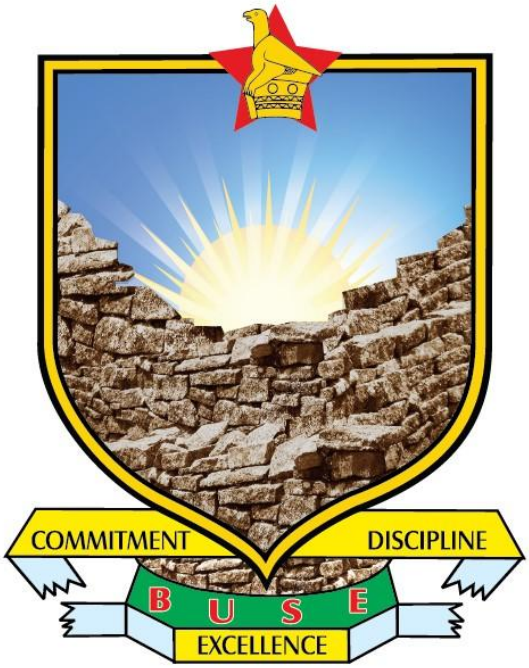
Bindura University of Science Education



Comparative assessment of antibiotic residues in poultry eggs from small-scale farmers and large-scale production systems

By

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B225279B

A research project submitted in partial fulfilment of the requirements for the Bachelor of Science Honors Degree in Biotechnology.

June 2026

Approval form

The undersigned certify that they have read the dissertation titled “Comparative assessment of antibiotic residues in poultry eggs from small-scale farmers and large-scale production systems” and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering, for assessment.

Signature of student:  Date: 15/06/2026

Signature of Supervisor:  Date: 15/06/2026

Signature of Chairperson of Department:  Date: 12 August 2026

Declaration

I, TAONA ZARANYIKA declare that this research herein is my own work and has not been plagiarized from another source(s) without acknowledgement of the concerned author(s) either electronically or otherwise

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Supervisor

I, J. Ndava, declare that I have supervised this thesis and I am satisfied that it can be submitted to the Biological Sciences Department, Faculty of Science and Engineering, at Bindura University of Science Education.

Signature:  Date: 15/06/26

Dedication

I dedicate this project to my family, whose unwavering support and encouragement made its completion possible.

Acknowledgements

I would like to express my sincere gratitude to Mr Ndava for his invaluable guidance, patience and academic mentorship throughout the research. His constructive feedback and expertise were instrumental in shaping this work. I also extend my appreciation to CVL staff for their cooperation, technical assistance and willingness to provide resources that made the completion of the study possible. Special thanks to Mr Musevedzera for his practical advice and motivation that kept me focused during challenging times. Finally, I am deeply thankful to my family and friends for their understanding and sacrifices that allowed me to dedicate the time and effort required for this dissertation.

List of abbreviations

AMR	Antimicrobial Resistance
ANOVA	Analysis of Variance
CFU/mL	Colony-Forming Units per millilitre
Df	Degrees of freedom
E. coli	*Escherichia coli*
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FAO	Food and Agriculture Organisation (of the United Nations)
FPT	Four-Plate Test
HPLC	High-Performance Liquid Chromatography
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
MCAZ	Medicines Control Authority of Zimbabwe
MRL(s)	Maximum Residue Limit(s)
PABA	para-Aminobenzoic acid
PBPs	Penicillin-Binding Proteins
WHO	World Health Organisation

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Abstract

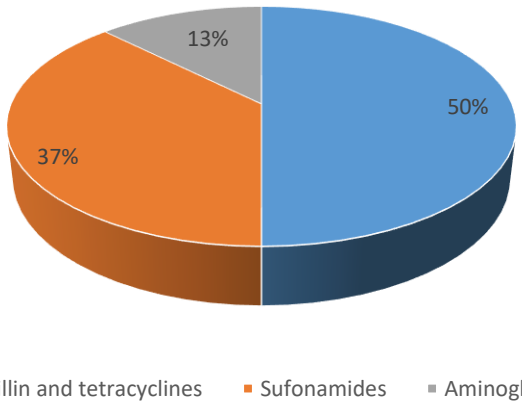
The extensive and often unregulated use of antibiotics in poultry production presents a significant public health risk due to residues found in animal-derived food products. This study compared antibiotic residues in eggs from small-scale farmers and large commercial farms in Harare, Zimbabwe. A sum of 30 composite egg samples were collected and examined for residues using the microbiological Four-Plate Test (FPT) with *Escherichia coli* at pH levels of 6.0, 7.2, and 8.0. Of these, 11 samples (36.7%) tested positive for antibiotic residues, 40% from small-scale farms and 33.3% from commercial farms containing Sulphonamides, Penicillins/Tetracyclines, and Aminoglycosides. Chi-square analysis showed no significant link between farming system and residue prevalence ($p > 0.05$), indicating that antibiotic misuse is a common issue across scales. Nonetheless, ANOVA results for aminoglycosides ($p = 0.047$) indicated a marginally significant difference in their occurrence between systems. These findings reveal a high prevalence of antibiotic residues in locally produced eggs, bringing attention to antimicrobial resistance (AMR) and possible hypersensitivity reactions among consumers. The study recommends stricter monitoring of withdrawal periods and enhanced farmer education to improve food safety and safeguard public health.

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Chapter 1

1.1 Background

The global prevalence of antibiotic traces in food products, particularly poultry eggs, constitutes a significant public health concern (Islam et al., 2020). Documented cases have been reported across multiple continents, including Italy, Chile, Pakistan, Saudi Arabia, Egypt, Ethiopia, Ghana, Kenya, Nigeria, South Africa, Sudan, and Tanzania (Cornejo et al., 2020). Developing countries are mostly at risk due to inadequate regulations governing antibiotic use in livestock, insufficient enforcement of maximum residue limits (MRLs), and limited detection infrastructure (Abdiukov, 2025). These factors exacerbate antimicrobial resistance (AMR) and pose direct risks to human health.

While developed countries like those in Europe and the USA have banned antibiotic growth promoters, the use of antibiotics for metaphylactic and prophylactic purposes, often at high doses, remains widespread in low-resource settings (Oliver et al., 2020). For instance, in Ghana, poultry farmers frequently use antibiotics such as penicillin, tetracyclines, fluoroquinolones, and aminoglycosides to treat diseases like coccidiosis, Newcastle disease, and E. coli infections (Owusu-Doubreh et al., 2023).

The global increase in poultry production systems has elevated the risk of disease spread, such as Gumboro disease and avian typhoid, due to high stocking densities. This often leads to financial losses from bird deaths and encourages antibiotic misuse. Such practices can result in harmful residues in eggs, risking consumer health through allergic reactions, antibiotic resistance, teratogenic effects, and carcinogenicity (Owusu-Doubreh et al., 2023). For example, β-lactams (like penicillin and cephalosporins) and sulphonamides in eggs may cause severe hypersensitivity, nephritis, and anaphylaxis. Moreover, ongoing exposure to low-dose antibiotics can promote antimicrobial resistance (AMR) and disrupt the gut microbiota.

