# ASSESSMENT OF ANTIBIOTIC USE IN DAIRY FARMING AND TETRACYCLINE AND SULPHONAMIDE RESIDUES IN FRESH MILK IN COAST REGION IN EASTERN TANZANIA

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**MSc (Epidemiology and Laboratory Management) Dissertation**
**Muhimbili University of Health and Allied Sciences**
**October, 2024**

**Muhimbili University of Health and Allied Sciences**

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# ASSESSMENT OF ANTIBIOTIC USE IN DAIRY FARMING AND TETRACYCLINE AND SULPHONAMIDE RESIDUES IN FRESH MILK IN COAST REGION IN EASTERN TANZANIA

**By**

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**A Dissertation Submitted in (Partial) Fulfilment of the Requirements for the Degree of Master of Science (Epidemiology and Laboratory Management) of**

**Muhimbili University of Health and Allied Sciences**
**October, 2024**

## CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled; **‘‘Assessment of Antibiotic Use in Dairy Farming and Tetracycline and Sulphonamide Residues in Fresh Milk in Coast Region in Eastern** Tanzania**’’,** in (partial) fulfillment of the requirements for the degree of Master of Science (Epidemiology and Laboratory Management) of Muhimbili University of Health and Allied Sciences.

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## DECLARATION AND COPYRIGHT

I, **Jasper Kimambo**, declare that this **dissertation** is my original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

**Signature**…… **Date**……...….....

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## ACKNOWLEDGMENT

Firstly, I would like to thank Almighty God for the strength and health within me the whole time of my studies. Secondly, I would like to sincerely thank Center for Disease Control and Prevention-Tanzania (CDC) through the Tanzania Field of Epidemiology and Laboratory Training Program (TFELTP) and the Ministry of Health (MoH) for their kind support in my studies including their financial support and provision of funds to purchase reagents for my dissertation.

Correspondingly, I would like to show sincere gratitude to all my supervisors Prof. Mecky Matee, Dr. Zuhura Kimera, Ms. Loveness Urio, and Dr. Elieshiupendo M. Niccodem for their support and valuable inputs in the design, data collection, analysis and writing of this dissertation.

Furthermore, I am grateful to the Kisarawe District Administrative and supportive staffs for their contributions during data collection. Finally, I appreciate the efforts done by all my colleagues at Muhimbili University of Health and Allied Sciences for the time we spent and shared learning experiences during the preparation of this dissertation.

## DEDICATION

This work is dedicated to all my family members; father and mother, wife and children, and brothers and sisters for their continuous love, support, and encouragement. Furthermore, I extend my sincere appreciation to my supervisor Prof. Mecky Matee of Muhimbili University of Health and Allied Sciences (MUHAS) Department of Microbiology and Immunology for his valuable contributions to this work.

## ABSTRACT

**Background:** The intensification in livestock production due to the increased demand for animal products has led to a significant rise in the use of antimicrobial agents, which threatens public health. Tetracycline and sulphonamides antibiotics are commonly used. The widespread and irrational usage of these antibiotics during livestock keeping is a significant factor to antimicrobial resistance (AMR). Little is known on farming practices and level of antibiotic residues in Tanzania.

**Methodology:** A cross-sectional study was conducted with 95 registered small-scale cattle farmers in Kisarawe district, Tanzania between January to March 2024 to assess their knowledge, attitudes, and practices (KAP) regarding the use of tetracyclines and sulphonamides in cattle farming. Data was collected through semi-structured questionnaires and milk samples were tested for antibiotic residues using Enzyme-Linked Immunosorbent Assay (ELISA). The KAP scores were assessed using modified Bloom's cut-off points, categorizing scores of 75% or above as indicative of adequate knowledge, positive attitude, and good practices, while scores below this threshold were deemed to reflect inadequate knowledge, negative attitude, and poor practices. Mann-Whitney statistical test and software like Stata and GraphPad Prism, explored the relationships between KAP scores and residue concentrations.

**Results:** Of the 95 dairy farmers enrolled, (62.1%) owned ≤ 5 cattle, 81.05% had inadequate knowledge of antibiotics, 34.74%% had negative attitudes toward antibiotics, 57.89% had poor practices and 63.16% did not observe a withdrawal period, 75.79% used antibiotics from unauthorised sources, 57.9% (55/95) had no training, only 38.9% (37/95) knew about antibiotic prophylaxis, and 24/95 (25.2%) were aware of AMR. 94.05% of milk samples contained sulphonamide residues, and 8.14% had tetracycline residues. 2.4% of milk samples had above the maximum residual limit (MRL) for tetracycline and 1.2% for sulphonamides. There was an association between farming practices and antibiotic residue for both tetracycline (*p-value* = 0.043) and sulphonamide (*p-value* = 0.023).

**Conclusions:** Dairy farmers in Kisarawe District have inadequate knowledge and attitudes, and exhibit poor practices on antibiotic use in cattle farming, necessitating awareness campaigns and training programs. The presence of antibiotic residues in milk calls for regular monitoring, enhanced extension services, and investigations into milk production practices to guide antimicrobial stewardship programs.

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## ABBREVIATIONS

| | |
|---|---|
| ADI | Allowable Daily Intake |
| AMR | Antimicrobial Resistance |
| AR | Antibiotic Resistance |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| FAO | Food and Agriculture Organization |
| LOD | Limit of Detection |
| MDR | Multidrug-Resistant |
| MoH | Ministry of Health |
| MRL | Maximum Residual Limit |
| MUHAS | Muhimbili University of Health and Allied Sciences |
| TFNC | Tanzania Food and Nutrition Centre |
| WHO | World Health Organization |

## DEFINITION OF TERMS

| TERM | DEFINITION |
|---|---|
| Allowable Daily Intake (ADI) | It is described as the highest level of a substance that can be taken every day for the rest of one's life without posing a significant risk to one's health. |
| Antimicrobial residues | Medicines or their active metabolites that remain in the tissues or by-products from treated animals, like meat, milk, and eggs |
| Antimicrobial Resistance | Ability of microorganisms to resist killing by antimicrobial agents |
| Maximum Residue Limit (MRL) | Is the highest level of residue in food goods that does not endanger human health |
| One Health Approach | A multisectoral, cross-disciplinary approach that emphasizes collaboration and acknowledges the linkages between humans, plants, animals, and their shared environment |
| Withdrawal Period | The safe interval between the final delivery of a veterinary medicine and the slaughter or manufacture of food from that animal |

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background

In dairy farming, antibiotics are used for the treatment, and prevention of infections as well as for growth promotion (1). The intensification in livestock production due to the increased demand for animal products has led to a significant rise in the use of antimicrobial agents (2). Antimicrobial use (AMU) in food animals has become an issue of global concern due to the presence of residues in food products, which threatens public health (3,4). In Tanzania, like any other low-middle-income country (LMICs), the use of antimicrobial agents in animal production is excessive (4). This is due to several reasons, including i) weak regulation and poor restriction (5), ii) lack of basic understanding of the concept of AMU (6), iii) mushrooming of the unregulated sales of pharmaceutical products (7), iv) lack of updated standard guidelines for the treatment of animal diseases (8) and v) the tendency for animal owners to stock drugs in their houses, as well as engaging unskilled people in treating animals (5). Other factors include lack of access to appropriate antimicrobial therapy, lack of continuing veterinary education on AMU for prescribers, and non-observance of withdrawal periods among livestock farmers (1,6,9). In Tanzania, the most commonly used antimicrobials in livestock production are tetracyclines, penicillin, aminoglycosides, macrolides, and sulphonamides (4,10,11).

A report estimated that between 2010 and 2017, 12,147,491 kg of antibiotics were used for veterinary purposes in Tanzania, with tetracyclines accounting for about 8,090,798 kg (66.6%) of antimicrobials consumed (10). The other agents included sulfonamides and trimethoprim, quinolones, aminoglycosides, beta-lactams, and antibacterial combinations (10).

Antibiotics in dairy farming led to the deposition of residues in meat and milk intended for human consumption (Kebede et al., 2014). Due to poor monitoring systems, developing countries are at a greater risk of exposure to residues in animal products than developed ones (12). To ensure consumer safety, antibiotic residues in animal food products are usually assessed using two international metrics: the maximum residue limit (MRL) and the allowable daily intake (ADI).

The Codex Committee on Pesticide Residues (CCPR) has established these metrics following suggestions from the Food and Agricultural Organization (FAO) and World Health Organization (WHO) Joint Meeting on Pesticide Residues (JMPR) (FAO, WHO 2013). For dairy milk, the MRLs for sulphonamide and tetracycline are set at 100 g/kg, while the ADI ranges for tetracycline and sulphonamide are set at 0–30 µg/kg and 0–50 µg/kg, respectively (13).

In Africa, Tanzania ranks second after Ethiopia in terms of cattle stockpiling. According to the 2019/20 National Sample Census of Agriculture, the country has an estimated 33.9 million cattle (14). Despite this large number of livestock and frequent use of antibiotics in dairy farming, studies that have monitored antibiotic residues in dairy milk are relatively few (4,15–19). Furthermore, most of the studies were conducted between 11 and 17 years ago (15–18). The recently published National Action Plan of AMR 2023-2028 strongly indicates the need for updated information (20).

This study was conducted in the eastern part of Tanzania. The region has a population of 673,991 cattle, comprising 633,942 indigenous, 32,508 dairies, and 7,541 beef breeds. Like in most parts of the country, dairy farming in the region is done at a small scale, and due to the limited use of vaccines and biosecurity measures, the use of antibiotics, especially tetracycline, and sulphonamides is widespread (10,11).

We aimed to determine the concentrations of tetracycline and sulphonamide residues in dairy milk and compare them with their corresponding MRL and ADI. We compared the presence of residues with the farmers' knowledge, attitudes, and practices (KAP) on antibiotic use and residues in dairy farming.

## 1.2 Problem statement

The increasing use of veterinary drugs, particularly antibiotics, in dairy cattle farming raises significant public health concerns, especially regarding the residues of these medications found in animal-derived products such as milk, meat, and eggs (21–23). Among the antibiotics frequently administered are tetracycline and sulphonamide, which play a crucial role in the treatment of both animal and human diseases (10). However, the irrational use of these antibiotics can lead to concentrations of antimicrobial residues exceeding the MRL established for animal products (24). This scenario not only poses a risk for food safety but also contributes to the growing issue of AMR (25), which the WHO identifies as one of the top ten global public health threats, resulting in approximately 1.27 million deaths annually (26).

In Tanzania, which is home to 32.2 million cattle, understanding the implications of antibiotic use in small-scale dairy farming is particularly critical (27). Regions like Kisarawe, with over 87,827 dairy cattle and an annual production of approximately 2,809,413.4 liters of fresh milk, present a relevant context for exploring this issue (28). Previous studies have highlighted concerning levels of antimicrobial residues in milk among small-scale farmers (29,30), particularly in rural parts of Tanzania (18,19,30). For example, over 80% of small-scale milk producers in Tanzania reported using antibiotics for medical purposes (31), while in 2012 in the same country, 21% of milk samples collected in coastal regions, including Kisarawe, tested positive for antibiotic residues (32).

Despite this increasing antibiotic use and its potential implications, there remains a significant gap in understanding the specific farming practices, the degree of knowledge, attitudes, and practices (KAP) of small-scale dairy farmers, and the actual levels of tetracycline and sulphonamide residues in fresh milk within Tanzania including Kisarawe district. Therefore, this study seeks to investigate the KAP of small-scale dairy farmers regarding veterinary drug use, alongside assessing the prevalence and concentration of tetracycline and sulphonamide residues in their milk. By addressing these knowledge gaps, the study aims to inform interventions and policy recommendations that could mitigate the risks associated with antibiotic use in livestock, ensure food safety, and contribute to the reduction of AMR in both animals and humans.

### 1.3 Conceptual framework

The two most commonly used antibiotics in livestock farming are tetracycline and sulphonamide. If these antibiotics are used inappropriately or if the correct drug withdrawal period is not followed, residues of these antibiotics may be found in animal products such as milk. Inaccurate use of these antibiotics can lead to high levels of residues exceeding the allowed limit, which can have public health implications such as AMR. The levels of these antibiotics in animal products can be detected in a laboratory using various methods, including ELISA (**Figure 1**).

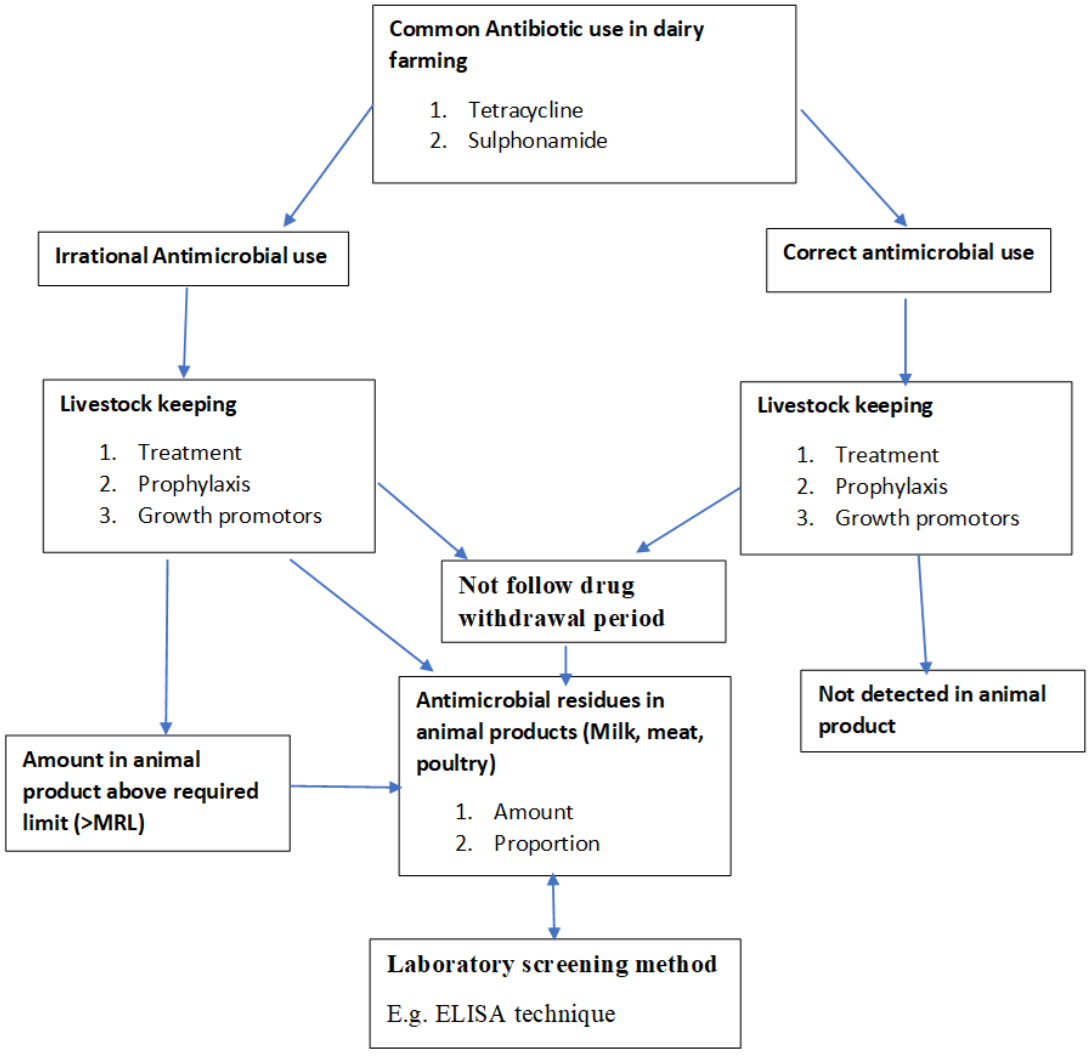



**Figure 1: Conceptual framework showing the effect of AMU in animal husbandry**

## 1.4 Rationale of the Study

The information provided on the percentage of milk in Kisarawe district that contained antibiotic residues and whether it exceeded recommended levels. The study will encourage decision-makers to monitor antibiotic use in animals to protect public health and suggest interventions to raise awareness among customers, farms, veterinary officers, and policymakers. Furthermore, it will shed light on information to ensure consumers whether milk is good or not therefore facilitating business and suggesting future training.

