ANTIBIOTIC RESIDUES IN RAW MILK PRODUCED IN COMMERCIAL FARMS UNGUJA, ZANZIBAR

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By

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A Dissertation Submitted in (Partial) Fulfillment of the Requirements for the Degree of Master of Science in Epidemiology and Laboratory Management of the Muhimbili University of Health and Allied Sciences

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CERTIFICATION FOR EXAMINATION

The undersigned certify that have read and hereby recommend for examination of the dissertation entitled **'**Antibiotic Residues in Raw Milk Produced in Commercial Farms Unguja, Zanzibar**.'',** in fulfillment of the requirements for the degree of Master of Science in Epidemiology and Laboratory Management of Muhimbili University of Health and Allied Sciences.

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DECLARATION

AND

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ABBREVIATION

FAO	Food and Agriculture Organization
JECFA	Joint Expert Committee on Food Additives
MVM	Milk Vendor Machine
OTC	Oxytetracyline
DVO	District Veterinary Officers
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

DEFINITIONS

Terminology	Meaning
Antibiotics	Substances either produced naturally by living organisms or synthetically in the laboratory which are able to kill or inhibit growth of microorganisms in small quantities
Antimicrobials	Substances that inhibit the growth or destroy bacteria and other types of microbes.
Antibiotic resistance	This refers to situations where antibiotics that were previously known to inhibit or destroy certain kinds of bacteria fail to express the desired effect
Antibiotic residues	Traces of antibiotics in edible materials
Composite sample	A milk sample containing a mixture of milk from the teats of the same cow.
Nitrocellulose	Is the globally preferred membrane substrate in diagnostic lateral flow assays where antigen-antibody binding occurs,
Colloidal particles	Are small solid particles that are suspended in a fluid phase.
Acceptable Daily Intake (ADI)	Is the amount of a veterinary drug, expressed on a body weight basis, that can be ingested daily over an entire human lifetime without any appreciable health risk

ABSTRACT

Background:

Dairy farming is one among the major sources of income for most of the people in Zanzibar. Although dairy production has been improved, there are a number of challenges facing milk industry in the island. There are no specific strategies to ensure that the milk at the farm level and that found at the marketing system is free from chemical agents. Equally, little is known regarding the level of knowledge to farmers and consumers on effects of antibiotic residues and resistance and factors associates with antibiotic residues in milk.

Objectives: To determine the proportion of antibiotic residues and its associated factors in raw milk production in commercial farms in Unguja, Zanzibar.

Methodology: A cross-sectional study was conducted in Unguja between March to June 2020.

A total of 136 farmers were included in this study from which 272 composite milk samples were collected from their farms. Information on farmers' knowledge to raw milk safety in particular antibiotic residues, practices related to raw milk safety and main factors associated to antibiotic contamination in milk was collected. Bivariate and multivariate analysis logistic regression was used to identify factors associated with antibiotic residues in raw milk.

Result. About half (49.6%) of the milk samples were positive with antibiotic residues. Only 25% percent adhere to withdrawal period. The risk of producing milk contaminated with antibiotic residues was almost 3 times more in farmers who do not have knowledge on withdrawal period compared to those who have knowledge although it was not statistically significant [AOR=2.82 95% CI=0.57-13.87, P- value 0.19].

Likewise, the risk of producing milk contaminated with antibiotic residues was almost three times higher in those farmers who obtained drugs from private drug sellers compared to those who obtained drugs from vet professionals although not statistically significant [AOR=2.81 95% CI=0.50-15.87, P-value 0.24]. Moreover, farmers who received training on drug

administration were 2 times less risky in producing antibiotic contaminated milk than those who did not receive training, although was not statistically significant AOR=2.10 [0.54-8.11, P-value 0.27].

Conclusion: About half of the milk samples obtained from famers had antibiotic residues. In addition, it was apparent that most farmers had limited knowledge and practices with regard to adhering to withdrawal period, understanding health effects on antibiotic residues consumption and milk safety and hygiene. Obtaining drugs from private drug sellers was also associated with antibiotic residues in milk. Specific strategies are therefore required to ensure that the milk at the farm level and that found at the marketing system is free from antibiotic residues.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Antibiotics are one of substances that are usually used for medicinal purposes in animal husbandry. They are used in higher doses for the purpose of treatment or sometimes used in small doses as prophylactic agents as well as growth promoters (1). Antibiotic contamination in milk not only the problem in many African countries but is also a major challenge and threat worldwide regardless of economic, geographical or legal differences which are existing among countries (2).

Centres for disease control and prevention (CDC) in the United States has recently reported that the current estimation of the antibiotic threat is higher (i.e. 2.8 million antibiotic resistant infections occur annually with an approximated number of deaths of 35,000 people). This is higher than what was estimated previously. However, there has been a decrease in number of deaths by 18% since the 2013 report (3).

The maximum residue limits (MRLs) for pesticide residues and residues of veterinary drugs are the maximum concentrations of residues to be permitted in or on food by national or regional legislation. MRLs are set by the Codex Alimentarius Commission (CAC), acting as the risk manager (4) .

Transfer of resistant bacteria species to humans due to residues of antibiotics in food can lead to pathological effects such as autoimmunity, immunopathological effects, carcinogenicity (sulphamethazine, oxytetracycline, furazolidone), mutagenicity, nephropathy (gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity (chloramphenicol) and allergy (penicillin) (5) . Despite the fact that there are several reasons that give rise to antimicrobial resistance in bacteria infecting humans, the main contributing factor is the use of antimicrobials in both humans and food producing animals (6).

Unless there is a change in the trend of the use of antibiotics/antibacterial drugs worldwide, there is a possibility on the emergence of many bacterial diseases due to increased resistant pathogenic environmental organisms in the coming years which may result to high number of deaths in both humans and animals, going back to the pre-antibiotic golden age (7).