In Zimbabwe, where both small-scale and large-scale poultry farming exist, antibiotic use is widespread but poorly monitored (Hakimzadegan et al., 2014). Small-scale farmers often lack veterinary oversight and resort to unregulated antibiotics, while commercial operations may prioritise productivity over residue management. Antibiotics like tetracyclines, macrolides, and fluoroquinolones are administered through feed, water, or injection and can accumulate in eggs due to their pharmacokinetic properties (absorption, distribution, and metabolism) as they deposit in the yolk and albumen during egg formation (Sánchez-Trasviña et al., 2021). The Four Plate Test (FPT) has shown that macrolides are predominant contaminants in egg yolks, although tetracyclines and penicillins are the most frequently used (Mohammadzadeh et al., 2022).

This dual burden underscores the urgent need for a One Health approach integrating human, animal, and environmental health to mitigate risks. Current strategies often overlook the interplay among different farming systems (small-scale vs large-scale), drug-residue dynamics, and AMR transmission (Mohammadzadeh et al., 2022). For example, mass medication in large-scale systems can amplify resistance genes, while small-scale practices may perpetuate unregulated residues. Addressing these gaps necessitates harmonised surveillance, context-specific MRLs, and interdisciplinary collaboration to protect food safety and curb zoonotic AMR.

1.2 Problem statement

Despite the recognised risks linked with antibiotic residues present in food, there is limited specific data in Zimbabwe comparing the occurrence of these residues in eggs from different poultry production systems. Small-scale farmers often operate without veterinary oversight and may use unregulated antibiotics, while large-scale commercial operations may prioritise productivity over residue management. The absence of systematic surveillance and weak enforcement of withdrawal periods creates a critical knowledge gap regarding the prevalence and concentration of antibiotic residues in eggs reaching consumers. This study addresses this gap by generating empirical data on residue occurrence in both farming systems in Harare.

1.3 Aim

The study aims to assess and compare the presence of antibiotic residues in eggs produced by small-scale individual farmers and large-scale commercial poultry systems, and to evaluate the potential risks to food safety and public health.

1.4 Objectives

1. To detect antibiotic residues in eggs from small-scale and large-scale poultry farms.
2. To compare residue patterns between small-scale and large-scale production systems.
3. To evaluate food safety and public health risks associated with these residues.
4. To assess the influence of farming practices on residue occurrence.
5. To provide evidence-based recommendations for improved regulation and antibiotic use.

1.5 Significance of the study

This study holds significant value as it addresses the pressing global issue of antimicrobial resistance (AMR), which is exacerbated by the misuse of antibiotics in agriculture. By directly comparing residue levels in eggs from small-scale and commercial farms, the research provides essential insights into how different production practices affect food safety. This will not only help protect consumers from potential health risks, such as allergic reactions and toxic effects, but also inform evidence-based policymaking in Zimbabwe, aiding regulators in strengthening monitoring and enforcing safe withdrawal periods. Furthermore, it benefits farmers by raising awareness and promoting sustainable alternatives to antibiotics, thereby contributing to the wider One Health framework that connects human, animal, and environmental wellbeing, and ultimately supporting safer food systems and the global fight against AMR.

1.6 Limitations

This study acknowledges methodological constraints that may affect the interpretation and applicability of its findings. The limited sample size of only six farms in Harare districts limits the representativeness of the results for Zimbabwe’s broader poultry sector. The geographic focus further limits generalizability to regions with different farming practices or regulations. Data on

antibiotic use, collected via questionnaires, may be compromised by recall or social desirability bias. Analytically, the targeted antibiotic panel and reliance on microbial inhibition assays, rather than advanced confirmatory techniques, provide a focused snapshot but not a comprehensive assessment of residue prevalence or resistance.

1.7 Delimitations of the study

This study is deliberately confined in scope to ensure focused and feasible investigation. It is geographically limited to Harare and examines only two farm categories: small-scale and commercial, excluding other production systems. The analysis focuses exclusively on poultry eggs, without extending to other products or environmental matrices. Furthermore, the research targets a specific panel of antibiotic classes based on common usage, and primarily employs screening methods for residue detection, acknowledging that advanced confirmatory techniques are beyond the project's current resource limitations.

1.8 Summary

This chapter introduces the global concern of antibiotic residues in food, particularly in poultry eggs, highlighting public health risks such as antimicrobial resistance, allergic reactions, and toxicity. It contextualises the problem within Zimbabwe's poultry sector, outlines the study's objectives, research questions, and hypotheses, and justifies the need for monitoring antibiotic residues in eggs from both small-scale and commercial farms.

Chapter 2

Literature review

2.1 Introduction

Antibiotic residues are leftover parent compounds or metabolites in edible tissues like eggs after treatment, mainly in poultry where antibiotics are vital for health and productivity (Booth et al., 2020). In hens, these drugs enter the bloodstream and deposit into yolk and albumen during follicular development. If the withdrawal period isn't followed, residues can enter the human food supply, risking toxicity, physiological effects, and antimicrobial resistance (AMR) (Amann et al., 2019). Health issues linked to penicillins, tetracyclines, and sulphonamides include allergic reactions, cancer risks, and anaphylaxis (Kirchelle et al., 2018). Organizations such as FAO/WHO's Codex Alimentarius and the EU set Maximum Residue Limits (MRLs) to control residues. Monitoring in developing countries is tough due to limited resources. Microbiological assays like the Four-Plate Test are common for screening because they are affordable and detect various inhibitory substances (Pihlstrom et al., 2021). This review focuses on antibiotic residues in Zimbabwean poultry eggs, drug classes used, their persistence mechanisms, and current screening methods.

2.2 Antibiotic uses in animal production

Antibiotics used in the poultry industry are classified primarily based on their chemical structure, which determines their spectrum of activity and their persistence in food products. In the context of egg production, the most significant classes include Tetracyclines, beta-lactams, Sulfonamides, and Aminoglycosides.

Tetracyclines

Tetracyclines, for instance, Oxytetracycline or Chlortetracycline are broad-spectrum agents that inhibit bacterial protein synthesis. They are among the most commonly used antibiotics in small-scale farming due to their low cost and efficacy against both Gram-positive and Gram-negative bacteria (Kirchelle et al., 2018). Because they bind readily to calcium, they tend to accumulate in the shells and yolks of eggs.

beta-Lactams (Penicillins and Cephalosporins)

These antibiotics interfere with bacterial cell wall synthesis (Kimera et al., 2020). While highly effective, they are notorious for causing hypersensitivity and allergic reactions in humans. In poultry, they are often used to treat systemic infections. Their presence in eggs is a high-risk factor for consumers with penicillin allergies (Islam et al., 2020).