The findings of this study will provide evidence on the scale and pattern of antibiotic use and residues in small-scale dairy farming in Tanzania, raising awareness among stakeholders (farmers, consumers and health authorities) necessary for driving the meaningful changes in the practices of farmers. The study aligns with the second national action plan against antimicrobial resistance (AMR) for 2023-2028, which underscores a need to use antimicrobial agents prudently, with consideration of AMR and AMU along the food chains.

## 1.5 Research questions

### General research question

What are the dairy practices regarding antibiotic use and amounts of tetracycline and sulfonamide residues in dairy milk from small-scale farmers in Coast region in Tanzania?

### Specific research questions

1. What is the knowledge, attitudes, and practices of small-scale dairy farmers regarding antibiotic use in farming?
2. To what proportion of dairy milk from small-scale farmers containing tetracycline and sulfonamide residues?
3. Do the amounts of tetracycline and sulfonamide residues exceed the MRL and ADI and therefore pose a public health risk?
4. Is there a relationship between the farming practices of dairy farmers and the concentrations of tetracycline and sulphonamide residues found in milk?

## 1.6 Objectives

### 1.6.1 Broad objective

To access knowledge, attitudes and practices of dairy farmers on antibiotic use and concentration of tetracycline and sulfonamide residues in dairy milk in the Kisarawe district of Tanzania.

### 1.6.2 Specific objectives

1. To determine knowledge, attitudes, and practices of small-scale dairy farmers regarding antibiotic usage in Kisarawe district, Pwani region.

2. To determine the proportion of diary milk containing tetracycline and sulfonamide antibiotic residues in Kisarawe district, Pwani region.

3. To determine the concentrations of tetracycline and sulfonamide residues in dairy milk in the Kisarawe District, Pwani region.

4. To determine the association between antibiotic residues concentration in milk and knowledge, attitude and practices of small-scale dairy farmers regarding antibiotic in Kisarawe District.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Overview of antimicrobial resistance

AMR poses a worldwide danger that impacts both the financial well-being and overall health of people (33). The FAO has implemented a health strategy to oversee the improper use of antibiotics and combat the inappropriate use of human medications for animal treatment (34). The Global AMR and Use Surveillance System (GLASS) and Europe both implemented a similar approach to combat AMR by monitoring the use of antimicrobial drugs (35). Europe started monitoring in 2011 before the Global Antimicrobial Plan (GAP) was initiated (33). In contrast, the United States saw a decrease of 38% in 2022 in the sales of antimicrobial drugs for food-producing animals due to effective monitoring (36). Food contamination during processing can lead to the transfer of resistance genes and contribute to AMR in humans (37).

The use of antimicrobial agents in LMICs is not well regulated, leading to an increase in AMR (38). In Africa, there is a high prevalence of antimicrobial overuse (78-100%) in animal husbandry, which contributes to AMR (9). A study in Kenya found that milk from both large-scale (18.0%) and small-scale farms (34.3%) had multidrug-resistant *Staphylococcus aureus* isolates (39).

The Tanzania National Action Plan for AMR (NAP-AMR) 2023 to 2028 aims to combat AMR in community health through animal sources and food chains (20). The excessive use of antibiotics by dairy farmers for therapeutic purposes (84.3%) and the lack of knowledge on withdraw period (34%) during animal care are contributing factors to AMR (4). A study on the resistance patterns of *E. coli* in dairy cattle found that tetracycline was a commonly resistant (50.4%) antibiotic (40). Tanzania's irrational use of antibiotics for livestock purposes has significantly contributed to the emergence and spread of AMR (11). There has been a rise in antibiotic-resistant bacteria, specifically Methicillin-resistant Staphylococcus aureus (MRSA) and Extended Spectrum Beta-Lactamase (ESBL), in the livestock industry in the country, and these bacteria are resistant to several types of antibiotics, including tetracycline (11). Therefore, interventions are necessary in animal food products to combat AMR.

## 2.2 Dairy farming knowledge, attitudes and practices in relation to the presence of antibiotic residues animal food products

In developing countries, a lack of information about antibiotic usage, AMR, and monitoring systems for drug residues leads to a widespread ignorance about the ongoing effects of antibiotics in the community (41). Controlling the use of antibiotics in various sectors, including animal farming, is necessary to prevent the rapid spread of antibiotic resistance (AR), which has become a significant public health concern over time (42).

A study in India found that only a limited number of farmers are aware of the "withdrawal period" associated with antibiotic use in livestock, indicating a gap in knowledge that may lead to the emergence of antibiotic-resistant bacteria (43). Another study conducted in Northcentral Nigeria found that 57% of cattle handlers are not aware of antibiotic residues, but also knowledge and practice of antibiotic use were poor (44)**.** Data analysis from Kenya reveals that as much as 33.3% of antibiotics were improperly used, and approximately 10.5% of milk contained tetracycline residues exceeding the MRL (45). The same country reported that, majority of these antibiotic residues are from the farm in rural settings (46).

Tanzania indicated that, livestock production, particularly dairy farming is crucial for enhancing the health and well-being of rural communities as it provides essential nutrients and offers a pathway out of poverty for numerous people (47). However, there is an increased usage of antimicrobials for prophylaxis, therapeutics and for growth promotion in livestock, aquaculture and crop production with reference to study conducted in the country(48). A study conducted in the same country shows that, milk was prepared, stored and handled poorly which is potential cause of presence of antibiotic residues in milk (49). Additionally, poor farming practices may lead into presence of residues in animal products, with higher levels of tetracycline and sulphonamide residues observed in milk collected from farmers than milk vendors (50).

Improving farming practices will increase policymakers' awareness of milk safety, potentially resulting in interventions like residue testing at milk collection centers and examining the production methods of smallholder farmers and market agents (15).

### 2.3 The proportion of dairy milk with sulfonamide or tetracycline residues

The prevalence of tetracyclines and sulphonamides residues varies globally, with occurrences ranging from 0% to 100% (6). Research indicates that a significant percentage of dairy milk contains trace amounts of tetracycline and sulfonamide, detected in 14.01% and 12.64% of samples, respectively (5). In China, report found that, up to 20.2% of milk samples tested positive for both tetracycline and sulfonamide residues (51), while in Iran, the situation is even more concerning, as 26% of raw milk samples contain these antibiotic residues (52).

In Africa, a Ethiopia found that, almost all beef farms (97.67%) used tetracycline, a small percentage (9.3%) used sulfonamide drugs, and none (100%) followed the recommended drug withdrawal period (53). Another study published in 2021 showed that milk, eggs, and beef were the most commonly contaminated foods with antibiotic residues (54). A recent study in Algeria found that, a high percentage (90.32%) of milk from the Northern region was contaminated with tetracycline residues (7). Somalia reported that, the proportion of antibiotic residues in raw milk from farmers (24%) was higher than that in raw milk from sale marketplaces (18%), with regional differences (55).

A separate study revealed that 60% of milk samples from East Africa contained sulfonamide residues (46). Various studies conducted in Kenya have pointed to several contamination sources in milk (56). Additionally, despite farmers receiving training in proper milking practices, 15.3% of milk collected from them still exhibited antibiotic residues (57). Another investigation found that, 10.5% of tested milk samples tested positive for antibiotic residues, with 3.2% specifically identified as tetracycline (58). Both tetracyclines and sulfonamides are commonly used antibiotics in animal farming (46).

The most commonly used antibiotics in Tanzania are tetracycline and sulphonamides (10). Multiple studies conducted between 1999 and 2011 have found antibiotic residues in cow's milk, with percentages ranging from 4.5% to 83% (59–62). Another study found that approximately 16.9% of the milk collected in three regions of the same country had tetracycline residues (63). In the Northern part of Tanzania, up to 25% of milk samples had tetracycline and Sulphonamides residues (64). In the Bagamoyo district in the same country, a study done in 2013 reported a 10% prevalence of tetracycline residues in raw dairy milk

being sold (65). It is necessary to implement measures to control these residues in animal food products.

### 2.4 Concentrations of tetracycline and sulfonamide residues in milk

Globally, the levels of antibiotic residues in food from animals vary significantly. The FAO/WHO Codex Alimentarius Commission has established MRL for tetracycline and sulfonamide in milk, set at 100 µg/kg and 25 µg/kg, respectively (66). The ADI for these antibiotics ranges from 0-30 µg/kg body weight (bwt) for tetracycline and 0-50 µg/kg bwt for sulfonamide (66). However, while these limits are established, actual findings in various countries often present concerning levels of antibiotic residues.

In China, studies have indicated that tetracycline and sulfonamide residues in milk often exceed the established MRL (67). Similarly, Bangladesh has reported tetracycline contamination in milk (7%) at levels that surpass the MRL (68). In Iran, a study found that tetracycline residues in dairy milk were 2.5 times higher than the MRLs, highlighting the variability and prevalence of antibiotic residues in different regions (69).

A study on African countries further revealed that levels of antimicrobial residues in milk remain above the MRLs set by the European Nations Codex Alimentarius (70). In Nigeria, residues of oxytetracycline were detected in both raw and fermented milk consumed in Maiduguri, although these levels were within the acceptable limits established by the Codex Alimentarius, suggesting that concentrations vary significantly across nations and geographical areas (71). A significant proportion from farms exceeded regulatory limits, with the highest percentages being 46.2% (46). Further investigation revealed that 10.8% of tested samples exceeded limits set by the Codex Alimentarius Commission and the European Union (72). Alarmingly, 60% of the samples tested positive for sulphonamides, with 71% of those exceeding allowed limits (46).

In Tanzania, the situation is similarly troubling. Antimicrobial residues were detected in the majority of milk samples collected from the Wete District in Zanzibar, totaling 83% of samples (60). Furthermore, different food products exhibited varying levels of antimicrobial residues across the country. A study conducted in the Morogoro region found that 71.1% of tested cattle meat contained traces of oxytetracycline, with 68.3% of those exceeding MRL (73). Meanwhile, in the Kilosa district in the same region, over two-thirds of cattle meat had

tetracycline residues above acceptable levels (73). The Dodoma region in the same country, 35% of beef samples had oxytetracycline residue, though none exceeded the MRL (74). In the Bagamoyo district, milk samples also displayed concerning levels of oxytetracycline, with all residue-positive samples exceeding regulatory thresholds (75). This landscape of antibiotic residues in milk and meat emphasizes the need for stringent monitoring and regulatory frameworks, particularly in developing countries.

### 2.5 Methods used for estimation of antibiotic residues in dairy milk

Various laboratory techniques, such as High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Tanden Mass Spectrometry (LC-MS), Enzyme-Linked Immuno-Sorbent Assay (ELISA), and liquid chromatography (LC), are employed to measure the amounts and levels of residues in milk (76,77). HPLC is commonly used as a secondary method to validate the presence of residues in food products after initial microbiological screening (78).

The Netherlands chooses to use a microbial screening test to monitor and control antimicrobial residues in animal food products, even though it is a time-consuming and expensive technique (76). In Iran, ELISA and HPLC analysis was used to measure the level of residues in milk Products (69,79). China uses ELISA to assess contamination levels in milk samples from various locations (80). In Punjab, India, a study found that 13.5% of milk samples contained tetracycline residues, and ELISA was used for monitoring (81). Although LC–MS is very useful for identifying antibiotic residues in veterinary plants (82).

A study in Kenya suggests that there is a need for improved methods to screen for antibiotic residues in milk to monitor food safety and promote responsible use of antibiotics (83). The same country used LC-MC to investigate antibiotic residues in milk collected from markets (72). The researchers used a method called HPLC-UV to measure the levels of antibiotics in milk (46).

A study in Tanzania conducted in 1999 and 2000 used the Charm-AIM screening kit to investigate antimicrobial residues in milk from markets (15). The same kit was used in a study in the same country, which found that 36% of the milk sold had antimicrobial residues, with 11 occurrences per month (15). Another study in the northern part of the country used microbiological techniques to assess tetracycline and sulphonamides residues in dairy milk

(50). Despite other techniques used, ELISA is a highly sensitive and specific method for detecting residues in milk, particularly useful for screening in the field or research purposes, with a lower limit of detection and quantification compared to other methods (24,81,84). It is found that, ELISA is mostly used technique to screen veterinary drug residues in various animal food products (85).

# CHAPTER THREE

## 3.0 METHODOLOGY

### 3.1 Study design

A cross-sectional study was conducted in small-scale dairy farms in Kisarawe District in the Pwani region from January to March 2024.

### 3.2 Study setting

Kisarawe District, located in Tanzania's Pwani region, lies along the coast between latitudes 6° 50° South and 7° 35° South and longitudes 38° 15° East and 39° 30° East (Figure 2). The district has a population of approximately 159,226 people and covers an area of 5,031 square kilometers (86). Kisarawe is notable for being one of the leading districts in Tanzania for milk production at the household level, with most farmers relying on indigenous cows rather than improved breeds (28).

The district is the second leading among coastal districts with the largest number of dairy cattle (87,827) and primarily engaged in livestock husbandry (87). The choice of Kisarawe District in Tanzania for the determination of tetracycline and sulphonamide residues in fresh milk from small-scale dairy farms was justified by several factors. Firstly, Kisarawe District is known for its significant agricultural activities, including dairy farming, which made it a relevant location for studying antibiotic residues in milk. Secondly, the district's proximity to urban centers like Dar es Salaam may increase the demand for dairy products, potentially leading to greater antibiotic use in dairy farming practices. Finally, focusing on Kisarawe allows for a targeted assessment of antibiotic residues in a specific geographic area, providing valuable insights into the local dairy farming practices and their implications for food safety and public health.

