	8	61.5 (31.6-86.1)	
Chilanga	0	0	0.0 (0.0-0.0)	8	2	25.0 (3.2-65.1)	
Overall total	4	2	50.0 (6.7-93.2)	56	17	30.4 (18.8-44.1)	

#### 4. Discussion

In this study, we report the detection of Enrofloxacin residues in both branded and unbranded table eggs from open markets and supermarkets in Lusaka province of Zambia. Of the total samples tested, 31.7% contained enrofloxacin residues. Our results were higher than broader surveys in other places such as China and Jordan, which reported enrofloxacin residues in market-sourced poultry eggs at contamination rates of 0.83% and 0.08%, respectively (3,24).

Conversely, a study in Iran reported a higher proportion (43.58%) of enrofloxacin residues in chicken eggs (23). Despite studies reporting these variable levels of residues, the mere detection of enrofloxacin in table eggs is a major public health concern because its use in laying hens is generally prohibited due to the potential risks of antibiotic residues entering the human food chain (20). The detection of enrofloxacin

residues in eggs characteristically suggests inappropriate use of enrofloxacin in laying hens, which raises concerns with adherence to food safety standards and drug withdrawal (1,2). However, it is important to understand that the detection of enrofloxacin residues in eggs may not entirely imply inappropriate antibiotic usage through therapeutic applications, as evidence suggests that indirect environmental mechanisms (such as contamination of soil, water sources, animal feed, and chicken droppings) may also play a role in the transmission of antibiotic residues into eggs (24,30,31). The role of environmental transmission, such as contaminated feed or water, warrants specific investigation in Zambia. Small-scale poultry farmers often keep layers in free-range systems and may use water from sources potentially contaminated with agricultural or human waste, providing a pathway for antibiotic exposure even without direct administration. Given these complexities, further research is necessary

to understand how environmental transmission contributes to the presence of antibiotic residues in table eggs, particularly those sold in both open and supermarket contexts.

The majority (84.2%) of enrofloxacin residues among the positive samples were detected in open markets compared to supermarkets. Similarly, most (89.5%) of enrofloxacin residues were detected in unbranded eggs compared to branded eggs. The differences in residue prevalence between market segments and the branding status may suggest variations in control and regulation. Unbranded eggs and eggs sold at open markets usually originate from less stringent production practices and less controlled sources where the use of antibiotics may be less monitored, leading to a higher prevalence of residues; whereas branded eggs and those from supermarkets may less likely contain residue contaminants because they are often produced by larger and more regulated establishments with tighter quality control (32–36). Further, supermarkets tend to have supplier qualification and monitoring programmes, which safeguard against the placement of low-quality products on their shelves. However, our finding that some supermarkets and branded eggs contained enrofloxacin residues suggests that contamination is not limited to open market and unbranded eggs, even though residues may be more prevalent in these segments. The presence of antibiotic residues in both branded and unbranded eggs, as well as eggs from the open and closed markets, highlights the need for strengthened monitoring and control across all segments.

Beyond the high prevalence of enrofloxacin residues, the concentrations detected (ranging from 0.16 to 1.52

µg/g) are also a cause for public health concern. While the maximum residue limits (MRLs) for enrofloxacin in eggs are established as zero in many jurisdictions, including the European Union, our detected values, particularly the high of 1.52 µg/g found in Chongwe, indicate a significant level of exposure for consumers. Future risk assessments should model the potential health impacts of chronic low-level exposure to these concentrations, especially for vulnerable populations. Some limitations should be taken into account in this study. We did not collect data to assess variables such as farm and antibiotic use factors that could influence the presence of enrofloxacin residues in table eggs. Furthermore, we did not meet the actual calculated sample size, and the application of a probability-based random sampling was not feasible due to resource and practical constraints on data collection, and the inclusion of market segments in our analysis was disproportionate. Therefore, caution must be taken when extrapolating the results.

Beyond these limitations, our findings are relevant and, to the authors' knowledge, these results provide the first evidence of enrofloxacin residues in eggs from open markets and supermarkets in Zambia. This data is useful for generating hypotheses on antibiotic residues and guiding future initiatives to reduce antibiotic residues in the food chain. Future research may focus on deciphering the role of environmental transmission pathways and variations related to branding and market segments in the contamination and persistence of residues in eggs, especially in resource-constrained contexts. In light of these findings, we recommend the development and implementation of systematic residue monitoring systems for table eggs meant for

human consumption, as well as extensive surveys to profile farm factors and antibiotic usage in poultry.

### Funding

This work was financially supported by the Africa Centre of Excellence for Infectious Diseases of Humans and Animals (ACEIDHA) Zambia Project.

### Author contributions

Vigirio Kalunga Mutemwa: conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft. George Nyamweya: data curation, formal analysis, investigation. Isaac Ssebulime: data curation, formal analysis, methodology, writing – review and editing. Felix Bongo: methodology, writing – review and editing. John Bosco Matovu: conceptualization, funding acquisition, methodology, project administration, resources, supervision, writing – review and editing. Kato M. Mugisha: conceptualization, methodology, project administration, supervision, writing – review and editing.

### Declaration of competing interest

The authors state no conflict of interest.

### Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

### Acknowledgements

The authors are grateful to the marketeers and retail shop owners who voluntarily and graciously participated in this study.

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