Both systematic reviews (8) and narrative literature reviews (9) have revealed that controlling the use of antimicrobials in food producing animals decreases the prevalence of their resistance in bacteria isolated from those animals that are, and can be spread to humans (6). Scientists worldwide have evidenced that resistant infections in humans can be the result of using antibiotics in food animals (10)

In Tanzania, there has been insufficient effort in either identifying the practices of the producers or market agents, or quantifying the prevalence of antibiotic residues in marketed milk throughout the country. The study which was carried out in Mwanza and Dar-es-salaam, revealed contamination of 36% of 986 raw milk samples in the marketing system (11). Screening of raw milk from Pemba, detected 83% of 98 samples contaminated with antimicrobial residues above the East African standards (12).

Therefore, the purpose of this study is to determine qualitatively the proportion of antibiotic residues in raw milk, knowledge and practices used by farmers as well as its associated factors, in Unguja island of Zanzibar.

1.2 Problem statement and justification

Dairy farming is one among the major enterprises, with an estimated 42% of the people in Zanzibar considered to be their main source of income (13) . Although dairy production has been improved, there are a number of challenges facing milk industry in the island. There have been no specific strategies to ensure that the milk at the farm level and that found at the marketing system is safe and is of the required standards.

Furthermore, there have been no sufficient efforts in monitoring milk safety concerning chemicals in animal products and protecting the consumers from unintentional consumption of their residues in milk and it's by products which may pose health risk to the public.

There is limited knowledge on magnitude and types of antibiotic residues in raw milk in Zanzibar. A study conducted in Pemba by S.H Gwandu et al, detected presence of antibiotic residues in raw milk but could not determine the types of the antibiotics. Therefore to add on what has already been established, this study aimed at determining presence and magnitude of contamination of raw milk with antibiotic residues and types of the antibiotic residues in raw milk in Unguja.

The information obtained from this study will be shared with relevant authorities and stakeholders so that appropriate measures can be taken to address issues related to antibiotic residues in raw milk in Zanzibar which in turn can contribute in protecting health of milk consumers and improve public health.

1.3 Conceptual frame work

The figure below illustrates the association of different factors to antibiotic residues in the farm milk. Different studies have shown that misuse of the drugs, failure to adhere to withdrawal periods, common diseases affecting dairy cows are among the major factors leading to contamination of the milk, making it of poor quality and unsafe for human consumption.

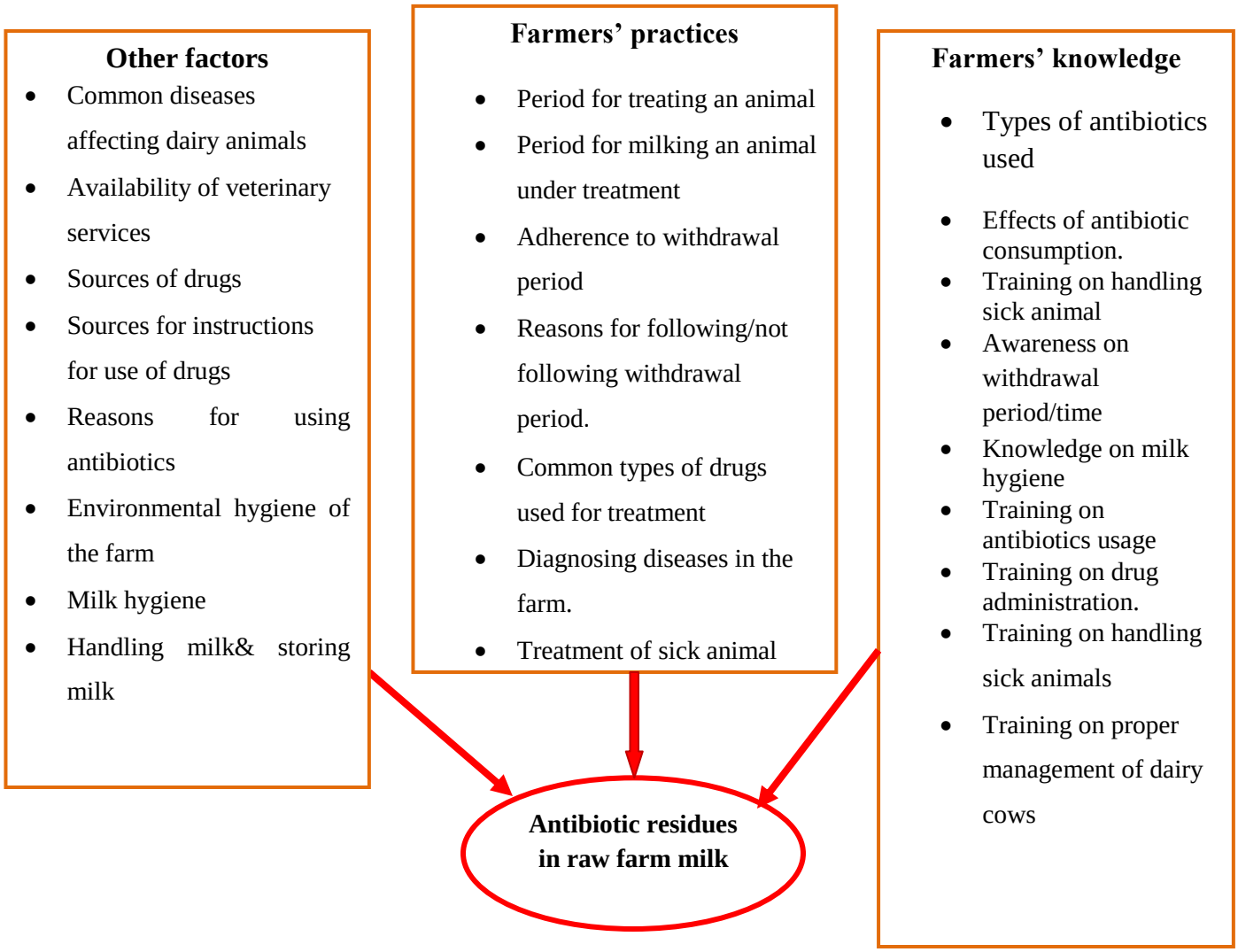

Figure 1: Interaction of various factors leading to milk contamination in the farm.