Sulphonamides

Sulphonamides are synthetic bacteriostatic agents that inhibit folic acid synthesis. They are frequently used in Zimbabwe for treating coccidiosis and respiratory infections in poultry. They have a high rate of excretion into the egg albumen rather than the yolk, making the entire egg a vehicle for residue exposure (Owusu-Doubreh et al., 2023).

Aminoglycosides

This class includes Streptomycin and Neomycin, which are used to treat aerobic Gram-negative infections. They are highly polar compounds, which can make their detection via standard solvent extraction difficult. They are known for potential nephrotoxicity and ototoxicity if consumed in high concentrations over time (Pihlström et al., 2021).

Macrolides and Others

Other classes such as Macrolides (Erythromycin) and Quinolones are also used but are often strictly regulated or banned for use in laying hens in many jurisdictions because they persist for long periods in the bird's system, extending the required withdrawal periods significantly (EU, 2019).

2.3 Mechanisms of Action of Antibiotic Residues

The efficacy of antibiotics in poultry production is derived from their ability to target specific biochemical pathways within pathogenic bacteria that are either absent or structurally different in eukaryotic (host) cells (Darwish et al.,2013). Understanding these mechanisms is crucial for interpreting screening results, such as the Four-Plate Test, which relies on the inhibition of specific test organisms like *Bacillus subtilis* and *Micrococcus luteus*.

Inhibition of Cell Wall Synthesis

Beta-lactam antibiotics, including penicillins and cephalosporins, act by binding to Penicillin-Binding Proteins (PBPs). These proteins are enzymes responsible for the final stages of peptidoglycan synthesis. By inhibiting the cross-linking of the bacterial cell wall, these drugs lead to osmotic instability and cell lysis (Islam et al., 2020). In residue screening, these are often detected at pH 6.0 using *B. subtilis*.

Inhibition of Protein Synthesis

Multiple classes of antibiotics utilised in Zimbabwean poultry production target the bacterial ribosome to inhibit translation. Tetracyclines bind reversibly to the 30S ribosomal subunit, thereby preventing the attachment of aminoacyl-tRNA to the mRNA-ribosome complex. This action inhibits the elongation of the polypeptide chain (Kirchelle et al., 2018). Aminoglycosides bind irreversibly to the 30S subunit, causing a misreading of the genetic code and the production of "nonsense" proteins that are lethal to the bacteria (Pihlstrom et al., 2021).

Inhibition of Nucleic Acid Synthesis

Quinolones and Fluoroquinolones interfere with DNA replication by inhibiting bacterial DNA gyrase (topoisomerase II) and topoisomerase IV (Pihlström et al., 2021).. This prevents the DNA from supercoiling, effectively stopping bacterial cell division. Residues of these drugs are particularly concerning due to their potential to induce long-term DNA damage in human gut microbiota (Owusu-Doubreh et al., 2023).

Interference with Metabolic Pathways

Sulphonamides are structural analogues of para-aminobenzoic acid (PABA) (Zhou et al.,2021). They competitively inhibit the enzyme dihydropteroate synthase, which is essential for the synthesis of folic acid. Since bacteria must synthesise their own folic acid while humans obtain it from their diet, this pathway is a highly specific target (EU, 2019). In the lab, these residues are specifically identified by their increased activity at pH 7.2.

2.4 Methods for the Detection of Antibiotic Residues

The detection of antibiotic residues in complex food matrices like eggs requires analytical methods that are both sensitive and specific. These methods are generally classified into two categories: rapid screening methods and high-performance confirmatory methods.

Microbiological Screening Assays

Microbiological assays are the most common primary screening tools. They rely on the principle of bacterial growth inhibition. A specific test organism (e.g., *Bacillus subtilis*) is seeded into an agar medium; if the egg sample contains antibiotics above a certain concentration, a "zone of inhibition" forms where the bacteria cannot grow (Pihlström et al., 2021).

The Four-Plate Test (FPT): This is a specialised microbiological assay that uses different pH levels (6.0, 7.2, and 8.0) and different test organisms to tentatively identify the class of antibiotic present. It is favoured in surveillance studies because it is cost-effective, requires minimal specialised equipment, and can detect a wide range of antibiotic classes simultaneously (Islam et al., 2020).

Immunological Assays (ELISA)

Enzyme-Linked Immunosorbent Assays (ELISA) use the high specificity of antibody-antigen interactions to detect specific drug residues.

Strengths

They are extremely sensitive and faster than microbiological methods.

Weaknesses

They are usually class-specific, meaning a different kit is required for each antibiotic class (e.g., one kit for Tetracyclines and another for Sulfonamides), making broad screening expensive (Owusu-Doubreh et al., 2023).

Physicochemical Confirmatory Methods

Methods like High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS/MS) are regarded as the gold standards for residue analysis. They work by separating the components of a sample and identifying them through their mass-to-charge ratio or UV absorbance (Castro et al., 2020). Although these techniques offer high accuracy and can quantify residues precisely (e.g., in µg/kg), they also require extensive sample preparation,

expensive high-purity solvents, and highly trained personnel. This often limits their practicality for routine monitoring, especially in developing regions (Robles-Jimenez et al., 2021).

Comparison of Methods

For a comparative assessment between production systems, microbiological screening (FPT) provides a qualitative answer and a tentative classification, which is sufficient for identifying prevalence trends. However, samples that test positive in screening ideally should be confirmed via LC-MS/MS to satisfy regulatory legal requirements (EU, 2019).

2.5 Antibiotic Use and Residues in the Zimbabwean Poultry Sector

In Zimbabwe, poultry production is a critical component of the national economy and a primary source of protein for the population. However, the sector faces significant challenges regarding the unregulated use of veterinary drugs, particularly among small-scale (backyard) farmers who contribute a substantial portion of the nation's egg production.

Patterns of Antibiotic Usage

Studies in Zimbabwe have indicated that tetracyclines, sulfonamides, and penicillins are the most frequently used antibiotics in poultry (Katsande et al., 2018). These drugs are often accessible over-the-counter without a veterinary prescription. In small-scale systems, antibiotics are frequently used prophylactically (to prevent disease) or as growth promoters, rather than strictly for treating diagnosed infections. This practice increases the likelihood of residues persisting in eggs sold at local markets.

Regulatory Framework and Challenges

The regulation of veterinary medicines in Zimbabwe is governed by the Medicines and Allied Substances Control Act [Chapter 15:03] and overseen by the Medicines Control Authority of Zimbabwe (MCAZ). While MRLs are theoretically aligned with international Codex Alimentarius standards, the enforcement of withdrawal periods remains a major challenge.

Small-scale vs. Commercial: Large-scale production systems in Zimbabwe generally adhere to stricter biosecurity and veterinary oversight. In contrast, small-scale farmers often lack the

technical knowledge regarding withdrawal periods which is the time required for a drug to clear the animal's system before food products can be safely harvested (Owusu-Doubreh et al., 2023).