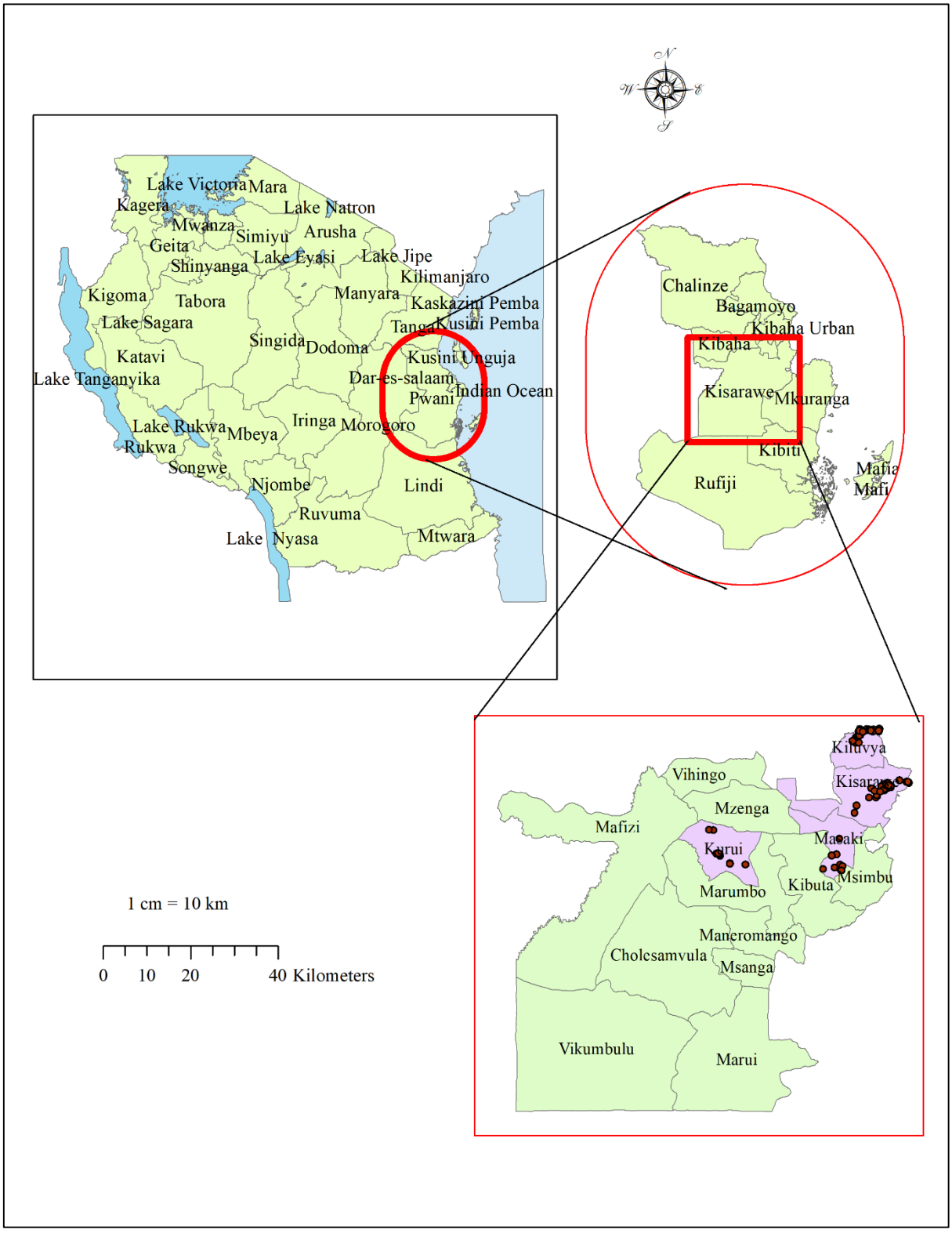



**Figure 2: A map displaying Kisarawe district wards where milk sample was collected**

### 3.4 Target population

The study recruited small holder cattle farmers from Kisarawe District, who are registered to provide dairy milk to designated milk centers. This study focused on a representative sample of small-scale dairy farmers in the Kisarawe district, considering a heterogeneous population with diverse gender and age groups.

### 3.5 Sampling Frame and Technique

The study's sample frame comprised all Kisarawe-based smallholder cow producers who were registered members of the farmers' union at the two operational dairy collecting sites. To find study participants, a random sampling technique was employed from the complete list of small-scale dairy farmers obtained from the district office. The sampling interval was calculated using a random number generator from the Microsoft Excel spreadsheet by considering the formula, k=n/N. The obtained population size (N) of farmers in Kisarawe was 283 and the desired sample size (n) was 70. I divided 283 by 70 to get an interval of approximately 4 which is equal to K. That means the interval from one farmer to another was 4. The researcher visited small-scale dairy farmers, assisted by district veterinary officer in locating and collecting milk samples and ensuring confidentiality and good communication skills.

### 3.6 Inclusion criteria

The study included small-scale dairy farmers from the Kisarawe District of Tanzania who were registered members of established farmers' unions and consented to participate in the research. Eligible participants were those who owned or managed dairy cattle and provided milk to designated milk collection centers at the time of sample collection.

### 3.7 Exclusion criteria

The study excluded farmers who were registered in farming union but had no fresh milk during data collection. Small-scale dairy farmers who were registered but during data collection they are not supplying milk to farming union were also excluded.

### 3.8 Study variables

In this study, the independent variables include the knowledge, attitudes, and practices (KAP) of small-scale dairy farmers regarding AMU, as well as socio-demographic factors such as age, sex, and education level. The dependent variables are the presence and concentrations of tetracycline and sulphonamide residues in dairy milk from small-scale dairy farms in Kisarawe District.

### 3.9 Sample size determination

Sample size calculation was based on the study conducted in Tanzania that showed the prevalence of antibiotic residues in milk supplied by small-scale dairy farms which were 4.5% (88). The formula and assumptions used to calculate the sample size are as follows: $n = Z^{2}P(1-P)^{2}/d^{2}$

Where;

Z=1.96, P=0.045, d=0.05, and n=sample size.

n= $(1.96)^{2}$*0.045(1-0.045)/ $(0.05)^{2}$

**n=63**

Using the formula for non-response rate (n/1-f), where, f= non-response rate of 10%= 0.1, we reached a minimum sample size of 70.

### 3.10 Data Collection Techniques and Procedures

#### 3.10.1 Determination of Knowledge, Attitudes, and Practices

A standard structured questionnaire was used to assess farmers' knowledge, attitudes, and practices on the use of antibiotics in dairy farming. Respondent's knowledge, attitudes, and practices on AMU and residues were assessed by directly scoring their answers to fifteen specific questions, with each correct answer earning 1 point and incorrect or unsure answers earning 0 points. The total score for each respondent was calculated by summing the scores of all fifteen questions. Each respondent could obtain a score of 0–13 for knowledge, 0–6 for attitude, and 0–16 for practice. For each component of the KAP component, the total

score was then converted to a percentage, with a score of 75% or higher classified as "adequate", and a score below 75% classified as "inadequate".

### 3.10.2 Collection and transportation of milk samples

Approximately 14 ml of milk samples were collected into 15 ml sterile Falcon tubes and kept in cool boxes at 4-8°C. The samples were then transported to the Microbiology and Immunology Laboratory of the Muhimbili University of Health and Allied Sciences (MUHAS) within four to six hours and stored at -20°C until processing.

### 3.10.3 The chemicals and reagents used in the procedure

For sulfonamide: 6x standard solution (1ml each) 0ppb, 1ppb, 3ppb, 9ppb, 27ppb and 81ppb, enzyme conjugate(7ml), antibody working solution (7ml), substrate A and B (7ml each), stop solution (7ml), 20x concentrated washing buffer (40ml) and 20x concentrated redissolving solution (50ml).

For tetracycline: 10x standard solution (1ml each) 0ppb, 2ppb, 6ppb, 18ppb and54ppb, enzyme conjugate (7ml), antibody working solution (7ml), substrate A and B (7ml each), stop solution (7ml), 20x concentrated washing buffer (30ml) and 20x concentrated redissolving solution (10ml).

### 3.10.4 Sample pre-treatment

The milk samples were pre-treated according to the instructions from the manufacturer (Shenzhen Lvshiyuan Biotechnology Company, Shenzhen, China).

For Sulfonamides, 20x concentrated redissolving solution was diluted at a ratio of 1:19 with deionized water (1 ml of 20x concentrated redissolving solution and 19 ml of deionized water). Then 20 µl of milk sample were mixed with 380 µl diluted redissolving solution by inverting to and from for 30 seconds, making a sample dilution factor of 20.

For Tetracyclines, 20x concentrated redissolving solution was diluted at a ratio of 1:19 with deionized water (1 ml 20x concentrated redissolving solution and 19 ml of deionized water). 50 µl of milk sample were added to 1950 µl of diluted redissolving solution to make a sample dilution factor of 40 and evenly vortexes strongly for 1 minute.

### 3.10.5 Extraction Procedures for Sulphonamides and Tetracycline

Milk samples were homogenized according to the instructions from the manufacturer (Shenzhen Lvshiyuan Biotechnology Company, Shenzhen, China) for each antibiotic kit.

For sulphonamides, 1 ml of milk was taken and combined with a diluted redissolved solution. This mixture was further diluted by adding 20 µl of the diluted redissolved solution to 380 µl of the mixture. After mixing for 30 seconds, 50 µl of the mixture was used for analysis using a sulfonamide ELISA kit to determine the amount of sulphonamide residue present.

For the tetracycline residue test, the milk sample was initially left at room temperature for 30 minutes. Then 50 ul milk sample was pipetted into 1950 ul diluted redissolving solution; and oscillated fully for 1 minute evenly. Finally, 50 µl of the mixture was tested immediately using a tetracycline ELISA quantitative kit to determine its concentration.

**3.10.6 Competitive Enzyme-linked Immunosorbent Assay (ELISA)**

The levels of tetracycline (TCs) and sulphonamide (SAMs) in milk samples were analyzed using an ELISA kit according to the instructions of the manufacturer (Shenzhen Lvshiyuan Biotechnology Company, Shenzhen, China) (89). The sensitivity, detection limit, and recovery rate for each kit were as follows: Tetracycline (TC) (sensitivity of 0.2 ppb, detection limit of 10 ppb, recovery rate of 90 ± 30%, linear range of 0 ppb to 54 ppb, and stability at 25 °C); Sulfonamides (SAs) (sensitivity of 1 ppb, detection limit of 20 ppb, recovery rate of 95 ± 25%, liner range of 1 ppb to 81 ppb, and stability at 25 °C). Each immunoassay kit contained sufficient material for 96 measurements. Each microtiter consisted of 96 wells, pre-coated with coupling antigens for respective antibiotics.

Before use, all reagents and samples were left at room temperature for 30 minutes and mixed well by shaking evenly, for tetracycline standards were diluted accordingly by adding 900µl of diluted redissolving solution while sulfonamides standards were not diluted. Each sample and standard solution were performed in duplicate wells. 50 µl of each standard solution or sample were added in duplicate to each well, followed by 50 µl of enzyme conjugate and 50 µl of the antibody working solution then the plate was mixed gently and sealed with a cover membrane and the microplate was incubated at 25 °C for 30 minutes. For tetracyclines plates were incubated at 25°C in a dark place for 30 minutes. Washing with 250 µl four times was performed with diluted 1:19 of 20x concentrated washing buffer with deionized water. For tetracyclines, 100 µl of the mixture of substrate A and B at a ratio of 1:1 was added and for sulfonamide 50 µl of substrate A was added, then substrate B was added in each well, incubated at 25°C at a dark place for 15 minutes. Finally, 50 µl of stop solution in each

well was added to stop the reaction and gently shacked manually, the absorbance at dual wavelength 450/630 nm was measured within 5 minutes. The concentration range was determined by comparing the average OD value of the sample with that of the standard solution.

### 3.11 Statistical Data Analysis

The data analysis of the milk samples was conducted using Stata version 17, Graph Pad Prism version 9.0.0 for Windows, and Green Spring Excel. The concentrations of sulfonamides and tetracycline were summarized as medians and interquartile ranges, and dot plots were generated to display the distribution of drug residue concentrations in the samples. Violin plots were used to visually compare the concentrations of sulfonamide and tetracycline with their ADI and MRL values. The presence of positive antibiotics was quantified and presented as bar charts and pie charts. The detection limits for tetracycline were set at ≥4 μg/kg (positive), <4 μg/kg (negative), and <0.1 μg/kg (not detected), while for sulphonamide they were set at ≥1 μg/kg (positive), <1 μg/kg (negative), and <0.1 μg/kg (undetected). The socio-demographic characteristics of the study participants were described using proportions for categorical variables and medians (IQR) for numerical variables since the numerical variables were not normally distributed. Items for knowledge, attitude, and practices were summed to their respective composite variables. A cut-off value of 75% was applied to these composite variables to classify participants with adequate knowledge, positive attitudes, and good practices. The association between farmers' knowledge and antibiotic residue concentrations in milk was analyzed by comparing the median concentrations of antimicrobial residues in the milk using the non-parametric Mann-Whitney U test. Cutoff percentages were based on previous research (90).

### 3.12 Ethical Issues

Before this study commenced, approval was obtained from the Research Committee at the MUHAS reference number DA.282/298/01.C/2027. Additionally, permission was granted from the Regional and District Administrative Offices. Informed consent was obtained in writing from the owners of the milk collection centers and farms. Confidentiality was maintained by using codes instead of names during sample collection, and all information

was kept secure on computers using specified passwords and shared only with authorized personnel whenever necessary.

### 3.13 Validity and Reliability of the Study

To ensure the validity of the study, a semi-structured questionnaire with both closed-ended and open-ended questions in both Swahili and English was used during data collection. The tool was revised by research supervisors from MUHAS to verify its contents, clarity, and comprehensiveness based on the study objectives. The modification of this tool was done to fit the objectives. The data collection tool was translated into Swahili to meet the cultural and social context of the study setting. To ensure the results were reliable, the proportions and concentrations were determined based on laboratory investigations of the milk samples following the established guidelines and standard operating procedures for ELISA. Furthermore, the MRL of the tetracycline and sulphonamides residues was adopted from the WHO/FAO Codex Alimentarius of 2021 (13) which was used to indicate the cutoff values of each residue when analyzing their concentrations.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Socio-demographic characteristics of study participants

Ninety-five registered dairy farmers were enrolled in this study, a response rate of 136% compared with the required minimum sample size of 70. Among the farmers, 57.9% were male, and the majority were aged between 36 and 60 years (48.4%), with a median age of 56 (*IQR*: 43-66) years. About one-third of them (33.7%) had primary school education and most of them (62.1%) owned ≤ 5 cattle (62.1%), with a median of 4 cattle (*IQR*: 3-10) (**Table 1**).