1.4 Rationale

Antibiotic contamination is a major challenge & threat worldwide (2). With reliable identification systems, antibiotics can be kept out of dairy milk and minimize the health and economic dangers posed by them (14). The findings of this study will provide significant information on the magnitude of the problem reflecting to the major milk production areas in the country as a whole.

Research findings will be forwarded to the Department of Livestock Development in Zanzibar as well as to farmers and other key stakeholders and will aid in planning appropriate interventions in curbing the presence of antibiotics in milk.

The study will also create awareness to farmers and the society in general about the potential hazards posed by antibiotic residues in milk and other food products in general.

1.5 Research questions

1. What are the main types of antibiotics present in dairy milk marketed in Unguja?
2. What are the main contributing factors to antibiotic contamination in milk?
3. What is the level of farmers' knowledge on effects of consuming antibiotic residues through animal products?

1.6 Objectives

1.6.1 Broad objective

To determine the proportion of antibiotic residues contamination in the raw milk and farmers' knowledge and practices that contribute to antibiotic residues in dairy milk in Unguja.

1.6.2 Specific objectives

1. To determine the proportion of farms with raw milk containing antibiotic residues.
2. To determine the main types of antibiotics in marketed milk in Unguja.
3. To determine farmers' knowledge and practices related with antibiotic residues in raw milk
4. To determine the main practices associated to antibiotic contamination in milk.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Antibiotic resistance threat

Antibiotic resistance is one of the biggest public health challenges in the world. Each year in the U.S., at least 2 million people get an antibiotic-resistant infection, and at least 23,000 people die (10). The excessive and abused uses of the drugs have resulted to a high diversity of pathogenic bacteria which are resistant to antimicrobials that can spread among a wide range of animals' species and to humans (6). The risk of developing resistance is higher in people who are under medical care or immune suppressed (3). Therefore fighting this threat is a public health priority that requires a collaborative global approach across sectors (10).

A lot of antibiotics that are utilized in food-producing animals are similar or so much linked to antibiotics used in humans (6).There is strong evidence from the scientists worldwide, that the use of antibiotics in food animals can result to resistant infections to humans (10). Reports show that about 75% of antibiotic drugs manufactured in the world are used for chemoprophylaxis and treatment of animals as well as growth promoters (7). Antimicrobial use in food producing animals can result to spreading of varieties of antimicrobial-resistant bacteria in food producing animals which can then be passed on to humans via food and other routes (5)

Many African countries have been recording extensive antibiotic residues levels in animal-derived foods exceeding the recommended WHO maximum residue limits (2).

In the environment point of view, extensive environmental pollution with antibiotics can slow down the natural processes taking place in various ecosystems and destroy their functioning systems, which can result to the emergence of pathogenic resistant bacteria. For a long period, humans and animals have been sharing and profiting from different population of microbes, particularly bacteria in our environment (7).

Pathological effects of antibiotic residues e.g. autoimmunity, immunopathological defects, carcinogenicity (e.g. sulphamethazine, oxytetracycline and furazolidone), mutagenicity, nephropathy (e.g. gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity (e.g. chloramphenicol) and allergic reaction (e.g. penicillin) can also result from the transfer of resistant bacteria species to humans from food containing antibiotics residues (5).

Antimicrobial resistant bacterial infections may have more serious complications in humans compared to infections caused by antimicrobial susceptible bacteria. These complications include failure in disease treatments, prolonged hospital stays and long duration of sickness (6).There is also a possibility for the world to step back to the pre-antibiotic golden age if the direction on the use of antibiotics is not changed which might result to the emergence of many bacterial diseases in the coming years (7).

Most countries have established regulations concerning acceptable and maximum residues levels of veterinary drugs in food. International and local bodies have been established to set various rules and regulations so as to observe adherence to withdrawal periods following antibiotic usage and to guarantee that the ideal procedures for handling and selling milk are practiced in order to protect the public from different potential hazards. Examples of international legislations are European Union Maximum Residues Limits and Codex Alimentarius Commission (15). In Tanzania, Tanzania Bureau of Standards formerly known as Tanzania Food and Drug Authority is the local body authorized for ensuring the acceptable and unacceptable standards. The TFDA was working based on the standards set by the Codex Alimentarius Commission. Currently there are drafted copies for the TBS and East African Standards for actual implementation. The Drafted MRL's for the new bodies shall comply with the current maximum residue limits for pesticides and veterinary drugs established in the Codex Alimentarius Commission CAC/MRL 2 (16–18).

According to the Codex Alimentarius Commission, the acceptable daily intake (ADI) in milk for the tetracycline group is 0-30µg/kg body weight while that of maximum residues limit (MRL) is 100µg/litre. The acceptable daily intakes for betalactams should be below 30µg/person/day for benzyl penicillin and procaine benzyl penicillin, amoxicillin 0-0.002

mg/kg body weight and ampicillin 0.003mg/kg body weight, the maximum residues limits for each is 4µg/liter. The acceptable daily intake for streptomycin and dihydrostreptomycin is 0-50µg/kg body weight and that of maximum residues limit for the group is 200µg/kg. The acceptable daily intake for gentamycin is 0-20µg/kg body weight and its maximum residues limit is 200µg/liter. The acceptable daily intake for sulphadimidine in milk is 0-50µg/kg body weight while the maximum residues limit is 25µg/liter (16).