Previous Research on Local Residues

Recent surveillance in Harare and surrounding provinces has highlighted a growing prevalence of residues. For instance, surveys have shown that a significant percentage of poultry meat and eggs from informal markets test positive for inhibitory substances when screened using microbiological methods (Islam et al., 2020). Despite these findings, there is a lack of recent, comparative data specifically contrasting the residue profiles of eggs from small-scale farmers against those from high-biosecurity large-scale systems.

Public Health Implications for Zimbabweans

The Zimbabwean context is unique due to the high consumption of eggs across all socio-economic groups. The presence of sub-therapeutic levels of antibiotics in daily diets contributes to the national burden of Antimicrobial Resistance (AMR). This complicates the treatment of common human infections, such as Salmonellosis and Typhoid, which are prevalent in urban areas (Kirchelle et al., 2018).

2.6 Gaps in Literature and Rationale for the Study

Despite the extensive global research on antibiotic residues, several critical gaps remain that justify the current investigation into the Zimbabwean poultry sector.

2.6.1 Lack of Recent Comparative Data

Most existing literature focuses either on large-scale commercial production or isolated surveys of informal markets. There is a notable scarcity of recent studies in Zimbabwe that perform a direct, side-by-side comparative assessment of eggs sourced from high-biosecurity commercial systems versus those from resource-limited small-scale farmers within the same geographical region (Harare).

2.6.2 Shift in Farming Practices

In recent years, economic shifts in Zimbabwe have led to an explosion of backyard poultry farming. Unlike established commercial entities, these farmers often operate without the oversight of a resident veterinarian. Literature has yet to fully document how this shift in the production landscape affects the prevalence of specific antibiotic classes like Macrolides or Aminoglycosides in the local food chain (Owusu-Doubreh et al., 2023).

2.6.3 Efficacy of Screening in Local Contexts

While the Four-Plate Test (FPT) is a recognised standard, its application in identifying the specific patterns of misuse in Zimbabwe remains under-reported. By utilising this biotechnological screening tool, this study seeks to provide a snapshot of current compliance with withdrawal periods across different production scales (Islam et al., 2020).

Rationale for the Study

The rationale for this research is rooted in public health safety. Eggs are a primary, affordable protein source in Zimbabwe. If residues are present, they pose a silent threat of chronic exposure, leading to the selection of multidrug-resistant bacteria. This study is therefore essential to:

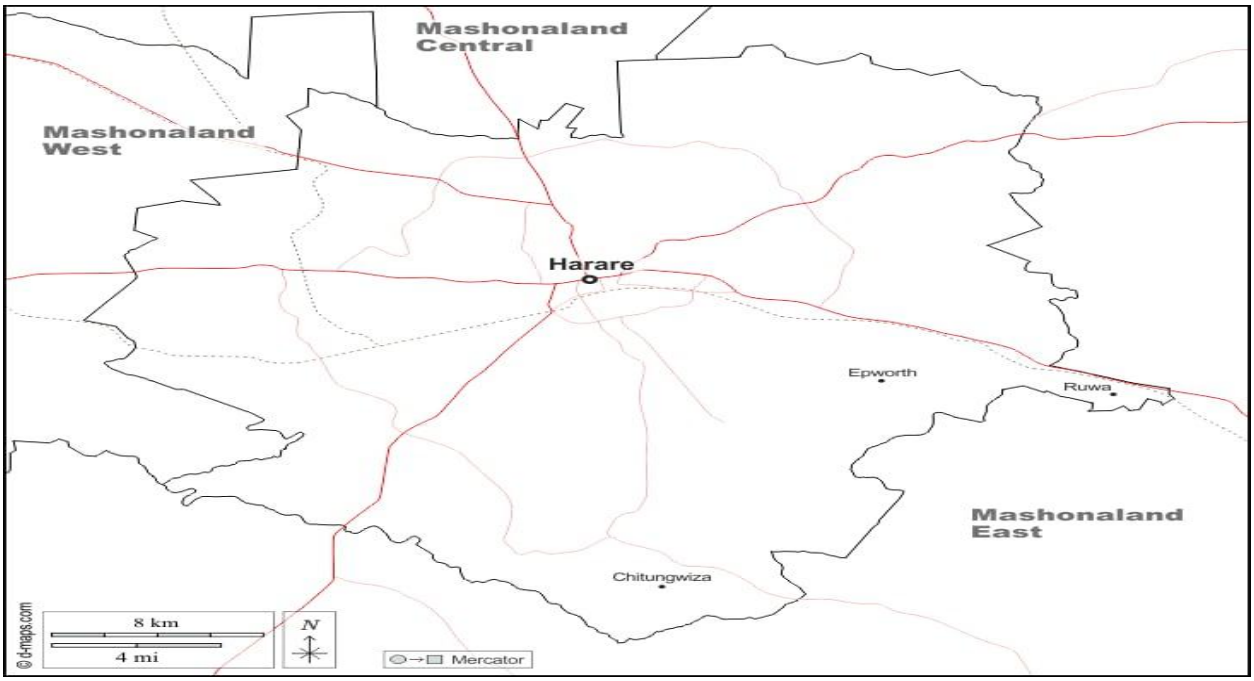
1. Provide evidence-based data to regulatory bodies like the Medicines Control Authority of Zimbabwe (MCAZ).
2. Identify which production system requires more urgent educational interventions regarding veterinary drug withdrawal periods.
3. Support the One Health approach by linking animal husbandry practices to human health outcomes (Kirchelle et al., 2018).

Chapter 3

Materials and methods

3.1 Study area

Figure 3.1 Map of Harare showing the study area and surrounding provinces



3.2 Research Design

This study employed a comparative cross-sectional design to assess and compare antibiotic residues in poultry eggs from small-scale and large-scale farming systems. The research integrated both laboratory-based analytical methods and field surveys to comprehensively evaluate residue levels, antimicrobial resistance patterns, and farming practices. The study was conducted over a period of 6 months from September 2025 to February 2026.

3.2.1 Target Population

The target population comprised poultry farms in Harare district, stratified into small-scale and large-scale commercial operations based on production system and flock size, ranging from backyard producers with limited biosecurity to intensive industrial units. From each farm, 10 fresh eggs with intact, clean shells were randomly collected across different batches and housing units within 24 hours of laying to ensure representativeness and minimise degradation of antibiotic residues.

3.2.2Composite sampling

A comparative cross-sectional study design was employed to assess the prevalence of antibiotic residues in poultry eggs, with a total of 30 egg samples collected from various production sites within the Harare metropolitan province. The samples were divided into two distinct categories based on the production system which are large-scale commercial systems (n=15), which were sourced from high-biosecurity facilities characterized by automated feeding systems, resident veterinary oversight, and bird populations exceeding 5,000 layers and small-scale backyard systems (n=15), sourced from peri-urban open-market suppliers and individual backyard farmers characterized by manual management, bird populations under 500 layers, and a lack of formal veterinary supervision (Codex Alimentarius, 2019).