**Table 1: Socio-demographic characteristics of the study participants**

| Variable | Frequency (n) | Percent (%) |
|---|---|---|
| **Age (years)** | | |
| ≤ 35 | 9 | 9.5 |
| 36 – 60 | 46 | 48.4 |
| >60 | 40 | 42.1 |
| *Median 56 (IQR:43-66)* | | |
| **Sex** | | |
| Male | 55 | 57.9 |
| Female | 40 | 42.1 |
| **Residence** | | |
| Kiluvya | 41 | 43.2 |
| Kisarawe | 20 | 21.1 |
| Kazimzumbwi | 15 | 15.8 |
| Kurui | 10 | 10.5 |
| Masaki | 9 | 9.5 |
| **Education level** | | |
| No formal education | 14 | 14.8 |
| Primary education | 32 | 33.7 |
| Secondary education | 21 | 22.1 |
| College or university | 28 | 29.5 |
| **Source of income*** | | |
| Farming livestock | 64 | 67.4 |
| Business | 27 | 28.4 |
| Crop cultivation | 9 | 9.5 |
| Formal employment | 9 | 9.5 |
| **Number of cattle own** | | |
| ≤ 5 | 59 | 62.1 |
| >5 | 36 | 37.9 |

*Participants may have more than one source of income

### 4.2 Knowledge of the dairy farmers

The majority of the farmers, 81.05% (77/95) were found to have inadequate knowledge of antibiotic use in dairy farming. Most farmers 57.9% (55/95) reported not having received training on commonly used veterinary drugs including proper handling and storage of veterinary medicines and awareness of potential risks associated with improper use of these drugs. With regards to knowledge of antibiotic prophylaxis, 38.9% (37/95) knew about antibiotic prophylaxis, and only 24/95 (25.2%) knew the term "antimicrobial resistance" (**Figure 3** and **Figure 4**).

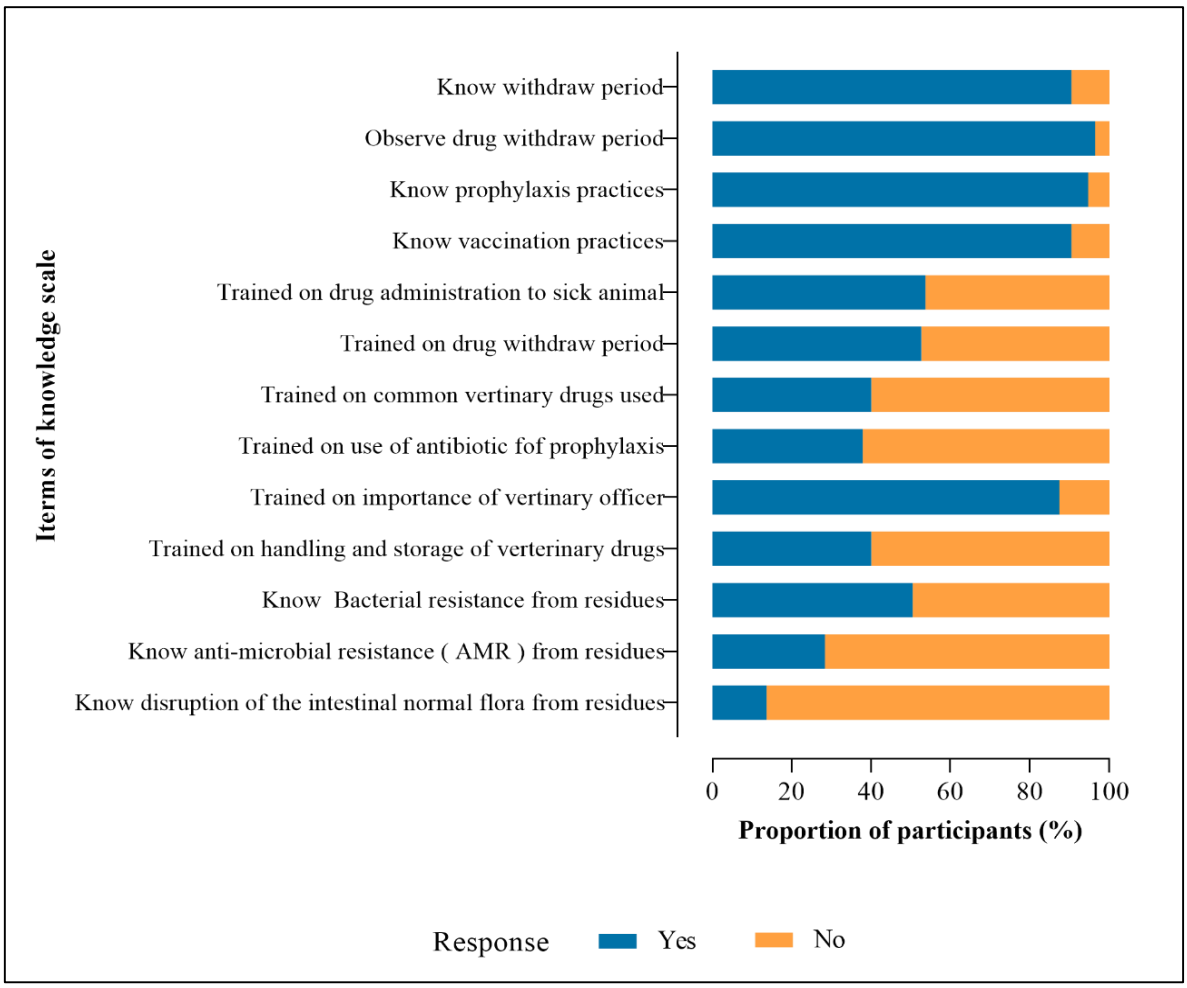



**Figure 3: Dairy farmers knowledge of the use and handling of antibiotics used to treat animals**

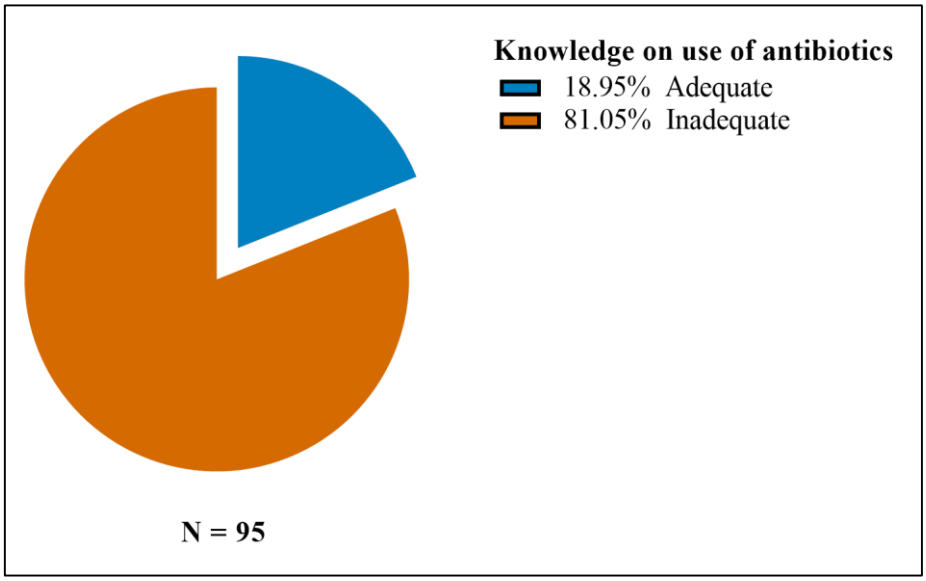



**Figure 4: Proportion dairy farmers with or without adequate knowledge of how to use antibiotics**

### 4.3 Attitude of dairy farmers on antibiotic residues

65.26% of dairy farmers were found to have positive attitudes toward antibiotics used to treat their cows, with more than 90% accessing extension services. However, only 50% of small-scale dairy cattle holders received the service regularly due to the inability to afford the cost (**Figure 5** and **Figure 6**).

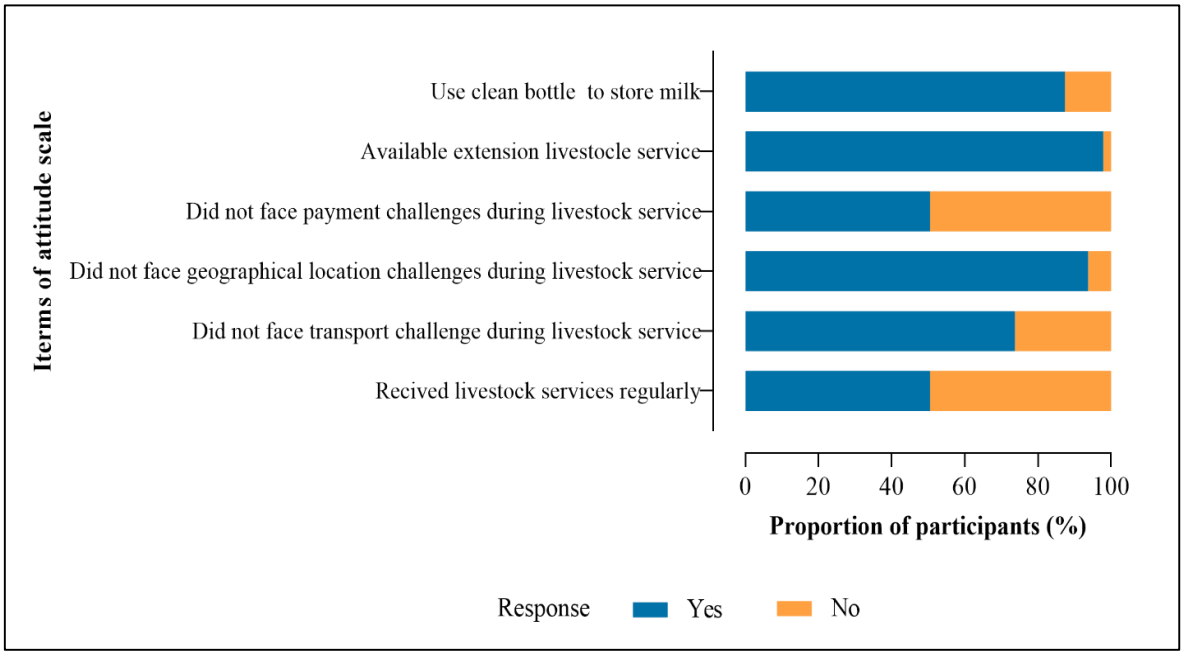



**Figure 5: Dairy farmers attitudes on antibiotic use and residues**

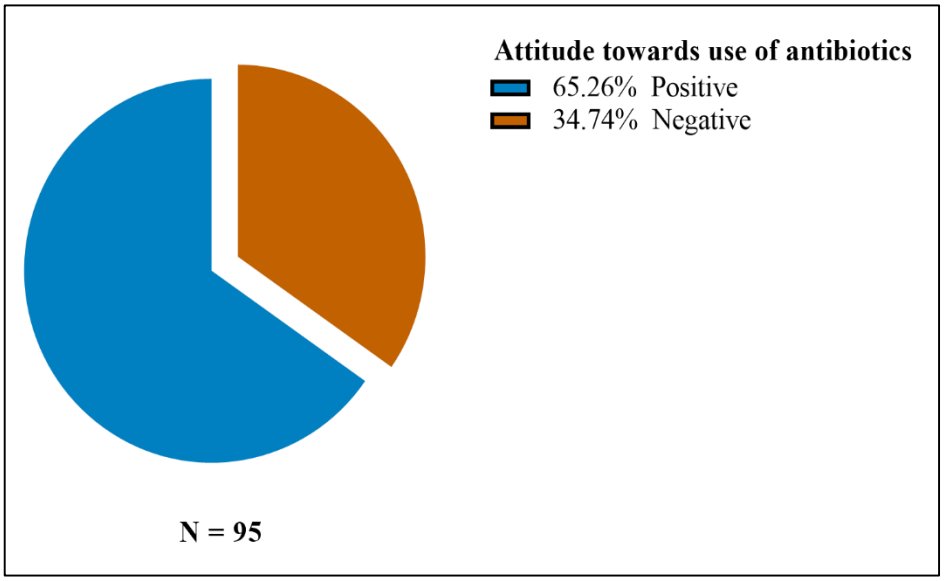



**Figure 6: Proportion dairy farmers with positive and negative attitudes towards the use of antibiotics.**

### 4.4 Practices of dairy farmers

Generally, 57.89% of enrolled participants had poor practices on antimicrobial drugs used to treat their cattle whereas 63.16% did not consider the drug withdrawal period. Also, 75.79% of the farmers did not use antibiotics from the recommended livestock office or livestock market clinics (**Figure 7** and **Figure 8**).