Similarly, according to the European Union, the maximum residues limits for betalactam e.g. benzylpenicillin, ampicillin and amoxicillin is 4µg/kg of milk. Others oxacillin, cloxacillin and dicloxacillin have 30µg/kg maximum residues limit. In the tetracycline group the maximum residues limit is 100µg/kg of milk for chlortetracycline, oxytetracycline and tetracycline .Unlike in the Codex Alimentarius Commission, the maximum residues limit for gentamycin in UE is 100µg/kg of milk. Streptomycin has the maximum residues limit of 200µg/kg of milk. All members of the sulphonamide group have the maximum residues limit of 100µg/kg of milk (19).

According to Article 6 of Regulation (EC) No 470/2009, Methodological principles for the scientific risk assessment referred to Annex I, section III.3. Metabolism and residue kinetics in the target species, subsection III.3.9 and III.3.10 and Article 7 of Regulation (EC) No 470/2009, Methodological principles for the risk management recommendations referred to Annex II, Elaboration of MRL's I, subsection I.1.2.a. The maximum residues limits (MRL's) are set at the levels below the acceptable daily intake (ADI) that will enable the consumer to be protected from being exposed to the residues of concern. Therefore acceptable daily intake (ADI) is taken as the starting point from which the MRL's shall be established (20)

2.2 The proportion of antibiotic residues in raw milk

At the present, antibiotic residues in food have become a worldwide problem. This is mainly due to its association with health conditions such as hypersensitivity reactions, antibiotic resistance, toxicity, teratogenicity, and carcinogenicity (21)

A study in Kenya where 1,600 milk samples were tested using improved two tube diffusion test and beta-lactam plate assay, 14.5% were tested positive to beta-lactam residues (22).

Similarly, in another study, a total of 229 samples in the rural dairy system revealed 72 (31.4%) positive samples while in peri-urban dairy system, 23 of 80 (28.8%) were also found positive. Number of farms with positive results was somehow higher in the rural areas compared to those in the peri-urban area. This indicates high intake of residues as consumers normally purchase the milk directly from the farm (23). On the other hand, a study which involved milk vender machine (MVM) and street vendors' milk samples in Eldoret and Nakuru counties, revealed the prevalence of 23.6% (13/55) and 28.8% (23/80) respectively (24). A small study in Sudan where 30 samples were collected from farms and 20 samples from milk markets also revealed a prevalence of 6% where 3 samples were found positive.

In Tanzania, a study which was carried out at Mwanza and Dar-es-salaam using a qualitative test of Charm AIM test, revealed high prevalence (36%) of antibiotic residues in milk which exceeded the maximum allowed concentration MRL's (11). Similar study conducted at Pemba also indicated the highest prevalence of 82.7% by a qualitative test Delvo SP test. This is probably due to small sample size used in the study (98) and also due to test limitation which is inhibited by milk natural inhibitors such as lactoferrin, lactoperoxidase, lysozyme, and N-acetyl-ß-D glucosaminidase, all having antimicrobial characteristics of the possibility of inhibiting the growth of test bacteria used in the Delvo SP test i.e. *Bacillus stearothermophilus var.calidolactis* (12).

Another study conducted at Bagamoyo in small holder dairy farmers, revealed a low prevalence of 10% contamination of oxytetracycline in the tested milk using a qualitative

microbial inhibition test (25). More interestingly, in another study performed at Arusha city and Meru district council, all milk samples (105) were found to be free from antimicrobial residues using Delvotest, this shows that there have been different findings on antimicrobial milk contamination in the country, varying from place to place concluding that they are still not representing the actual magnitude of the problem in the country (26).

On the other hand, a quantitative method (High performance liquid chromatography) which was carried out from the 11 positively oxytetracycline screened milk samples using microbial inhibition test in Bagamoyo district, were all (100%) confirmed positive by HPLC, at a level above oxytetracycline maximum residue's limits (MRL's) of 100µg/l. This makes an average of 766.278µg/l (i.e. the average of the positive ones) which is nearly eight times above the suggested codex maximum residue's limits for milk (25).

High performance liquid chromatography is one of the most popular and powerful tools in analytical chemistry that has the capacity to separate detect and quantitate any dissolved substance in a fluid. Nowadays even trace concentrations of substances to a lowest level e.g. parts per trillion (ppt) can be simply detected (27). Similar suggestions have been reported by (28–30). More studies are needed to reveal the actual situation and to find out the mechanisms of solving the problem of antibiotic residues contamination, particularly at the farms where regulations such as adherence to withdrawal periods after treatment is usually not practiced (11).

Other tests used include microbial growth inhibition assays e.g. Delvotest P, Brilliant Black Reduction Test, Disk Assay for Milk. Microbial receptor assays e.g. the CHARM II tests and I. Enzymatic colorimetric assays e.g. the P enzyme test. Receptor binding assays e.g. the SNAP and Delvo-XPress tests and immunoassays e.g. enzyme immunoassay (EIA), enzyme-linked immunosorbent assay. These tests are either qualitative, quantitative or semi-quantitative (31).

Qualitative assays: Use specific fixed cut-off value to grade the samples into positive or negative depending on the actual drug concentration, Examples of these assays are microbial

growth inhibition assays, enzymatic colorimetric assays, microbial receptor assays, receptor binding assays etc. (31).

Quantitative assays: Require that positive controls covering a wide range of drug concentrations be tested with each sample set, thus permitting residue quantitation by extrapolation from a standard curve.

Such assays require precise instrumentation to measure the test response and determine the standard curve. Examples of these assays are chromatographic methods (31).

Semi quantitative assays: These are the same as quantitative assays but differ in that the interpretation of results is based on ranges of drug concentrations (e.g. negative, low positive, high positive). This is due to the reflection of positive controls used in running the test samples. Examples are ELISA tests (31).

The choice of a suitable screening test for antimicrobial residues in milk will depend on awareness of the commonly used antimicrobials in dairy farms (32). None of these tests can satisfactorily determine all antibiotics and therefore a direct confirmation to a family antibiotic level can be carried out by adding a post–screening test, which can be an immunological test such as Enzyme-linked immunosorbent assay, which are normally quite costly. Antibodies production for screening the whole family of antibiotics is the drawback of these methods (33). Residues assays for field use are mainly qualitative or semi-quantitative and are normally referred as screening assays (31).