3.2.3 Metadata Collection

During egg collection, structured questionnaires were administered to farm owners or managers to gather comprehensive data on farm demographics, antibiotic usage history, observance of withdrawal periods, biosecurity practices, waste management methods, sources of veterinary advice, and production objectives. The questionnaires were conducted in the participants' preferred language, either English or Shona, to facilitate accurate and complete responses, and all information was anonymised using farm codes to ensure confidentiality.

3.3 Sample Collection and Handling

Freshly laid eggs were collected aseptically from each farm using gloved hands, selecting only those with intact, clean shells from nest boxes or collection points, and placed in sterile, labelled cardboard trays to prevent breakage and cross-contamination. Each composite sample was assigned a unique identifier indicating farm type, farm code, and sample number. The samples were transported in cool boxes maintained at 4°C ± 2°C to the Central Veterinary Laboratory in Harare within four hours of collection, with temperature monitored continuously using digital data loggers to preserve sample integrity and prevent degradation of antibiotic residues.

3.3.1 Storage

Upon arrival at the laboratory, samples were logged into the laboratory information management system. Eggs designated for immediate analysis were processed within 24 hours. Samples requiring storage were refrigerated at 5°C ± 2°C for a maximum of 72 hours to prevent residue

degradation while maintaining sample stability, following protocols established by the Codex Alimentarius Commission (2019) and guidelines from the European Union Reference Laboratory for antimicrobial residues**.**

3.4 Research procedure

3.4.1 Separation of Egg Components

Each egg was aseptically processed after equilibration to room temperature, with hands washed and sterile gloves worn throughout. Eggshells were cleaned with 70% ethanol swabs and air-dried before being aseptically broken using sterile separators to isolate yolk and albumen into separate 50 mL polypropylene centrifuge tubes. Each component was homogenised individually using a sterile laboratory blender at normal speed for two minutes to ensure uniform consistency, and samples were stored at -20°C if not analysed immediately. Separation of yolk and albumen was performed to evaluate residue distribution patterns, as different antibiotic classes exhibit preferential partitioning based on their physicochemical properties (Kan, 2000).

3.5 Preparation of culture medium

3.5.1 Four-Plate Test Protocol

The four-plate test (FPT) was performed following the protocol described by Okerman et al. (2007) with modifications for egg analysis. Preparation of culture media: Mueller-Hinton agar was prepared according to the manufacturer's instructions and adjusted to three different pH levels: 6.0, 7.2, and 8.0 using 1M acetic acid and 1M sodium hydroxide with a digital pH meter. Media were sterilised by autoclaving at 121°C for 15 minutes and cooled to 45-50°C before pouring into sterile Petri dishes (90 mm diameter) at a volume of 20 mL per plate.

Bacterial Inoculation

Escherichia coli, obtained from the Central Veterinary Laboratory bio bank, was used as the test organism. The bacterium was cultured on nutrient agar, and a bacterial suspension was prepared in sterile saline solution at a concentration equivalent to 0.5 McFarland standard (1.5×10^8 CFU/mL), following established protocols (Berna et al., 2008).

Inoculation

Each plate was inoculated by flooding with 2 mL of the bacterial suspension, excess suspension was removed, and the plates were dried at 37°C for 30 minutes. Wells of 8 mm diameter were cut into the agar using a sterile cork borer, and the agar plugs were removed with a sterile needle.

Sample application

Egg yolk samples (100 µL) were placed into the wells of each pH plate. Positive controls using standard antibiotic solutions and negative controls using antibiotic-free egg samples were included in each batch.

Incubation and reading

Plates were incubated at 37°C for 18-24 hours. Following incubation, zones of inhibition were measured using a digital calliper. Any clear zone around the well, excluding the well diameter, was considered positive. The diameter of inhibition was recorded in millimetres.

3.6 Interpretation

Antibiotic classes were inferred based on inhibition.

Table 3.6: antibiotic classes identified across different Ph levels of the culture medium

ph. of culture medium	Antibiotics identified
Inhibition at pH 6	Penicillins or tetracyclines
Inhibition at pH 7.2	sulphanomides
Inhibition at pH 8	Aminoglycosides or macrolides

3.7 Ethical considerations

This study was carried out following ethical guidelines for research involving both human participants and animal products. All farm owners and managers received comprehensive information regarding the study's aims, procedures, and their rights. Before conducting questionnaires and collecting samples, written informed consent was obtained. Farm identities were anonymised through unique codes to ensure confidentiality. All collected data were securely stored and accessible only to the research team. Sample collection involved only egg gathering

and did not require handling live birds or invasive techniques. Standard biosecurity measures were observed during farm visits to reduce the risk of disease transmission. Participating farms will receive summary reports with findings and suggestions to enhance antibiotic management

CHAPTER 4

Results interpretation and analysis

4.1 Detection of Antibiotic Residues in Egg Samples

The inhibition zones observed across all positive samples ranged from 0 to 3 mm, with 11 out of 30 composite samples (36.7%) showing evidence of antibiotic residues. Residues were detected in eggs from both small-scale and commercial farms, indicating that neither production system is free from contamination. Specifically, small-scale farms contributed 6 positive samples out of 15 (40%), while commercial farms yielded 5 positive samples out of 15 (33.3%). Some samples exhibited inhibition at multiple pH levels (e.g., pH 6 and pH 7.2, or pH 6 and pH 8), suggesting the presence of more than one antibiotic class in a single egg sample. A chi-square test of independence was performed to assess whether farm type was associated with the occurrence of antibiotic residues. The calculated chi-square value was 1.142, which is less than the critical value of 9.48 at 4 degrees of freedom ($p > 0.05$). Therefore, the null hypothesis was accepted, concluding that there is no statistically significant association between farm type (small-scale vs. commercial) and the accumulation of antibiotic residues in poultry eggs. This implies that both farming systems contribute similarly to the risk of residue contamination in Harare.

Table 4.1: Inhibition Zone Diameters and Inferred Antibiotic Class

Farm Type	Sample ID	pH 6 (mm)	pH 7.2 (mm)	pH 8 (mm)	Inferred Antibiotic Class
Small-scale Farm A	1	0	3	0	Sulphonamides
	2	0	0	0	None
	3	0	2	2	Sulphonamides, Aminoglycosides

Farm Type	Sample ID	pH 6 (mm)	pH 7.2 (mm)	pH 8 (mm)	Inferred Antibiotic Class
	4	0	0	0	None
	5	0	0	0	None
Small-scale Farm B	1	3	0	0	Penicillins, Tetracyclines
	2	0	0	0	None
	3	0	0	0	None
	4	0	2	0	Sulphonamides
	5	2	2	0	Penicillins, Tetracyclines
Small-scale Farm C	1	2	0	0	Penicillins, Tetracyclines
	2	0	0	0	None
	3	2	0	2	Aminoglycosides
	4	0	0	0	None
	5	0	0	0	None
Commercial Farm A	1	0	0	0	None
	2	3	0	0	Penicillins, Tetracyclines
	3	2	0	0	Sulphonamides
	4	0	0	0	None
	5	0	2	0	Sulphonamides
Commercial Farm B	1	0	0	0	None
	2	0	0	0	None
	3	0	0	0	None
	4	1	0	1	Penicillins, Tetracyclines, Aminoglycosides