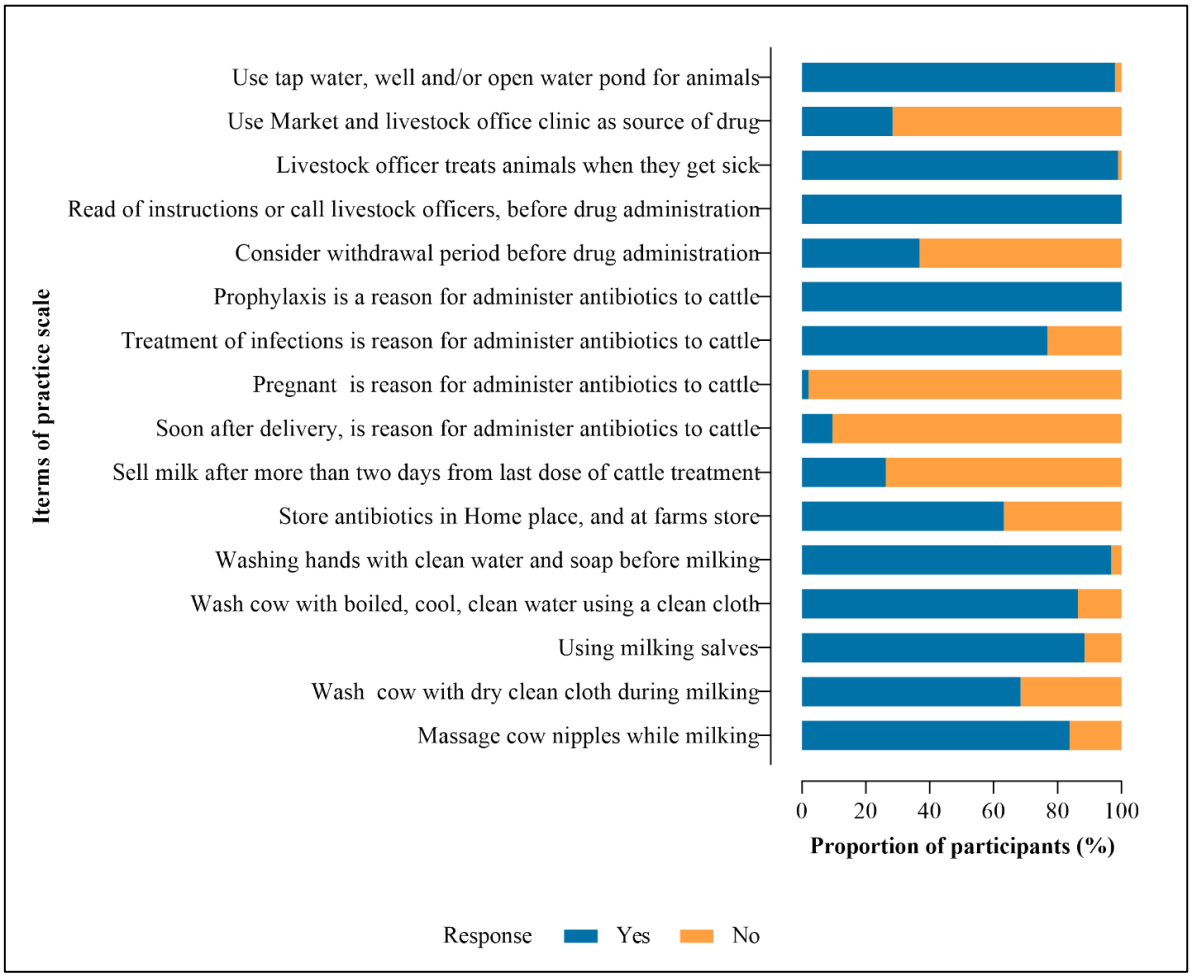



**Figure 7: Dairy farmers practices during animal husbandry**

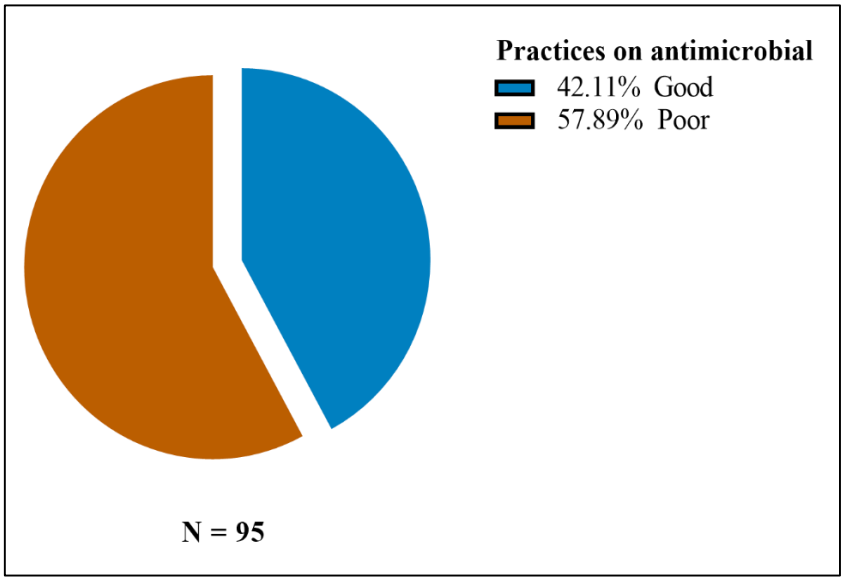



**Figure 8: Practices of small-scale dairy cattle holder on antimicrobial use in dairy farming**

### 4.5 Proportion of milk samples with drug residues obtained from Kisarawe district

Most of the milk samples 79 (94.05%) contained sulphonamide residues, with a smaller proportion having tetracycline residues 6 (8.14%). All samples from three wards were positive for sulphonamides. Also, there was a significant difference in tetracycline antibiotic residues within wards (*p-<0.001*) (**Table 2** and **Figure 9**).

**Table 2: Proportion of milk positive for sulfonamides and tetracycline in Kisarawe wards, Tanzania 2024**

| Sample site (Wards) | Drugs | *Positive n (%) | *Negative n (%) | P-value |
|---|---|---|---|---|
| Kazimzumbwi | Tetracycline | 1 (7.1) | 13 (92.9) | <0.01 |
| | Sulfonamides | 14 (100) | 0 (0.0) | |
| Kiluvya | Tetracycline | 3 (7.5) | 37 (92.5) | <0.01 |
| | Sulfonamides | 35 (87.5) | 5 (12.5) | |
| Kisarawe | Tetracycline | 1 (5.0) | 19 (95.0) | <0.01 |
| | Sulfonamides | 20 (100) | 0 (0.0) | |
| Masaki | Tetracycline | 1 (11.1) | 8 (88.9) | <0.01 |
| | Sulfonamides | 9 (100) | 0 (0.0) | |
| Kurui | Tetracycline | 0 (0.0) | 1 (100) | <0.01 |
| | Sulfonamides | - | - | - |

*Note: positive ≥ 10 µg/kg, negative < 10 µg/kg for tetracycline; Positive ≥ 20 µg/kg, negative < 20 µg/kg for sulfonamides

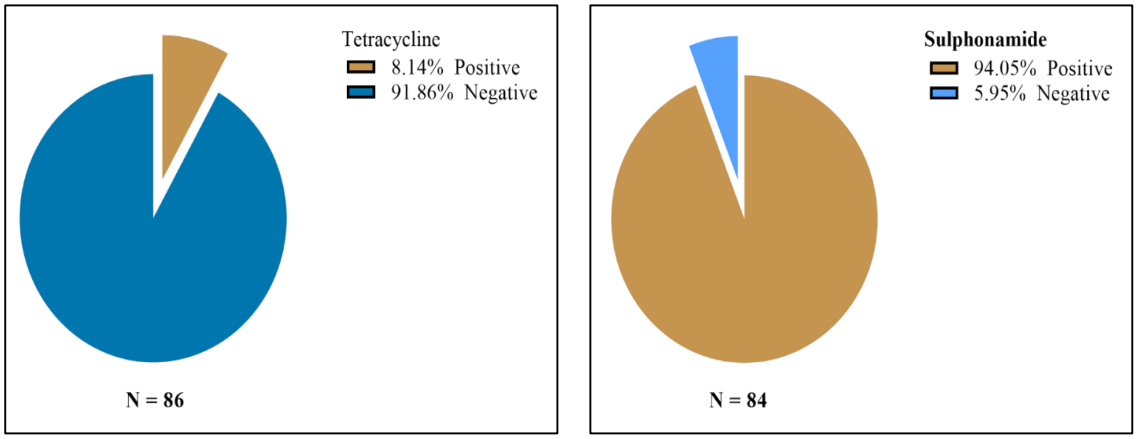



**Figure 9: Proportion of tetracycline and sulphonamide residues in dairy milk**

### 4.6 Residue Concentrations Relative to Allowable Daily Intake (ADI)

Of the tested milk samples, 95.35% (n=82) had acceptable levels of tetracycline, compared to 64.29% (n=54) of the samples tested for sulphonamides (**Figure 10**).

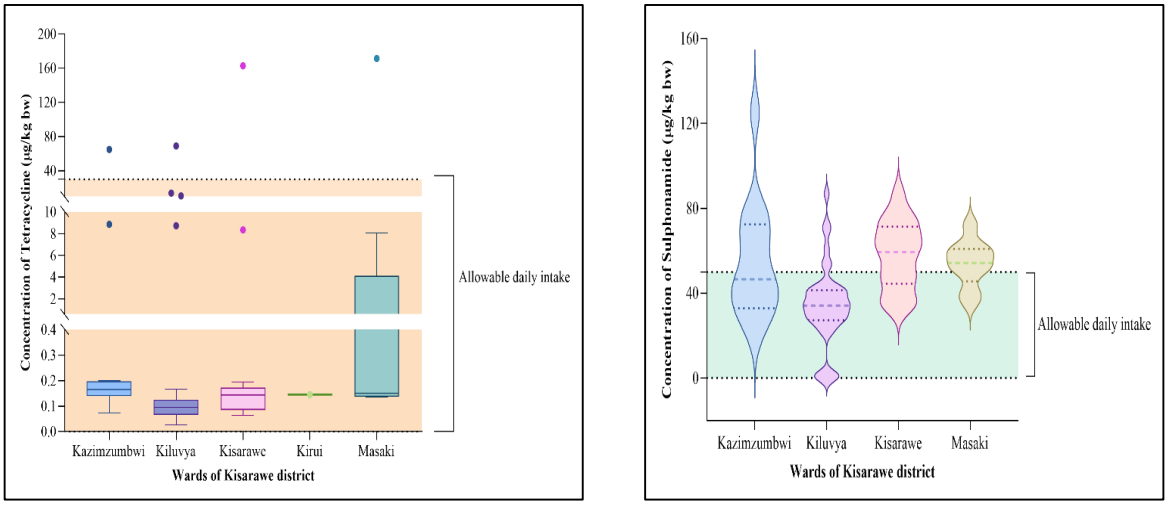



**Figure 10: Concentration of Tetracycline and Sulphonamides residues versus Allowable Daily Intake (ADI)**

### 4.7 Residue Concentrations Relative to Maximum Residue Limits (MRLs)

Of the tested milk samples, 2 (2.4%) had tetracycline levels above MRL, and 1 (1.2%) contained sulphonamide residue levels above the MRL. The highest concentrations of these residues exceeded the MRL by 60% and 25% respectively. The median concentration and

interquartile ranges of tetracycline and sulphonamide residues across the wards were statistically significant (p <0.001) (**Figure 11** and **Table 3**).

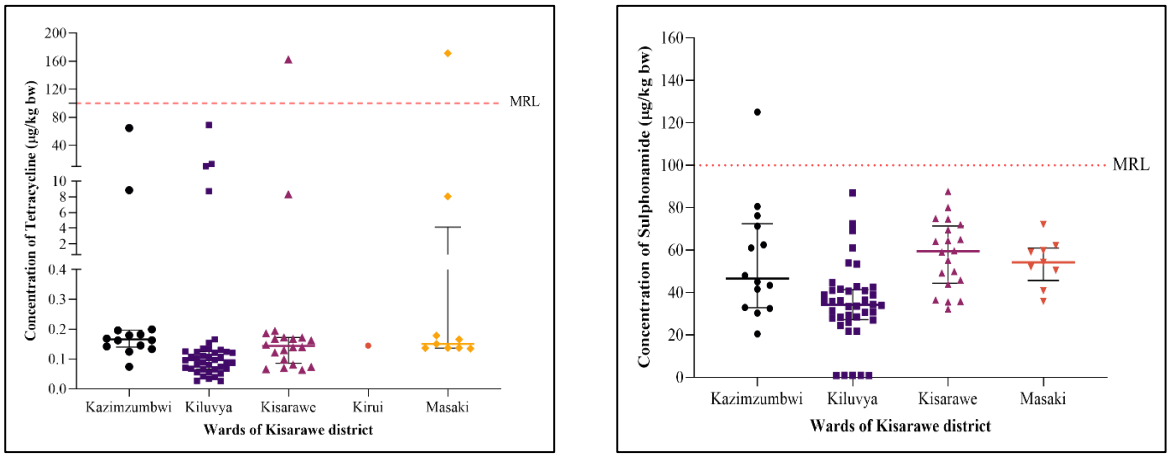



**Figure 11: Concentration of Tetracycline and Sulphonamides residues versus Maximum Residue Limits (MRLs)**

**Table 3: Median concentrations and interquartile ranges of Tetracycline and Sulphonamides residues**

| **Median Concentration (IQR)** | | | | | |
|---|---|---|---|---|---|
| **Milk farm ward** | **Kisarawe** | **Kiluvya** | **Kazimzumbwi** | **Masaki** | ***p-value*** |
| Tetracycline | 0.144 (0.086,0.172) | 0.097 (0.066,0.125) | 0.166 (0.140,0.197) | 0.151 (0.137,4.12) | < 0.01 |
| Sulphonamides | 59.331 (44.41,71.41) | 34.165 (27.23,41.35) | 46.525 (32.84,72.48) | 54.199 (45.64,60.91) | < 0.01 |

### 4.8 Association between farmers knowledge and antibiotic residues concentration in milk

We found no association between dairy farmers' knowledge and antibiotic residue concentration in milk for both tetracycline (p = 0.720) and sulphonamide (p=0.326) (Figure 12).

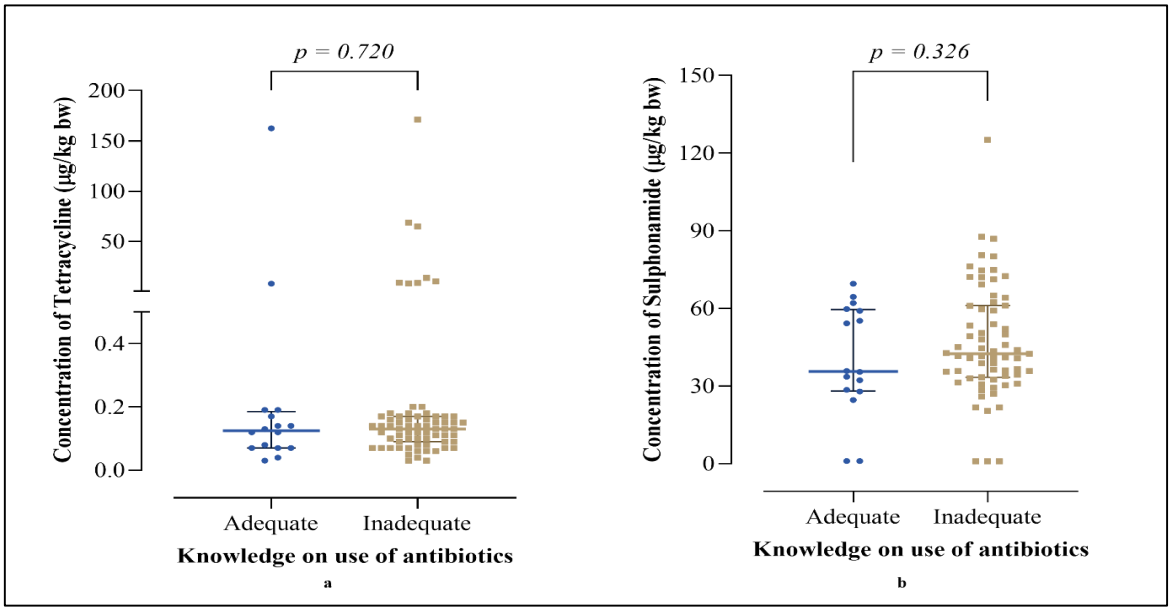



**Figure 12: Farmer's knowledge and antibiotic residues concentration in milk**

**4.9 Association between farmers' attitude and antibiotic residue concentration in milk.**

We found no significant association between farmers' attitudes and antibiotic residue concentration in milk for both tetracycline (p-value 0.512) and sulphonamide (0.308) (**Figure 13**).

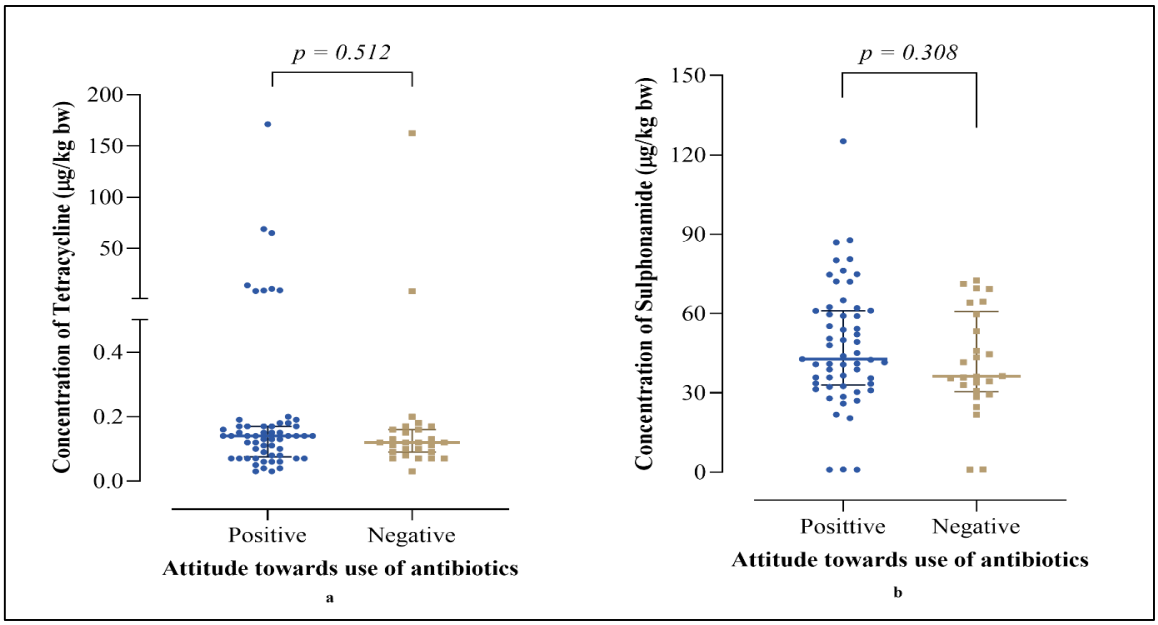



**Figure 13: Farmers attitudes and antibiotic residues concentration in milk**

### 4.10 Association between farming practices and antibiotic residue concentration in milk

We found a significant association between farming practices and antibiotic residue concentration in milk for both tetracycline (p-value 0.043) and sulphonamide (0.023) (**Figure 14**).