2.3 Main types of antibiotics present in milk

The main types of antimicrobials used in dairy production are oxytetracycline which is commonly used, followed by beta-lactams e.g. pen-strepto also sulphonamides and aminoglycosides e.g. gentamycin, streptomycin, neomycin (11,12,26) which are among the commonly used drugs in the world. Aminoglycoside group gentamicin and streptomycin were reported to be the major antibiotics used in livestock production in Lebanon (34)

A study at Kiambu, Nairobi and Nakuru districts detected beta lactam, sulphonamide, aminoglycoside, tetracycline and macrolide in milk at the level above the European Union maximum residues limits (35). In another study carried out rural and peri urban areas in Kenya detected sulfachloropyradizine, sulfadiazine, sulfadimidine, sulfaquinoxaline, sulfamerazine, sulfathiazole , sulfamethoxazole , sulfadoxine and sulfamethazine (23).

In Tanzania oxytetracycline, penicillin and streptomycin, sulphonamides and gentamycin are reported to be the common drugs used in dairy farming (11,12,25,26). In a study at Bagamoyo, oxytetracycline ranked to be number one, followed by pen & streptomycin, sulphonamides and gentamycin (25). Similarly, a study conducted at Tanga, Coast and Morogoro regions revealed that tetracycline and sulphur were mostly used by farmers and that 33% of 328 milk samples were contaminated with the two drugs (36).

2.4 Farmers' knowledge on raw milk safety and antibiotic residues

Antibiotic residues in milk can come from different channels that farmers should be aware (11). Milk production starts at the farm, antibiotics residues normally get into the milk at the farm level and therefore it is the farmers' responsibility to ensure that the milk produced and sold from his/her farm is free from such residues (37). A study conducted in Bagamoyo indicated 91.8% of the farmers were aware of the effects of consuming antimicrobial residues in raw milk and 96.4% awareness on withdrawal periods. Another study conducted at Kinondoni and Morogoro districts, reported lack of knowledge on effects of antibiotics consumption to human health, when they are used for different purposes in animal production (38). On the other hand it was revealed that 71.4% of farmers were not aware on the antimicrobial types used for treatment (26). Also most of them either neglect or are unaware on the effects of antibiotic/antimicrobial residues in milk and its products (12).

In Tanga dairy co-operative union (TDCU) where training programmes that involve all stakeholders in the milk production value chain are usually conducted, no antimicrobial residues were detected in Tanga milk samples (36). Therefore increased awareness to farmers

on the antimicrobial residues and their consequences in animal foods and by products is required and also on their restricted use in animal production (12).

2.5 Farmers' practices on raw milk safety and antibiotic residues

Studies have shown a higher problem of antibiotic residues in the developing poor countries where veterinary services have been privatized and are expensive (7). In addition, another major cause of unaccepted residues of veterinary drugs in animals' products, is the breakdown to adhere to the recommended withdrawal periods (12). Generally it is the mandate of the veterinarian to take care of the sick animal and farmers should only treat their animals with antibiotics/antibacterial drugs after being advised by the veterinarians, unfortunately most of these drugs have been misused by veterinarians and many farmers (7). In addition, implementation of strict legislation in order to reduce the misuse of antibiotics should be practiced (21).

Increased antibiotic levels in milk means that there is irregular utilization of antimicrobials in production activities and that farmers do not follow withdrawal conditions (12). Most of the drugs are applied by veterinary extension officers with the exception of a few like kanamycin which is used for intramammary infusions (26).

Different routes used to administer antibiotics to the animal's body, may result to the appearance of residues in foods of animal origin such as milk, meat and eggs, these include injection routes such as intramuscular, intravenous, subcutaneous, orally in the food and water and topically on the skin, intramammary and intrauterine infusions (31). The type of drug used, dosage, route of administration, and animal species are the basis for determining the withdrawal periods (12).

2.6 Factors associated to antibiotic contamination in milk

Many countries have been using antibiotics in the treatment and prevention of udder complications as their main mastitis control strategy (39).

The informal milk marketing systems in some of developing countries thought to be existing because they serve as social and profitable sources to smallholder producers, small market agents and consumers in terms of cheaper prices, income generating activities and competition of prices for consumers (40). The price for the formal and pasteurized milk in the market is higher compared to that of the raw milk which can be 20-50% lower; in addition the unprocessed milk can be purchased at any quantity which gives the poor people a chance to buy the product at an affordable price (41).This seems to speed up the informal marketing system.

According to Kurwijilla et al, (11), contributing factors to high prevalence of antimicrobial residues are mastitis treatment, vector borne diseases and addition of preservatives as an alternative of unavailability of refrigeration. Mastitis was again reported by Ridhiwani followed by east coast fever, anaplasmosis, trypanosomiasis, and foot and mouth disease as common diseases in dairy husbandry (25). All these diseases contribute to the appearance of antimicrobial residues in milk.

On availability of antibiotics for animal treatment, some studies revealed high proportion of farmers (80.9%) having access to them and (64.4%) not trained on handling and uses of those drugs and thus rely on drug sellers for the instructions of use. Other sources are pharmacy (41.1%), veterinary officers (48.2%) and lastly 2.7% from livestock markets (25). All these have association to increased contamination of milk with antibiotic residues. On observation, poor environmental conditions on cow sheds and milking areas, poor milking practices and milkers' hygiene, poor cleanliness of the milking and milk storage equipment and also poor handling and storage of milk were among the associated factors leading to poor milk quality in Pemba (12).

Limited knowledge among stakeholders e.g. in observing good handling practices and in improved ways on milk collection, storage and marketing is also a contributing factor in which creation of awareness is a very important option (36).