Farm Type	Sample ID	pH 6 (mm)	pH 7.2 (mm)	pH 8 (mm)	Inferred Antibiotic Class
y	5	0	0	0	None
Commercial Farm C	1	0	0	0	None
	2	3	0	0	Penicillins, Tetracyclines
	3	0	0	0	None
	4	0	2	0	Sulphonamides
	5	0	0	0	None

4.2 Prevalence of Antibiotic Classes

Table 4.2: Prevalence of Antibiotic Residues by Class

Antibiotic Class	Positive (n)	Negative (n)	Total (n)	p-value
Penicillins & Tetracyclines	8	22	30	0.27
Sulphonamides	6	24	30	0.20
Aminoglycosides	2	28	30	0.06

Figure 4.2 Prevalence of inferred Antibiotic residues classes in poultry eggs during the study

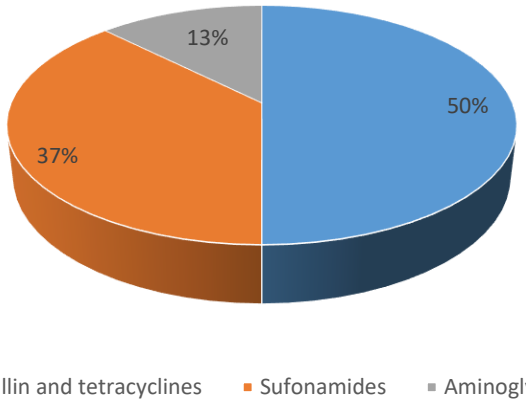


Table 4.3 ANOVA Results for Antibiotic Residues

Penicillins and tetracyclines were the most common antibiotic classes detected, found in 8 out of 30 samples (26.7%), while aminoglycosides were the least detected, present in only 2 samples (6.7%). Sulphonamides appeared in 6 samples (20%), showing an intermediate prevalence. ANOVA analysis assessed differences in residue presence across farms for each antibiotic class. The p-values for penicillins and tetracyclines were 0.599; for sulphonamides, 0.409. None of these values indicated significant differences at the $p < 0.05$ threshold, suggesting uniform occurrence among farms. However, the p-value for aminoglycosides was 0.0474, approaching significance, implying a possible farm-related variation that needs further study with a larger sample to identify specific patterns in antibiotic use.

(a) Sulphonamides

Source	df	Sum Sq	Mean Sq	F value	p-value
Between	1	0.701	0.701	0.85	0.409
Residual	4	3.299	0.825		

(b) Penicillins & Tetracyclines

Source	df	Sum Sq	Mean Sq	F value	p-value
Between	1	0.300	0.300	0.324	0.599
Residual	4	3.700	0.925		

(c) Aminoglycosides

Source	df	Sum Sq	Mean Sq	F value	p-value
Between	1	2.667	2.667	8.00	0.047
Residual	4	1.333	0.333		

4.4 Summary of all results

Overall, 36.7% (11 out of 30) of the analysed composite egg samples contained antibiotic residues, indicating considerable contamination in eggs from both small-scale and commercial farms in Harare. Statistical tests showed no significant link between farm type (small-scale versus commercial) and residue presence ($\chi^2 = 1.142$, $p > 0.05$), implying that both production systems pose similar risks of contamination. The most common antibiotic classes detected were penicillins and tetracyclines, representing 8 out of 30 positive samples (26.7%), followed by sulphonamides (20%) and aminoglycosides (6.7%). Notably, aminoglycoside presence varied significantly between farms ($p = 0.047$), suggesting farm-specific factors influence their occurrence. Multiple residues were also identified in several samples, where inhibition at two different pH levels indicated contamination with two antibiotic classes in the same egg. This raises concerns about possible additive or synergistic health effects for consumers and the development of antimicrobial resistance.

CHAPTER 5

DISCUSSION

5.1 Prevalence of Antibiotic Residues in Poultry Eggs

The study found that 36.7%of the egg samples contained antibiotic residues. This indicates a relatively high level of contamination and suggests that consumers may be exposed to antibiotic residues through egg consumption. These findings are consistent with studies conducted in other developing countries, where limited regulation and monitoring contribute to higher residue prevalence. Owusu-Doubreh et al. (2023) reported similar prevalence rates in Ghana, while Cornejo et al. (2020) found that all egg samples tested in their Chilean study were positive for at least one antimicrobial residue.

The detection rate of 36.7% is concerning from a public health perspective, as it suggests that a significant proportion of eggs reaching consumers in Harare may contain antibiotic residues. This is particularly problematic given that eggs are a widely consumed animal protein source in Zimbabwe, often eaten by vulnerable populations, including children, pregnant women, and the elderly (Islam et al., 2020).

The prevalence observed in this study is higher than that reported in some developed countries where stricter regulations and monitoring systems are in place. Saluti et al. (2021) found lower residue prevalence in Italian egg samples, highlighting the disparity between regions with robust regulatory frameworks and those with limited enforcement capacity. This comparison underscores the importance of strengthening monitoring systems in developing countries like Zimbabwe.

5.2 Comparison Between Farming Systems

Among the 30 composite samples analysed, small-scale farms accounted for 6 positive samples out of 15 (40%), while large-scale farms had 5 positive samples out of 15(33.3%). Although the chi-square test ($\chi^2 = 1.14$, $p > 0.05$) indicated no statistically significant association between farm

type and residue occurrence, the higher proportion of positives in large-scale farms deserves consideration.

This finding contrasts with the common assumption that small-scale farmers, due to a lack of veterinary oversight and unregulated antibiotic use, would have higher residue levels. Several factors may explain this observation:

First, large-scale commercial operations often practice mass medication through feed and water, administering antibiotics to entire flocks for prophylactic purposes (Erofeeva et al., 2021). This practice increases the likelihood of residues accumulating in eggs, particularly when withdrawal periods are not strictly observed.

Second, the study by Mohammadzadeh et al. (2022) noted that intensive production systems with high stocking densities face greater disease pressure, potentially leading to more frequent antibiotic use. The commercial farms in this study may have been treating flocks more regularly than small-scale farmers, who often wait until birds show clinical signs before intervening.

Third, small-scale farmers in this study may have been less likely to use certain antibiotics that persist longer in eggs, or their scavenging feeding systems might result in lower and more variable drug intake compared to the consistent medicated feed and water provided in commercial systems (Cornejo et al., 2020).

The lack of statistical association between farm type and residue occurrence ($p > 0.05$) suggests that both production systems contribute similarly to the risk of antibiotic residues in eggs. This finding has important implications for regulatory approaches, indicating that monitoring efforts should target both sectors rather than focusing exclusively on one.

The small sample size (n=30 composite samples) may have limited the statistical power to detect true differences between groups. A larger study with more farms and samples might reveal significant associations that this study could not detect. This limitation should be considered when interpreting the results and designing future research.