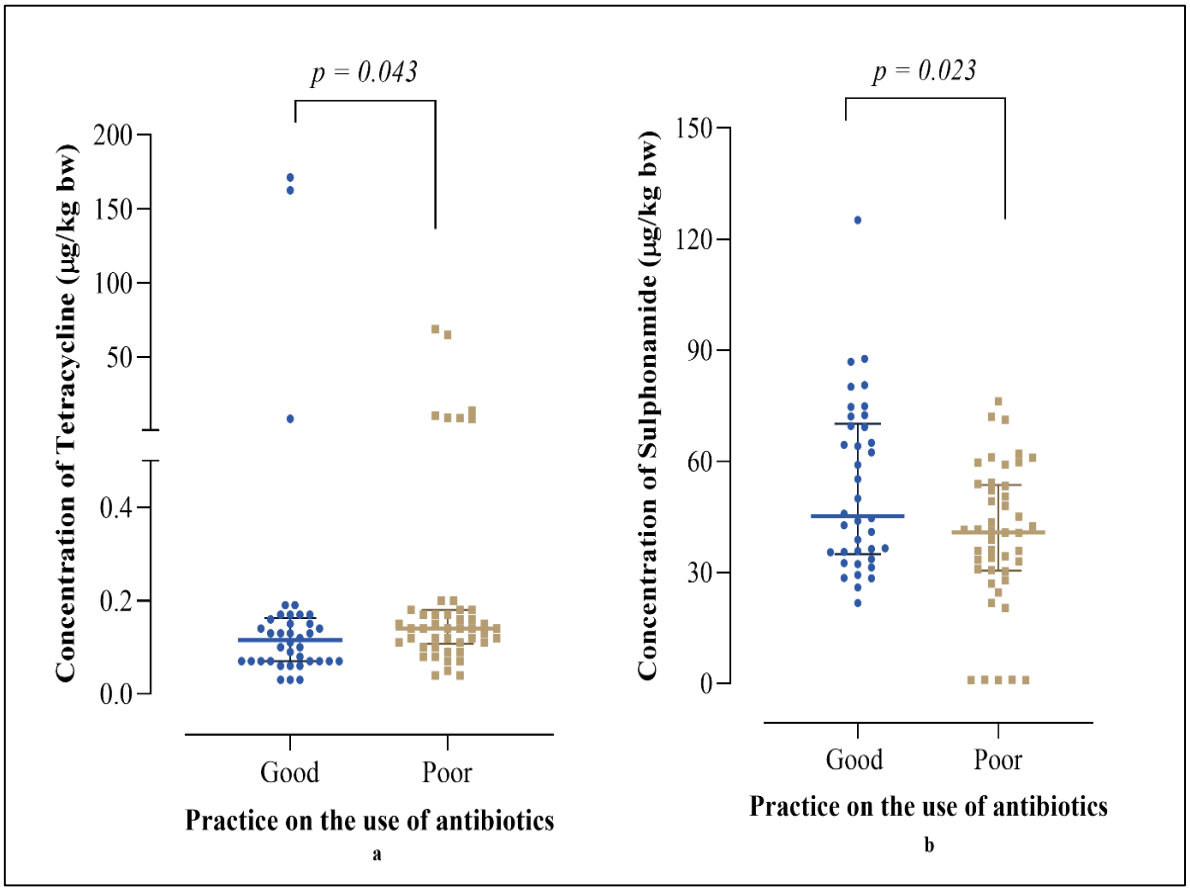



**Figure 14: Farming practices and concentration of antibiotic residues in milk**

# CHAPTER FIVE

## 5.0 DISCUSSION

This study was conducted in Kisarawe District in Tanzania, to determine dairy farmers' knowledge, attitudes, and practices regarding AMU in farming, and measure the levels of tetracycline and sulphonamide residues in dairy milk. The farmers were mostly male (57.9%), aged 36 to 60 years (48.4%), with one-third of them (33.7%) having primary school education. Farming was mostly done on a small scale, with 62.1% of the farmers owning ≤ 5 cattle, with a median of 4 cattle (*IQR*: 3-10). Overall, 77 (81.05%) of the farmers were adjudged to have inadequate knowledge of antibiotics, 57.9% were not trained on commonly used veterinary medicines, and very few (25%) were aware of anti-microbial resistance.

Regarding their attitudes towards antibiotic use, 65.26% of dairy farmers were found to have positive attitudes, with more than 90% having access to extension services. However, only 50% of them received the service regularly due to inability to afford costs, which seem to be a significant barrier to seeking professional advice. As a result, farmers prefer the advice of untrained animal health workers or para-veterinarians because they charge lower fees or rely on peer advice and experience instead of professional guidance (91).

Concerning practices on AMU, 57.89% of the farmers were adjudged to have poor practices, with 63.16% not observing the withdrawal period, a finding that has been associated with either ignorance of the associated public health risks, but mainly perceived economic losses (92,93).

Furthermore, we found that more than three-quarters (75.79%) of the farmers were using medicines not prescribed by veterinary officers, due to the tendency of farmers to self-medicate, and in some cases involve farm attendants and peer groups in treatment decisions. Unfortunately, the Veterinary Act No. 16 of 2003 (94), which regulates veterinarians and paraprofessionals also provides circumstances under which unqualified personnel may perform some of the duties (11).

Analysis of the milk samples revealed that 79 (94.05%) contained sulphonamide residues and 6 (8.14%) contained tetracycline residues. Significantly, we found that 2 (2.4%) samples had tetracycline levels above MRL, and 1 (1.2%) contained sulphonamide residue levels above the MRL. Previous studies conducted in Tanzania showed variable levels of residues

in dairy milk. In one study, 7% of raw milk marketed by milk selling points (MSPs) in Dar es Salaam was positive for antimicrobial residues (16). Using the Charm-AIM screening test kit in Tanzania, (15) detected antimicrobial residues in 36% of marketed milk samples in the Mwanza and Dar es Salaam regions, while a study done in the same country in 2009 using the Delvotest found antimicrobial residues in 4.5% of milk samples in smallholder dairy farms in Mvomero and Njombe districts (59,95). Our findings are also higher than those of a relatively more recent study in Tanga municipality, Tanzania that found a residual positivity of 42.7% (96). These variations could be due to the differences in i) the time when the studies were conducted since some were performed some time ago when the consumption of antibiotics was still low (59,61), ii) the performance of the tests used for screening residues, we used ELISA technique while others used different technologies such liquid chromatography-mass spectrometry (74), the Charm-AIM screening test (15), and the Delvotest (59), iii) farming practices iv) clinical conditions of cattle since some studies were conducted in animals with subclinical and clinical mastitis (59). Compared to studies done in other parts, the proportion of samples with antibiotic residue found in this study is above the 24% reported in Somalia, 21 % in Kenya, 36% in Ethiopia, 9% in Niger, and 35% in Ghana (39,97–100). Unfortunately, most of these studies used non-quantitative methods such as microbial growth inhibition assays, microbial receptor assays, receptor binding assays, immunologic assays, enzymatic assays, chromatographic analysis, and the Delvotest. They therefore did not indicate the percentage of samples that exceeded MRL.

We found a few recent studies that determined the concentration of residues and compared them against respective MRLs. A study conducted in Northeastern Nigeria found oxytetracycline residues, found all 172 (86 raw and 86 fermented) samples to be within the acceptable limits (71), while in neighboring Kenya found in 109 milk samples that 0.9% of the samples had tetracycline residues that exceeded MRL and (2.8%) had oxytetracycline residue above MRL, these levels that are comparable to our findings (101). Other studies found higher concentrations of antibiotic residues. For instance, Kenya using the Charm TRIO® test kit and liquid chromatography (LC) coupled to the triple quadrupole mass spectroscopy (MS) (LC-MS/MS) found between 3 and 10.5% of samples in Nyandarua, Meru, and Kiambu counties to have tetracycline residues above the recommended MRLs (45). Assessment of the presence of amoxicillin, cloxacillin, tetracycline, sulfamethoxazole,

and trimethoprim residues in milk sold in Juja and Githurai markets in Kenya, finding 10.8% of the 65 samples having at least one antibiotic residue above respective MRLs (72). Again, these variations could be explained by differences in the performance of laboratory test methods used in the different studies (36-38), farm management systems, and differences in extension services and the extent of enforcement of legislation and regulations concerning antimicrobials in dairy farming (9).

Regarding farmers' knowledge, attitudes, and practices, we found a significant association between farming practices and antibiotic residue concentration in milk for both tetracycline (p-value=0.043) and sulphonamide (p-value=0.023), but not between dairy farmers' knowledge with the presence of tetracycline (p = 0.720) or sulphonamide (p-value=0.326), nor was there an association between farmers' attitudes with the presence of tetracycline (p-value=0.512) or sulphonamide (p-value=0.308) residues. Collectively, these findings indicate that the practice of using antibiotics may not necessarily be associated with the knowledge or attitudes of the farmers as observed by others (102). Of particular note, 57.89% of the farmers had poor practices on antimicrobials, 63.16% did not consider the drug withdrawal period and 75.79% did not use antibiotics from the recommended livestock office or livestock market clinics. The findings suggest that, many farmers rely on unqualified advice and prioritize economic considerations over safety regulations, leading to continued poor practices in antibiotic use despite positive attitudes (65.26%) towards veterinary guidance.

This study has strength in that it enrolled many small-scale dairy farms to ensure representativeness in providing updated information on the KAP, and determined the presence and concentration of tetracycline and sulphonamide residues in comparison with the two international metrics, the ADI and MRL. These metrics are used globally as health-based benchmarks in nutrition research studies that consider the safety evaluation is carried out following international guidelines. The ELISA technology used in this study is much cheaper, faster, and technically less demanding than liquid chromatography-mass spectrometry and can be used in most laboratories in LIMCs while producing comparable results (103).

This study screened for only tetracycline and sulphonamide residues. Although these are two most frequently used antibiotics, the study provides a partial picture of antibiotic residues in dairy milk in the area since other antibiotics such as beta-lactams, quinolones, aminoglycosides, and various other antibacterial agents are also used (104). Secondly, the study was supposed to be random in design, giving equal opportunity to all farmers to be included in the study. However, some farmers could not be assessed due to logistical challenges such as inaccessible roads.

# CHAPTER SIX

## 6.0 CONCLUSIONS AND RECOMMENDATIONS

### 6.1 Conclusion

Our study indicates inadequate knowledge, attitudes, and poor practices of dairy farmers on AMU in farming in the Kisarawe District in eastern Tanzania, and the presence of antibiotic residues (especially sulphonamides) in milk, prompting calls for observation of withdrawal period, promoting behavioral change through public communication, regular monitoring and increased extension services, and antimicrobial stewardship programs.

### 6.2 Recommendations

The presence of antibiotic residues in diary milk observed in this study poses a public health concern for consumers. A number of measures are required to ensure public health safety. There is a need to sensitize both farmers and the general public on the dangers associated with antibiotic residues in milk. Farmers, in particular, should be educated on prudent use of antibiotics and the need to observe withdrawals periods. There is need to reduce the use of antibiotics by the use of vaccines and improving biosecurity measures. Public health campaigns are also needed by use of various media outlets. Furthermore, there is need of testing milk for residues using simple and rapid technologies that can be employed at points of sale. The government of Tanzania, together with other stakeholders, should address the issue of residues in the food chain, as stipulated in the second action plan on antimicrobial resistance 2023-2028.

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# APPENDICES

## Appendix 1: Informed Consent Form (English version)

INFORMED CONSENT FORM

**PARTICIPANT NUMBER: _____ _____ _____**

**Objective:** To obtain scientific information on the presence of human antibiotic residues, namely, tetracycline and sulfonamide, in samples of milk produced by dairy cattle from small holder dairy farmers in the coastal region and used as food for residents and neighboring regions. Additionally, we checked to what extent these medicinal products can affect the health of consumers based on the Codex Alimentarius standards set by the Food and Agricultural Organization and the World Health Organization (FAO/WHO). Along with that, several questions are expected to be asked to obtain more insightful information from the breeder himself/herself.

**Researchers: Jasper Kimambo**

**Sponsor:** PEPFAR under the Centre for Disease Control and Prevention (CDC) through the University of Mzumbe in collaboration with Muhimbili University of Health and Allied Sciences.

**Harm:** There was no harm from this research to any person except his/her time to help complete this research.

**Benefits:** The results of this research will help the government determine to what extent milk can cause effects on humans to plan a strategic plan to address the control and inspection of milk from breeders to meet the health needs of consumers.

**Confidentiality:** I guarantee the firm maintenance of the participants' secrets, as only the number of participants will be recognized and not the full name of the participant; additionally, the participants' information will be kept strictly confidential and used only for research purposes and not otherwise.

**The right to participate:** This study is voluntary. One or more dairy farmers were allowed to participate or not participate in this study according to their wishes. However, your participation is a great patriotism to help the country and researchers solve human health challenges.

**Contact:** For more comments or questions about this study, you can contact the MUHAS research ethics directory P.O. BOX 65000 or the Principal Researcher named Jasper Kimambo by phone number +255764828066 or through email at kimamboj86@gmail.com of the Muhimbili University of Health and Allied Sciences P.O. BOX 65000 Dar es Salaam. Or you can contact the Supervisor of this study Prof. Mecky Matee by phone number +255713081162 or through email at mateemecky@yahoo.com of Muhimbili University of Health and Allied Sciences P.O. BOX 65000 Dar es Salaam.

**Consent:** I have agreed/not agreed to participate in this study with my consent, as I have understood/not understood the purpose and importance of this study.

**Participant's signature:** _____ or Fingerprint signature_____.

In case you are not able to sign someone as your witness may sign here on behalf of the witness: _____.