Other factors include poor animal health delivery systems and limited extension services (12). Also farmers' economic status is the major factor of antibiotic residues presence because for many of them to discard the contaminated milk, will lead to loss of income (42).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study design and duration

This was a cross- sectional analytical study involving dairy farmers from four districts of Unguja that was conducted from March 2020 to June 2020.

3.3 Study setting

The study was conducted in four districts West A, West B, Central and North B in Unguja Island. The districts were purposely selected based on their high number of dairy farmers and more milk production.

3.4 Study population

Healthy lactating cows of at least 2 weeks after calving and milked for the purpose of milk marketing or consumption.

3.5 Sample size determination

Sample size was calculated using the following formula $n = Z^2 p \frac{(1-p)}{d^2}$ (43)

Where Z^2 = confidence limit 1.96^2

P = Prevalence (36%) 0.36 (11)

d^2 = 6 % Precision (0.06)

A Sample size was = 245 lactating cows. With an assumption of a response rate 90% (0.9), the sample size was 272 lactating cows.

3.6 Sampling method

The study involved 2 stages of selection. The first stage involved purposive selection of districts, based on milk production and availability of dairy farmers in those areas. The second stage involved random selection of farms from each district. Four out of seven districts were selected due to budget constraints. Purposive selection of these four districts based on the fact that they are the leading districts in the number of dairy farmers and production in Unguja. The selected districts are Central, North B, West A and West B. A list of dairy farms from department of livestock development was used as a sampling frame to select farms in those districts. Simple random selection was used to select 136 dairy farms, 34 farms from each of the four districts. From each of the selected dairy farm, 2 lactating cows were randomly selected making a total of 272 lactating cows. From each of the selected cow $5cm^3$ of milk sample was taken for investigation. An on-line random generator programme was used for random selection (Http://stattrek.com/statistics/random-number-generator.aspx). 136 farmers (1 from each farm) were interviewed on the risk factors for contamination of raw milk with antibiotic residues.

3.5 Inclusion criteria

Any healthy lactating cow of at least 2 weeks after calving, which was being milked for the purpose of milk marketing or consumption.

3.6 Exclusion criteria

Non-lactating cow or any lactating cow, which was under treatment and that, the farmer has decided not to milk the cow for marketing or consumption.

3.7 Study variables

3.7.1 Dependent variables

The outcome variable is a dichotomous variable that is presence or absence of antibiotic residues in raw milk. Presence or absence of the antibiotic residues was detected by test strips impregnated with specific antibiotic group that can detect both presence/absence and the type of the antibiotic. The screening test results were then analysed and interpreted**.**

3.7.2 Independent variables

The following were the independent variables in the study such as major diseases affecting dairy cows, drugs used for disease treatment, sources of drugs, sources for instructions for use of drugs, equipment used for milking and storing milk, availability of veterinary services, understanding milk hygiene, awareness on effects of antibiotic residues consumption in humans, awareness on withdrawal periods for antibiotic uses, trainings on proper management of dairy animals, proper management practices, handling sick animals, drug administration, treatment of sick animals etc Interviews using semi structured questionnaires and observations helped us gain the actual insight on farmer's attitude, understanding and practices as well as the general environmental condition of the farms.

3.8 Data collection methods

Questionnaires (semi-structured), interviews with selected farmers and direct observations in all farms were used to collect information linked to causal factors. More focus was on major drugs used for treatment, disease control and prevention, frequency of use, sources of drugs and instructions for use, antibiotics withdrawal periods, common diseases which necessitate farmers' use of drugs, milk handling/storage, awareness on antibiotic residues effects etc. Questionnaires were translated in Swahili for easy understanding of the questions. Research assistants (districts' veterinary officers) were trained on how to use the questionnaires and on proper sampling procedures. Data was collected at the farm site of each individual farm.

The types of antibiotics analysed in the samples were betalactams (e.g. penicillin, cloxacillin, dicloxacillin), others were tetracyclines (e.g. tetracycline, oxytetracycline, chlortetracycline), aminoglycosides (e.g. gentamycin, streptomycin) and sulphonamides (e.g. sulphamethazine etc).

Milk collection and antibiotic residues testing

Milk collection:

Composite milk samples were collected aseptically from lactating cows in selected farms. At least half a universal bottle containing a mixture of milk from all the teats was taken from each cow. The bottles were then labeled properly and transported in a cool box to the Central Veterinary Laboratory at the Department of Livestock Development Maruhubi, Zanzibar to be tested.

Laboratory investigations

The test strips of KonRun Biological Technology Company limited from China were used to screen for ß-lactams, tetracyclines, sulphonamides, streptomycin and gentamycin in raw milk according to manufacturer's instructions. The kit was based on the specific reaction of antibody-antigen and immunochromatography. Group of antibiotics in the samples compete for the antibody with the antigen coated on the membrane of the test strip, then after a specified time period a colour reaction occurs, the result can be observed using naked eyes.

Immunochromatographic tests depend on the principle of capturing the target antigen or antibody from different samples. The procedure uses a nitrocellulose membrane or a paper strip as an immobile capture antibody test area which is mounted with antibodies/antigen. The colloidal gold or coloured microparticle-labeled antibody conjugate is moved through the capillary flow which is then attached to the target antigen/antibody in the mobile phase while moving to the captured antibody/antigen in the immobile phase. The capture of the moving labeled antigen/antibody complex by a second immobilized anti-species antibody in the test area result to the formation of a coloured line which indicates a positive result. A control line

is formed by another control antibody conjugate attached to the extra colloidal dye conjugate and functions as an indicator for the test validity (44).

The test kit used was for qualitative testing and could only detect the presence or absence of antibiotic residues in the milk samples, hence it could not discriminate between the samples with acceptable antibiotic residues levels from unacceptable levels.

Testing procedures

Milk of about 200ul was dispensed into the microwells and pipetted up &down for10 times to mix the reagent completely. The mixture was kept for 5 minutes and then test strips were inserted (fully dipped) into the microwells and the results were read within 5 minutes based on the manual instruction.