5.3 Distribution of Antibiotic Classes

5.3.1 Penicillins and Tetracyclines

Penicillins and tetracyclines were the most frequently detected antibiotic classes, appearing in 8 out of 30 samples (26.7%). This finding is consistent with the literature, as these are among the most commonly used antibiotics in poultry production globally (Owusu-Doubreh et al., 2023; Mohammadzadeh et al., 2022).

In Zimbabwe, these antibiotics are readily available over-the-counter in many agricultural supply stores, making them accessible to farmers without a veterinary prescription. The questionnaires administered during egg collection confirmed that tetracycline-containing products were frequently used by both small-scale and large-scale farmers for disease treatment and prevention.

The detection of penicillins in eggs is particularly concerning from a public health perspective. β-lactam antibiotics are known to cause hypersensitivity reactions in sensitive individuals, ranging from mild skin rashes to life-threatening anaphylaxis (Arsène et al., 2022). Chronic exposure to low levels of these antibiotics through food consumption may also contribute to the development of antimicrobial resistance.

5.3.2 Sulphonamides

Sulphonamides were detected in 6 out of 30 samples (20%), making them the second most common residue class. Inhibition at pH 7.2, which is characteristic of sulphonamides, was observed in samples from both farm types. This finding aligns with studies by Yang et al. (2020) in China, where sulphonamides were among the most frequently detected residues in eggs.

Sulphonamides are widely used in poultry production for treating coccidiosis and bacterial infections. Their relatively long half-life and tendency to accumulate in eggs make them a particular concern for residue monitoring programs (Kan, 2000). The detection of sulphonamides in this study suggests that farmers may not be adhering to recommended withdrawal periods before egg collection and sale.

5.3.3 Aminoglycosides

Aminoglycosides were detected in only 2 out of 30 samples (6.7%), representing the least frequently observed residue class. However, the statistical analysis revealed a significant

difference in aminoglycoside residues between farms ($p = 0.047$), suggesting that their occurrence is not uniform across production systems.

This finding may reflect variations in antibiotic use practices, with some farms using aminoglycosides more frequently than others. Aminoglycosides such as gentamicin and neomycin are sometimes used in poultry for treating gram-negative bacterial infections, but their use may be less common than tetracyclines or sulphonamides due to cost or availability factors.

The low detection rate for aminoglycosides may also relate to their pharmacokinetic properties. Some aminoglycosides do not partition readily into eggs or may degrade more quickly during egg formation and storage (Kan, 2000). This could result in lower residue levels that fall below the detection limit of the microbial inhibition assay used in this study.

5.3.4 Mixed Residues

Several samples showed inhibition at multiple pH levels, indicating the presence of more than one antibiotic class. Sample S3-5 from a small-scale farm, for example, showed inhibition at both pH 6 and pH 7.2, suggesting co-contamination with penicillins/tetracyclines and sulphonamides. Similarly, sample S4-4 showed inhibition at pH 6 and pH 8, indicating possible contamination with penicillins/tetracyclines and aminoglycosides.

The presence of multiple residues in single samples is particularly concerning, as it may lead to additive or synergistic effects on human health and antimicrobial resistance development. Combined exposure to different antibiotic classes could potentially increase the selective pressure on bacteria, accelerating the emergence of multidrug-resistant strains (Ahmed et al., 2020).

5.4 Testing and farm practices

5.4.1 Methodological Considerations

The four-plate test (FPT) used in this study is a microbiological inhibition assay that relies on the growth suppression of *Escherichia coli* at different pH levels to infer the presence of specific antibiotic classes. The test is widely used for screening purposes due to its simplicity, low cost, and ability to detect multiple antibiotic classes simultaneously (Okerman et al., 2007; Koenen-Dierick et al., 1995).

However, the FPT has limitations that should be acknowledged when interpreting results. The test provides qualitative or semi-quantitative data rather than precise concentrations of antibiotics. It

cannot distinguish between different antibiotics within the same class, and may have varying sensitivity for different compounds. Some antibiotics may produce false negatives if present at concentrations below the detection threshold, while others may produce false positives due to non-specific inhibition.

The use of a single bacterial strain (E. *coli*) in this study, rather than the multiple strains sometimes employed in FPT protocols, may have affected the range of antibiotics detectable. Some protocols use *Bacillus subtilis* or *Micrococcus luteus* in addition to *E. coli* to broaden detection capabilities.

5.4.2 Antibiotic Use Practices

The questionnaires administered during egg collection provided valuable context for understanding residue occurrence. Farmers in both sectors reported using antibiotics for therapeutic, prophylactic, and in some cases, growth promotion purposes. This finding aligns with global concerns about antibiotic misuse in livestock production (Landers et al., 2012; Kumar et al., 2020).

Several large-scale farmers reported adding antibiotics to feed or water regularly as a disease prevention strategy, particularly during periods of perceived disease risk. This practice, known as metaphylaxis, increases the likelihood of residue accumulation in eggs, especially when withdrawal periods are not observed before egg collection (Erofeeva et al., 2021).

Small-scale farmers, while using antibiotics less consistently, often relied on informal sources for drug procurement, including agricultural supply stores and open markets where veterinary oversight is minimal. This may result in inappropriate drug selection, incorrect dosing, and a lack of awareness about withdrawal periods.

5.5 Withdrawal Period Compliance

Non-compliance with withdrawal periods emerged as a potential factor contributing to residue detection. Several farmers, particularly in the small-scale sector, demonstrated limited awareness of withdrawal requirements for eggs. Some reported selling eggs from treated flocks immediately after medication to avoid economic losses, a practice documented in other developing country contexts (Arsène et al., 2022).

In large-scale operations, while farmers were generally aware of withdrawal periods, economic pressures to maintain egg supply may have led to premature collection from treated flocks. The

high stocking densities and continuous production cycles in commercial systems make it logistically challenging to segregate eggs from treated birds for the required withdrawal duration.

Biosecurity and Disease Management

Farms with better biosecurity practices appeared to have lower reliance on antibiotics, though this relationship was not systematically quantified in the current study. Farms that implemented strict cleaning and disinfection protocols, controlled visitor access, and sourced replacement stock from reliable suppliers reported less frequent disease outbreaks and consequently lower antibiotic use.

This observation supports the One Health approach advocated by WHO and FAO, which emphasises disease prevention through improved management practices as a strategy to reduce antibiotic dependence (Allen et al., 2014). Investing in biosecurity infrastructure and training for farmers could yield dual benefits of improved animal health and reduced residue risks.

5.6 Broader impacts and regulations

5.6.1 Antimicrobial Resistance

The detection of antibiotic residues in eggs destined for human consumption has direct implications for antimicrobial resistance (AMR). Chronic exposure to sub-therapeutic levels of antibiotics through food can select for resistant bacteria in the human gut microbiome, reducing the effectiveness of these drugs for treating infections (Ahmed et al., 2020).