**Appendix 2 : Fomu ya ridhaa (Swahili version)**

**FOMU YA KUKUBALI KUSHIRIKI KATIKA UTAFITI**

**Namba ya fomu_____ _____ _____**

**Lengo la utafiti huu :** Kupata taarifa za kisayansi juu ya uwepo wa mazalia ya dawa za binadamu ambazo ni tetracycline na sulphonamide kwenye sampuli za maziwa yanayozalishwa na wafugaji wadogo wa maziwa ya ng'ombe katika mkoa wa Pwani wilaya ya Kisarawe na kutumiwa kama chakula kakazi na makazi ya jirani. Pia kuangalia ni kwa kiwango gani mazalia hayo ya dawa yanaweza kuathiri mlaji kiafya kwa kuzingatia viwango vilivyothitibishwa na taasisi ya chakula na shirika la afya duniani (WHO/FAO). Sambamba na hilo maswali kadhaa yanategemewa kuulizwa ili kupata taarifa Zaidi za ufahamu kutoka kwa mfugaji mwenyewe.

**Jina la mtafiti** : **Jasper Kimambo**

**Mfadhili :** PREPFAR chini ya taasisi ya kupambana na magonjwa ya binadamu (CDC) kupitia chuo kikuu cha Mzumbe, wakishirikiana na Chuo kikuu cha Tiba na Afya Shirikishi Muhimbili.

**Madhara** : Hakuna madhara yatokanayo na utafiti huu kwa mhusika yeyote isipokuwa muda wake tu kusaidia kukamilisha utafgiti huu.

**Faida ya utafiti huu** : Matokeo yatokanayo na utafiti huu yatasaidia serikali kujua ni kwa kiwango gani maziwa yanaweza kusababisha athari kwa binadamu, ili kupanga mpango mkakati kukabiliana na uthibiti na ukaguzi wa maziwa kutoka kwa wafugaji ili yakidhi matakwa ya kiafya kwa mtumiaji.

**Usiri** : tunakuhakikishia utunzaji thabiti wa siri za mshiriki kwani namba pekee ndio itatambulika na sio jina kamili ya mshiriki, na pia taarifa zake zitatunzwa kwa usiri mkubwa na kutumika kwa lengo la utafiti tu na sio vinginevyo.

**Haki ya kushiriki au kutokushiriki** : Utafiti huu ni wa hiari. Mfugaji wa ng'ombe wa maziwa mmoja au Zaidi anaruhusiwa kushiriki au kutoshiriki katika utafiti huu kulingana na matakwa yake. Aidha kushiriki kwako ni uzalendo mkubwa kuisaidia nchi na watafiti kutatua changamoto za kiafya kwa binadamu.

**Mawasiliano** : Kwa maoni au maswali Zaidi juu ya utafiti huu unaweza kuwasiliana na usimamizi wa tafiti chuo kikuu cha tiba Muhimbili kwa S.L.P 65000 au MTAFITI MKUU jina Jasper Kimambo kwa namba za simu 0764828066 au kwa barua pepe kimamboj86@gmail.com wa Chuo Kikuu cha Afya na Sayansi Shirikishi Muhimbili. Au unaweza kuwasiliana na Kurugenzi ya Utafiti na machapisho, Chuo Kikuu cha Afya na Sayansi shriikishi Muhimbili S.L.P 65000, Dar es Salaam. Au unaweza kuwasiliana na Msimamizi mkuu wa utafiti huu Prof. Mecky Matee kupitia simu namba +255713081162 au kwa kupitia barua pepe mateemecky@yahoo.com wa Chuo Kikuu Kishiriki cha Afya Muhimbili S.L.P 65000 Dar es Salaam.

**Ridhaa** : Nimekubali/sijakubali kushiriki katika utafiti huu kwa ridhaa yangu mwenyewe kwani nimeelewa/sijaelewa lengo na umuhimu wa utafiti huu.

**Sahihi ya mshiriki :** _____ au alama za kidole _____.

Kama huwezi kuandika au kusaini kwa alama za kidole unaweza kuruhusu mshiriki mwingine akusaidie kusaini hapa. **Sahihi ya shahidi:** _____.

**Appendix 3: Semi-Structured Questionnaire for Small-Scale Dairy Farmers in Kisarawe District, Pwani region of Tanzania (English version):**

**Determination of Antibiotic Residues in Dairy Milk in Kisarawe District, Pwani Region, in Tanzania, 2024**

**SECTION ONE: LOCATION**

1. **GPS CODE (open-source electronic)**
2. Ward Name _____
3. Date of Interview _____

**SECTION TWO: SOCIO-DEMOGRAPHIC INFORMATION**

4. Respondent number_____ _____ _____

5. A respondents' gender
   (1) Male
   (2) Female sex

6. Age of respondent _____

7. Education level of respondent?

   i) No formal education
   ii) Incomplete primary
   iii) Primary school education ()
   iv) Incomplete secondary
   v) Secondary school education ()
   vi) College OR University education ()

8. What is your primary source of income for your household?

   i) Dairy farming ()
   ii) Crop cultivation ()
   iii) Farming and livestock ()
   iv) Business ()
   v) Formal employment

9. How many cattle do you have? _____

10. How long have you been keeping cows? (Mention the year and/or month you began livestock keeping)

Year _____

Month _____

**SECTION THREE:**

**GENERAL KNOWLEDGE ATTITUDE AND PRACTICES OF FARMERS ON THE USE OF VETERINARY DRUGS, ACCESS TO VETERINARY SERVICES AND ANTIBIOTIC RESIDUES.**

## I. PRACTICE

11. What do you use as primary source(s) of water during your animal husbandry?

i) Tap water
   Yes ()
   No ()

ii) Wells
   Yes ()
   No ()

iii) Open water ponds
   Yes ()
   No ()

iv) Other sources
   Yes ()
   No ()

12. Where do you normally obtain drugs for animals?

i) Market, e.g., cattle market
   Yes ()
   No ()

ii) Private Pharmacy

Yes ()

No ()

iii) From a livestock office clinic

Yes ()

No ()

iv) Any other sources (neighbor, locally)

Yes ()

No ()

13. Who treats your animal when they get sick? Select all that apply

i) Livestock officer ()

ii) Health workers ()

iii) Myself ()

iv) Local healers ()

14. If you treat animal yourself, do you practice the following before drug administration?

i. Reading of instructions

Yes ()

No ()

ii. Self-awareness knowledge

Yes ()

No ()

iii. Asking neighborhood friends

Yes ()

No ()

iv. Communication to livestock officers

Yes ()

No ()

v. Information from TV, radio, etc.?

Yes ()

No ()

15. Why do you administer antibiotics to your cattle?

i) Prophylaxis

Yes ()

No ()

ii) Treatment of infections/diseases

Yes ()

No ()

iii) Growth promotion

Yes ()

No ()

iv) When they are pregnant

Yes ()

No ()

v) Soon after delivery

Yes ()

No ()

vi) To increase productivity

Yes ()

No ()

16. When do you sell milk after the last dose of cattle treatment?

i. Immediately after the last dose ()

ii. Two to seven days ()

iii. One to two weeks ()

iv. After two weeks ()

v. Three to four weeks ()

vi. After one month or longer ()

17a. Are you aware of drug withdraw period?

i) Yes

ii) No

17b. If you are aware of the drug withdrawal period, do you always observe the period?

i) Yes ()

ii) No ()

18. Where do you store antibiotics?

i) At farms

Yes ()

No ()

ii) Cowshed

Yes ()

No ()

iii) Home place

Yes ()

No ()

iv) Other places

Yes ()

No ()

## II. KNOWLEDGE OF FARMERS ON VETERINARY DRUGS AND HUSBANDRY

19. Do you know about the following animal husbandry practices?

i. Quality food products

Yes ()

No ()

ii. Cleanliness

Yes ()

No ()

iii. Prophylaxis

Yes ()

No ()

iv. Vaccination

Yes ()

No ()

20. Do you normally wash your hands with clean water and soap before milking?

i. Yes ()

ii. No ()

21. During milking, do you wash your cow with boiled, cool, clean water using a clean cloth?

i. Yes ()

ii. NO ()

22. Have you ever used milking salves?

i. Yes ()

ii. No ()

23. Do you wash your cow with dry clean cloth during milking?

i. Yes ()

ii. No ()

24. Do you massage cow nipples while milking?

i. Yes ()

ii. No ()

25. Do you know the meaning of the drug withdrawal period?

i. Yes

ii. No

26. Have you got to know the following human health risks, which may be caused by antibiotic drug residues in milk?

i) Allergic reactions to sensitive individuals

Yes ()

No ()

ii) Development of antimicrobial drug resistance

Yes ()

No ()

iii) Disruption of the intestinal normal flora

Yes ()

No ()

iv) Bacterial resistance to antibiotics

Yes ()

No ()

v) Direct toxic effects

Yes ()

No ()

vi) Carcinogenicity

Yes ()

No ()

vii) Mutagenicity

Yes ()

No ()

27. Have you received any training on how to handle veterinary drug administration and handling sick animals? Put a tick (√)

i) Yes ()

ii) No ()

28. Have you ever received any training on the following animal husbandry practices?

i) Withdrawal period and its importance

Yes ()

No ()

ii) Handling and storage of veterinary drugs

Yes ()

No ()

iii) Types of veterinary drugs used to treat animals

Yes ()

No ()

iv) Use of antibiotics for prophylaxis in animal husbandry

Yes ()

No ()

v) Importance of using veterinary officers to treat animals or cattle

Yes ()

No ()

29. If you treat animal yourself, do you consider the following before drug administration?

i) Proper dose to be administered

Yes ()

No ()

ii) Age and weight of the animals/cows

Yes ()

No ()

iii) Health status of the animals/cows

Yes ()

No ()

iv) Type of drugs to be administered

Yes ()

No ()

v) Withdrawal period

Yes ()

No ()

vi) Date of drug use

Yes ()

No ()

## III. **ATTITUDING OF FARMERS**

30. Do you receive livestock services regularly?

i) Yes

ii) No

31. Do you face the following challenge(s) while accessing livestock services?

i) Transportation

Yes ()

No ()

ii) Geographical location

Yes ()

No ()

iii) Lack of payment fees

Yes ()

No ()

iv) Don’t know where to access these extensions

Yes ()

No ()

v) The extensions are not available in our settings.

Yes ()

No ()

**Appendix 4: Fomu Ya Kukusanyia Taarifa kutoka kwa wazalishaji wa maziwa wilaya ya Kisarawe, Mkoa wa Pwani Tanzania (Swahili version)**

**Ukusanyaji Wa Taariza Za Uwepo Wa Mabaki Ya Antibiotiki Katika Maziwa Kwenye Mashamba Ya Ng'ombe Kisarawe, Mkoa Wa Pwani Tanzania, 2024**

**SEHEMU YA KWANZA: MAHALI ANAPOPATIKANA MTEJA**

1. **GPS CODE (open-source electronic)**
2. Jina la Kata _____
3. Tarehe ya mahojiano _____

**SEHEMU YA PILI: TAARIFA BINAFSI**

5. NAMBA YA MSHIRIKI

_____ _____ _____

6. Jinsia
   i) Kiume ()
   ii) Kike ()

7. Una umri gani? _____

8. Una kiwango gani cha elimu?
   i) Sijasoma ()
   ii) Sijamaliza shule ya msingi ()
   iii) Elimu ya msingi ()
   iv) Hajamaliza Sekondari ()
   v) Elimu ya sekondari ()
   vi) Kidato cha sita ()
   vii) Chuo/chuo kikuu ()

9. Nini chanzo kikuu cha mapato kwenye kaya yako? (Chagua kila inayohusika)
   i) Ufugaji/Ufugaji wa ng'ombe wa maziwa ()
   ii) Kilimo cha biashara ()

iii) Kilimo na ufugaji/ufugaji wa ng'ombe ()

iv) Biashara ()

v) Mwajiriwa ()

10. Unamiliki ng'ombe wangapi? _____

11. Umefuga ng'ombe kutokea lini? (Taja mwaka na mwezi alioanza ufugaji, kama hakumbuki mwezi taja mwaka tu)

Mwezi _____

Mwaka _____

**SEHEMU YA TATU :**

**UELEWA, TABIA NA VITENDO VINAVYOFANYWA NA WAFUGAJI JUU YA MATUMIZI YA DAWA ZA KUTIBU MIFUGO NA MABAKI YA DAWA ZA ANTIBIOTIKI**

**I. MABO ANAYOPENDELEA KUFANYA MFUGAJI**

12. Naweza kuviona vifaa hivyo vya kukamulia/kuhifadhia maiwa tafadhali? Gradi

i) Visafi ()

ii) Vichafu ()

13. Je? Ni chanzo/vyanzo gani vya maji kati ya zifuatazo huwa unatumia kwa shughuli zako za ufugaji?

i) Maji ya bomba
   Ndio ()
   Hapana ()

ii) Maji ya Kisima
   Ndio ()
   Hapana ()

iii) Maji kutokana na hodhi au mifereji

Ndio ()

Hapana ()

iv) Vyanzo vinginevyo

Ndio ()

Hapana ()

14. Kwa kawaida unapataje dawa za kutibia wanyama wako kati ya zifuatazo?

i. Maeneo ya sokoni au masoko ya vyakula vya mifugo?

Ndio ()

Hapana ()

ii. Maduka ya dawa binafsi?

Ndio ()

Hapana ()

iii. Kliniki ya Idara ya Mifugo?

Ndio ()

Hapana ()

iv. Kwengineko (jirani, kienyeji)?

Ndio ()

Hapana ()

15. Ni nani anaekutibia wanyama wako wanapoumwa?

i) Afisa mifugo ()

ii) CAHWs ()

iii) Mwenyewe ()

iv) Wengineo ()

16. Iwapo unatibu mwenyewe, ni vipi unakisia kiwango cha dawa kinachohitajika kwa matumizi katika sababu zifuatazo?

i) Kwa kusoma maelekezo

Ndio ()

Hapana ()

ii) Kwa uzoefu binafsi wa elimu

Ndio ()

Hapana ()

iii) Kwa kuuliza majirani/rafiki?

Ndio ()

Hapana ()

iv) Kwa kuwasiliana na daktari wa mifugo?

Ndio ()

Hapana ()

v) Kwa Kusikia Kutoka Vyanzo Vingine Vya Taarifa (tv, radio n.k.)