3.9 Data Analysis

Data was handled using Microsoft Excel and Epi info 7. Parameters such as proportions, mean, standard deviation were calculated.

Proportions were calculated to determine proportion of antibiotic residues in raw milk samples. Bivariate and multivariate analysis was done to determine association between famers' knowledge and practices related with milk contamination with antibiotic residues at 95% confidence intervals. Figures and tables were used for illustration of important information.

3.10 Ethical issues

Farmers were explained on the importance of participating in the study and that their participation was highly voluntary. They were ensured on confidentiality of the study findings and that the results will be only shared with the Livestock Development Department authorities for the purpose of assisting farmers and improving the situation. The permission to carry out this study was granted by the District commissioners, the Director of Livestock Development Department. Ethical clearance was obtained from ethical review board MUHAS (HD/MUH/T.618/2018).

CHAPTER FOUR

4.0 RESULTS

4.1 Background information of study population

4.1.1 Socio - demographic characteristics

A total of 136 study participants were enrolled and consented to participate in the study with a response rate of 100%. The mean age of study participants was 43 with standard deviation of ± 9.4 years, majority 45 (33.1%) being in the age range of 40-49 years. Majority of them were males 126 (92.7%). More than two third 97 (71.3 %) had attained a secondary level of education. Interviewing was focused to the farm owners or farm attendants (Table 1).

Table 1: Socio-demographic characteristics of the study participants (N=136)

Variable	Number	Percent (%)
Age group		
< 30	11	8.1
30-39	40	29.4
40-49	45	33.1
50+	40	29.4
Mean age	43± 9.4	
Sex		
Male	126	92.7
Female	10	7.3
Level of education		
Primary	34	25.0
Secondary	97	71.3
Tertiary	5	3.6
None	0	0.0
District		
Central	34	25
North B	34	25
West A	34	25
West B	34	25

4.2 The proportion of antibiotic residues in screened raw milk in selected farms

This study determined the proportion of antibiotic residues contamination in raw milk from farms in the West A, West B, Central and North B districts in Unguja. Out of 272 milk samples collected, 49.6% were positive for antibiotic residues.

The proportion of farms produced contaminated milk varied from one district to another. The district with highest level of contamination was North B (85%), while that with lowest level of contamination was West A (56%).

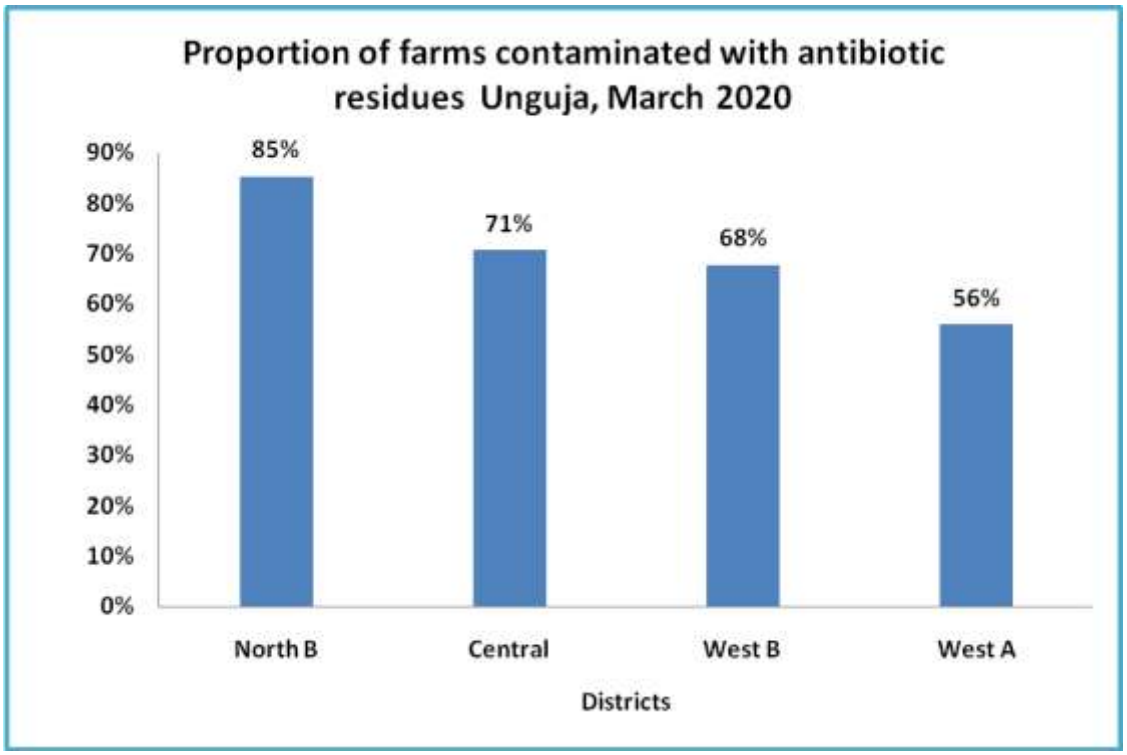

Fig. 2: Proportions of farms with raw milk containing antibiotic residues by District (N=136)

4.3 The types of antibiotics found in milk samples

The types of antibiotic detected in the screened milk samples was Betalactams 41% (N=272) followed by tetracyclines (7%), streptomycin (6%) and sulphonamides (3%), while gentamycin was not detected in any of the milk samples.