Of particular concern is the detection of multiple antibiotic classes in some samples, as this may promote the development of multidrug resistance. The World Health Organisation has identified AMR as one of the top ten global public health threats, with foodborne transmission of resistant bacteria representing a significant pathway for resistance spread (Amann et al., 2019).

The residues detected in this study include antibiotics classified by WHO as critically important for human medicine, including Penicillins and Sulphonamides. The presence of these drugs in food undermines their therapeutic value and may contribute to treatment failures in clinical settings.

5.6.2 Toxicological Effects

Beyond AMR, antibiotic residues in food pose direct toxicological risks to consumers. Penicillins and sulphonamides are known to cause hypersensitivity reactions in sensitised individuals, with symptoms ranging from urticaria to life-threatening anaphylaxis (Arsène et al., 2022). While such reactions are relatively rare, they can be severe and require immediate medical attention.

Chronic exposure to antibiotic residues has been associated with other adverse effects, including disruption of the intestinal microbiome, which plays crucial roles in digestion, vitamin synthesis, and immune function (Kirchelle et al., 2018). Long-term consumption of residues may also contribute to organ toxicity, with some antibiotics having documented nephrotoxic or hepatotoxic potential at high doses.

5.7 International Trade Implications

The presence of antibiotic residues in eggs has implications beyond domestic public health, potentially affecting international trade. Countries importing food products require compliance with their own residue standards, which may be more stringent than those in Zimbabwe. Detection of residues in export consignments can lead to rejection of shipments, economic losses, and damage to trade relationships (Pihlström et al., 2021). Strengthening residue monitoring programs would not only protect domestic consumers but also enhance access to international markets, supporting the growth of Zimbabwe's poultry sector.

5.8 Study Limitations

Sample size and geographic scope

The study included only six farms in the Harare district, limiting the generalizability of findings to other regions of Zimbabwe. A larger, more representative sample would provide more robust estimates of residue prevalence.

Screening method constraints

The four-plate test provides qualitative detection and class identification but does not quantify residue concentrations or confirm specific compounds. Confirmatory analysis using LC-MS/MS would strengthen the validity of findings and enable comparison with MRLs.

Temporal factors

Sampling was conducted at a single time point, providing a snapshot of residue occurrence rather than information about seasonal or temporal variations. Longitudinal studies would better characterise residue patterns over time.

Questionnaire limitations

Self-reported data on antibiotic use may be subject to recall bias and social desirability bias, potentially underestimating actual antibiotic use practices.

Lack of confirmatory testing

Without confirmatory analysis, the inferred antibiotic classes should be considered presumptive rather than definitive identifications.

5.9 Recommendations and conclusion

1. Conduct larger-scale surveys: Undertake comprehensive residue surveys across multiple districts and farming systems to establish baseline prevalence data for Zimbabwe.

2. Perform confirmatory analysis: Utilise LC-MS/MS methods to confirm residue identities, quantify concentrations, and assess compliance with MRLs.

3. Investigate resistance patterns: Study the relationship between antibiotic residues in food and the prevalence of resistant bacteria in human and animal populations.

4. Evaluate intervention effectiveness: Assess the impact of regulatory interventions, training programs, and alternative disease management strategies on residue occurrence.

5. Explore consumer awareness: Investigate consumer knowledge about antibiotic residues and their preferences regarding food safety, to inform risk communication strategies.

6. Conduct risk assessment: Perform quantitative risk assessments to estimate the public health burden associated with antibiotic residues in eggs and other foods.

Conclusion

This study provides the first comparative assessment of antibiotic residues in eggs from small-scale and large-scale poultry farms in Harare district, Zimbabwe. The detection of residues in 36.7% of samples indicates a substantial food safety concern, with penicillins, tetracyclines, and sulphonamides being the most frequently identified antibiotic classes.

While no statistically significant association was found between farm type and residue occurrence ($p > 0.05$), the higher proportion of positives in small-scale farms (40%) compared to large-scale farms (33.3%) challenges assumptions about which sector poses greater risk. Both production

systems contribute to the residue problem, highlighting the need for comprehensive interventions targeting all farm categories.

The detection of multiple residues in some samples and the significant variation in aminoglycoside occurrence between farms ($p = 0.047$) underscore the complexity of residue dynamics and the importance of tailored approaches to risk mitigation.

From a public health perspective, the findings raise concerns about antimicrobial resistance development, direct toxicological effects on consumers, and potential trade implications. The study supports the One Health approach to addressing antibiotic resistance, recognizing the interconnections between human health, animal production, and environmental contamination.

Addressing the challenge of antibiotic residues requires coordinated action from regulatory authorities, farmers, veterinary professionals, and researchers. Strengthening monitoring systems, enforcing withdrawal periods, promoting biosecurity, and raising awareness among producers and consumers are essential components of a comprehensive strategy to ensure the safety of eggs and protect public health in Zimbabwe.

The study contributes valuable baseline data to inform policy development and risk management decisions, while highlighting the need for continued research and surveillance in this critical area of food safety and antimicrobial stewardship.

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CODEX Maximum Residual Limits (MRLs) and Risk Management Recommendations (RMRs)

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Appendices

Analysis of the Association between Farm level type and the accumulation of antibiotic residues in poultry eggs.

Table 4.1.1 Contingency Table for the association between Farm level type and the accumulated antibiotics in eggs.

Farm type	Total number of positives pulled samples (Observed)	Expected Values pos	Total number of negative samples (Observed)	Expected negative	Totals
Small farm	6	3.67	9	6.3	15
Commercial	5	3.67	10	6.3	15
Totals	11		19		30

Test of independence

H0: There is no association between farm level and accumulation of antibiotic residues in poultry eggs

H1: There is an association between the farm level and accumulation of antibiotic residues in poultry eggs.

@ level df =0.05

X^2 -Chi square calculations = $(Observed-Expected)^2/Expected$

$= 2(3-3.67)^2/3.67 + (5-3.67)^2/3.67+2(7-6.3)^2/6.3 +(5-6.3)^2/6.3$

$=0.245+0.481+0.156+0.26$

= 1.142 compared against df (n-1)(r-1)= 1 at 5% which is 3.841

Conclusion: Accept Ho and conclude that there is no association between antibiotic residues and the farm level

4.2 Analysis of the antibiotic residues between and among farms using R package

A. Df Sum Sq Mean Sq F value Pr(>F)

sulfo 1 0.701 0.7013 0.85 0.409

Residuals 4 3.299 0.8247

Interpretation P>0.05 No significant difference across farms for residues for farms

B. Df Sum Sq Mean Sq F value Pr(>F)

pen 1 0.3 0.300 0.324 0.599

Residuals 4 3.7 0.925

Interpretation P>0.05 No significant difference across farms for penicillin residues and tetra cyclines

C Df Sum Sq Mean Sq F value Pr(>F)

amino 1 2.667 2.6667 8 0.0474 *

Residuals 4 1.333 0.3333

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Interpretations : P<0.05, there was a significant difference in the antibiotic residues related to antibiotic use for aminoglycosides for farms