Ndio ()

Hapana ()

17. Je, ni sababu zipi zinazopelekea kuwapa mifugo yako dawa za antibiotiki?

i. Kukinga dhidi ya magonjwa

Yes ()

No ()

ii. Kutibu magonjwa ya kuambukiza

Yes ()

No ()

iii. Kwa ajili ya kukuzia

Yes ()

No ()

iv. When they are pregnant

Yes ()

No ()

v. Mara tu baada ya kuzaa

Yes ()

No ()

vi. Kwa ajili ya kuongeza uzalishaji wa maziwa

Yes ()

No ()

18. Je ? Ni wakati gani unauza maziwa baada ya dozi ya mwisho?
    i) Muda mfupi tu baada ya dozi kuisha ()
    ii) Baada ya siku mbili hadi saba ()
    iii) Baada ya wiki moja hadi mbili ()
    iv) Baada ya wiki mbili ()
    v) Baada ya wiki tatu hadi nne ()
    vi) Baada ya mwezi mmoja au zaidi ()

19. Iwapo unafahamu muda mnyama anapaswa kukamuliwa baada ya kutumia dawa, Je ? unafuata muda huo ?

    i) Ndio
    ii) Hapana

20. Je? ni vipi unatunza/wapi unapoweka madawa yanayotumika kutibia/wanyama wako

    i) Stoo ya shambani
       Ndio ()
       Hapana ()
    ii) Zizi la ng'ombe
       Ndio ()
       Hapana ()
    iii) Nyumbani
       Ndio ()
       Hapana ()
    iv) Sehemu nyingine
       Ndio ()
       Hapana ()

## II. ELIMU YA WAFUGAJI JUU YA DAWA ZA MIFUGO NA UFUGAJI

21. Uliwahi kusikia juu ya huduma bora za utunzaji wa ngombe wa maziwa, kati ya zifuatazo?

i) Lishe bora
   Ndio ()
   Hapana ()

i) Usafi bora
   Ndio ()
   Hapana ()

ii) Kinga dhidi ya magonjwa
   Ndio ()
   Hapana ()

iii) Chanjo
   Ndio ()
   Hapana ()

22. Je kila mara unaosha mikono yako kwa maji na sabuni kabla ukamuaji?

iii. Ndio ()
iv. Hapana ()

23. Je, kila unapokamua maziwa unaosha ngómbe kwa kutumia maji ya uvuguvugu kwa kitambaa safi?

iii. Ndio ()
iv. Hapana ()

24. Uliwahi Kutumia Milkoring Salve?

iii. Ndio ()
iv. Hapana ()

25. Je, huwa unashafisha mfugo wako kwa kitambaa safi kikavu wakati wa kukamua maziwa?

iii. Ndio ()
iv. Hapana ()

26. Je huwa unabinya chuchu wakati wa ukamuaji?

iii. Ndio ()

iv. Hapana ()

27. Je? unafaham kile kipindi mnyama anapomaliza tiba ya mwisho hadi kukamuliwa tena maana yake ni nini?

i) Ndio ()

ii) Hapana ()

28. Je, uliwahi kusikia juu ya madhara yafuatayo ya kiafya kwa binadamu yatokanayo na mabaki ya dawa za antibiotiki katika maziwa?

i) Kupata muwasho, vipele na uvimbe wa mwili

Ndio ()

Hapana ()

ii) Usugu wa madawa ya antibiotiki

Ndio ()

Hapana ()

iii) Kupungua Kwa Bakteria kinga wa Wei Wei

Ndio ()

Hapana ()

iv) Madhara ya sumu mwilini

Ndio ()

Hapana ()

v) Kupata maradhi ya kansa

Ndio ()

Hapana ()

vi) Kubadilika Kwa Selii Za Wei

Ndio ()

Hapana ()

29. Je ulishapata mafunzo ya namna ya kuhifadhi, kutumia au kuwatibia ng'ombe wako kwa kutumia dawa za antibiotiki? Weka vema (√)

i) Ndio ()

ii) Hapana ()

30. Je, uliwahi kupata mafunzo yafuatayo yanayohusiana na namna ya ufugaji?
    i) Muda gani wa kukamua ng'ombe baada ya matibabu ya dawa na faida zake
       Ndio ()
       Hapana ()
    ii) Utumiaji na namna ya kuhifadhi dawa za mifugo
       Ndio ()
       Hapana ()
    iii) Aina ya dawa za mifugo zinazotumika kutibu Ng'ombe
       Ndio ()
       Hapana ()
    iv) Matumizi ya dawa za mifugo kama kinga
       Ndio ()
       Hapana ()
    v) Umuhimu wa kuwatumia madaktaki wa mifugo kwenye matibabu ya mifugo
       Ndio ()
       Hapana ()
31. Kama unatibu mifugo yako mwenyewe, Je unazingatia yafuatayo kabla ka kutoa dawa?
    vii) Dozi husika ya dawa?
       Ndio ()
       Hapana ()
    viii) Umri/uzito wa mfugo/ng'ombe?
       Ndio ()
       Hapana ()
    ix) Hali ya kiafya ya mfugo/ng'ombe wako?
       Ndio ()
       Hapana ()
    x) Aina ya dawa inayotakiwa kutolewa?
       Ndio ()
       Hapana ()
    xi) Muda maalumu kusubiri hadi dawa ya awali uishe?
       Ndio ()
       Hapana ()

xii) Kuchina/kuisha mda wa matumizi ya dawa?

Ndio ()

Hapana ()

## III. MTAZAMO WA WAFUGAJI WA NG'OMBE

32. Je? unapata huduma za tiba ya mifugo kutoka kwa wataalamu mara kwa mara?

i) Ndio ()

ii) Hapana ()

33. Je, unapata changamoto zifuatazo unapotaka huduma za tiba ya mifugo yako kutoka kwa maafisa mifugo?

i) Huduma ya usafiri?

Ndio ()

Hapana ()

ii) Mazingira uliyopo ni vigumu kufikika?

Ndio ()

Hapana ()

iii) Kukosa fedha za malipo ya dawa na mtaalamu?

Ndio ()

Hapana ()

iv) Hujui wapi pa kupata huduma hizi za mifugo?

Ndio ()

Hapana ()

v) Huduma hizi hazipo kabisa kwenye eneo lenu?

Ndio ()

Hapana ()

**Appendix 5: Approval for ethical clearance**

**UNITED REPUBLIC OF TANZANIA**

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

**OFFICE OF THE DIRECTOR – RESEARCH, PUBLICATIONS AND INNOVATION**

Ref. No.DA.282/298/01.C/2027 Date: 14/02/2024

MUHAS-REC-02-2024-2027
JASPER KIMAMBO
SCHOOL OF PUBLIC HEALTH AND SOCIAL SCIENCES- MUHAS

**RE: APPROVAL FOR ETHICAL CLEARANCE FOR A STUDY TITLED: DETERMINATION OF TETRACYCLINE AND SULPHONAMIDE RESIDUES IN FRESH MILK OBTAINED FROM DAIRY FARMS IN KISARAWE DISTRICT AT PWANI REGION, TANZANIA, USING ELISA TECHNIQUE**

Reference is made to the above heading,

I am pleased to inform you that the Chairman has on behalf of the University Senate, approved ethical clearance of the above-mentioned study, on recommendations of the Senate Research and Publications Committee meeting accordance with MUHAS research policy and Tanzania regulations governing human and animal subjects research.

APPROVAL DATE: 14/02/2024
EXPIRATION DATE OF APPROVAL: 14/02/2025

**STUDY DESCRIPTION:**
**Purpose:**

"The purpose of this cross-sectional study is to determine the presence and concentrations of tetracycline and sulphonamide residues in dairy milk between January and March of 2024 in the Kisarawe district of Tanzania"

The approved protocol and procedures for this study is attached and stamped with this letter, and can be found in the link provided: https://irb.muhas.ac.tz/storage/Certificates/Certificate%20-%2014554.pdf and in the MUHAS archives.

**The PI is required to:**

1. Submit bi-annual progress reports and final report upon completion of the study.
2. Report to the IRB any unanticipated problem involving risks to subjects or others including adverse events where applicable.
3. Apply for renewal of approval of ethical clearance one (1) month prior its expiration if the study is not completed at the end of this ethical approval. You may not continue with any research activity beyond the expiration date without the approval of the IRB. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.
4. Obtain IRB amendment (s) approval for any changes to any aspect of this study before they can be implemented.
5. Data security is ultimately the responsibility of the investigator.
6. Apply for and obtain Data Transfer Agreement (DTA) from NIMR if data will be transferred to a foreign country.
7. Apply for and obtain Material Transfer Agreement (MTA) from NIMR, if research materials (samples) will be shipped to a foreign country.
8. Apply for and obtain permission to publish the results from National Institute for Medical Research. The application should be before submitting to a publisher and not concurrently through the email publications@nimr.or.tz.
9. Any researcher, who contravenes or fail to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III section 10 (2)
10. The PI is required to ensure that the findings of the study are disseminated to relevant stake holders.
11. PI is required to be versed with necessary laws and regulatory policies that govern research in Tanzania. Some guidance is available on our website https://drp.muhas.ac.tz/.

Muhimbili University of Health and Allied Sciences
DIRECTOR
Research, Publications & Innovations
Box 65001

Dr. Nahya Salim Masoud,
**CHAIRPERSON, MUHAS RESEARCH AND ETHICS COMMITTEE**

## Appendix 6: Introduction letter

**UNITED REPUBLIC OF TANZANIA**
MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY
MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
**OFFICE OF THE DIRECTOR – POSTGRADUATE STUDIES**

In reply quote;
Ref. No. HD/MUH/T.846/2022 21st February, 2024

District Executive Director,
P.O. Box 28001,
**KISARAWE.**

**Re: INTRODUCTION LETTER**

The bearer of this letter is Jasper Kimambo (HD/MUH/T.846/2022), a student at Muhimbili University of Health and Allied Sciences (MUHAS) pursuing MSc Epidemiology and Laboratory Management.

As part of his studies he intends to do a study titled: **"Determination of Tetracycline and Sulphonamide Residues in Fresh Milk Obtained from Dairy Farms in Kisarawe District at Pwani Region, Tanzania, Using Elisa Technique."**

The research has been approved by the Chairman of University Senate.

Kindly provide him with the necessary assistance to facilitate the conduct of his research.

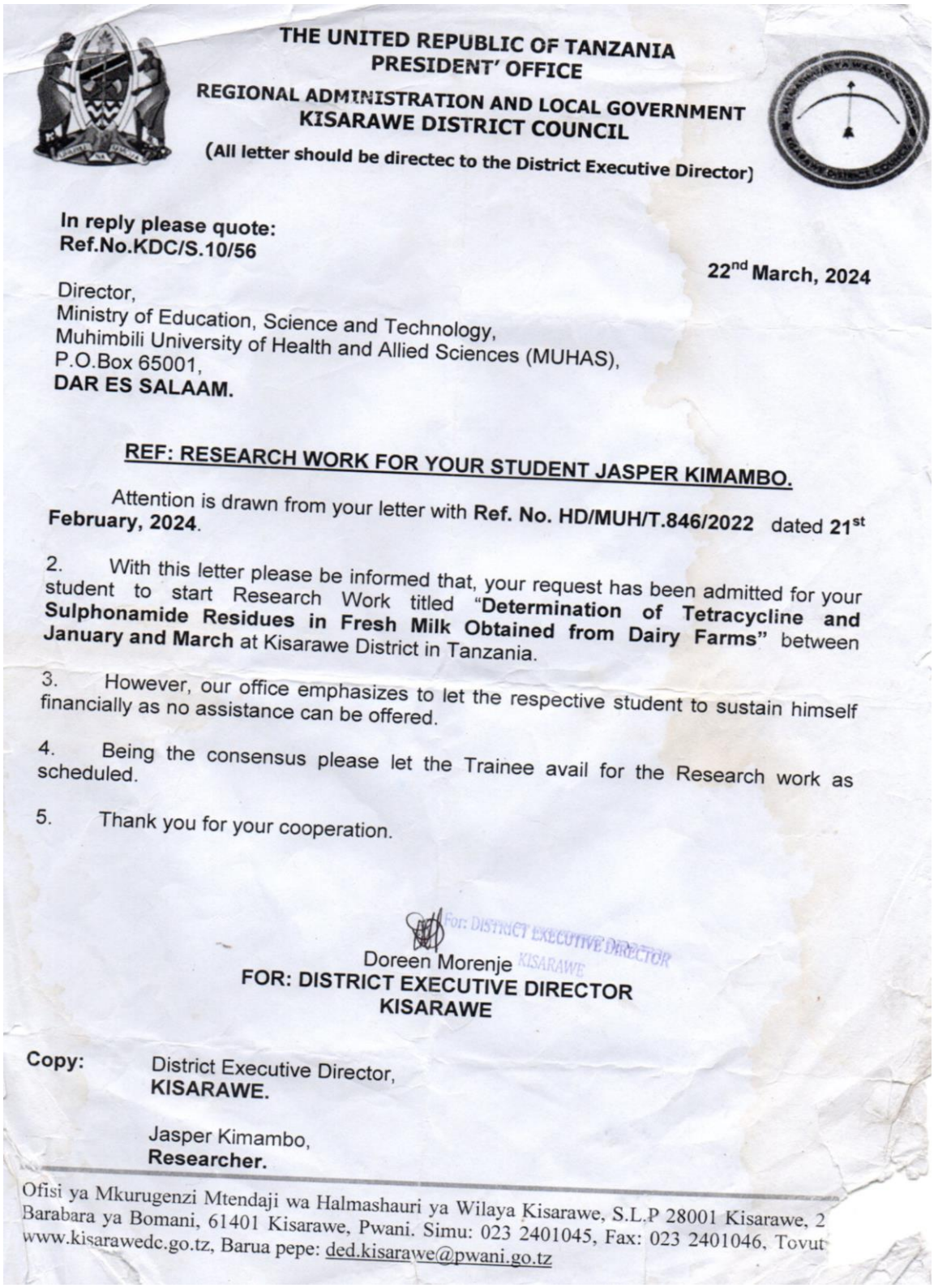
We thank you for your cooperation.

*Ms. Victoria Mwanilwa*
**For: DIRECTOR, POSTGRADUATE STUDIES**

c.c. Jasper Kimambo

9 United Nations Road; Upanga West; P.O. Box 65001, Dar Es Salaam: Tel. G/Line: +255-22-2150302/6; Ext. 1015; Direct Line:+255-22-2151378;Telefax:+255-22-2150465;E-mail:dpgs@muhas.ac.tz;Web:https://www.muhas.ac.tz

**Appendix 7: Permission letter**



THE UNITED REPUBLIC OF TANZANIA
PRESIDENT' OFFICE
REGIONAL ADMINISTRATION AND LOCAL GOVERNMENT
KISARAWE DISTRICT COUNCIL
(All letter should be directec to the District Executive Director)

**In reply please quote:**
**Ref.No.KDC/S.10/56**

**22nd March, 2024**

Director,
Ministry of Education, Science and Technology,
Muhimbili University of Health and Allied Sciences (MUHAS),
P.O.Box 65001,
**DAR ES SALAAM.**

**REF: RESEARCH WORK FOR YOUR STUDENT JASPER KIMAMBO.**

Attention is drawn from your letter with **Ref. No. HD/MUH/T.846/2022** dated **21st February, 2024**.

2. With this letter please be informed that, your request has been admitted for your student to start Research Work titled "**Determination of Tetracycline and Sulphonamide Residues in Fresh Milk Obtained from Dairy Farms**" between **January and March** at Kisarawe District in Tanzania.

3. However, our office emphasizes to let the respective student to sustain himself financially as no assistance can be offered.

4. Being the consensus please let the Trainee avail for the Research work as scheduled.

5. Thank you for your cooperation.

For: DISTRICT EXECUTIVE DIRECTOR KISARAWE

Doreen Morenje
**FOR: DISTRICT EXECUTIVE DIRECTOR**
**KISARAWE**

**Copy:** District Executive Director,
**KISARAWE.**

Jasper Kimambo,
**Researcher.**

Ofisi ya Mkurugenzi Mtendaji wa Halmashauri ya Wilaya Kisarawe, S.L.P 28001 Kisarawe, 2 Barabara ya Bomani, 61401 Kisarawe, Pwani. Simu: 023 2401045, Fax: 023 2401046, Tovut www.kisarawedc.go.tz, Barua pepe: ded.kisarawe@pwani.go.tz