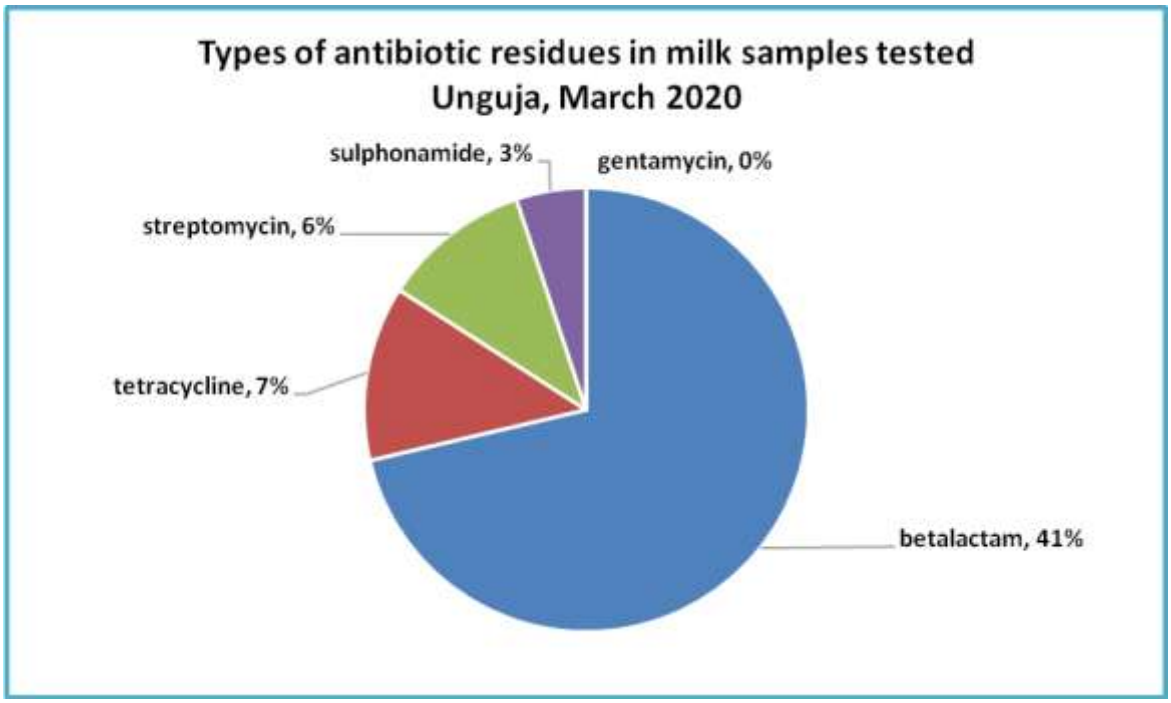

Fig. 3: Types of antibiotics residues in tested raw milk samples (N= 272)

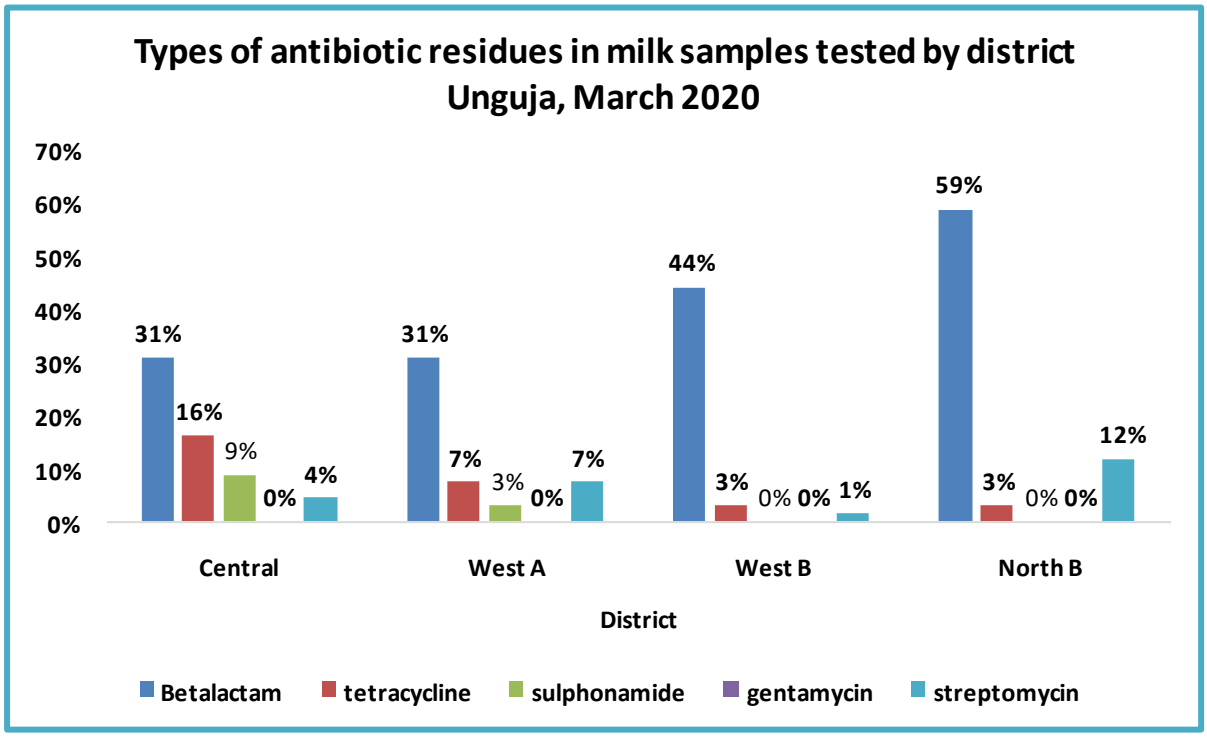

Fig. 4: Types of antibiotic residues in raw milk by district (N=5)

The types of antibiotic residues detected in all districts in tested milk samples was betalactam (31%-59%), followed by tetracycline (3% -16%). The least featured antibiotic was sulphonamide (3% -9%), while gentamycin was not found in any district.

4.4 Farmers' training, knowledge and practices related with antibiotic residues in raw milk

4.4.1 Farmers' training on practices related to antibiotic residues in raw milk

Less than 50% of interviewed farmers received any training on milk safety. Majority (42%) recived training on proper management of cow diseases while 13% received training on handling and administration of vet drugs. Figure 4 below summarizes training findings.

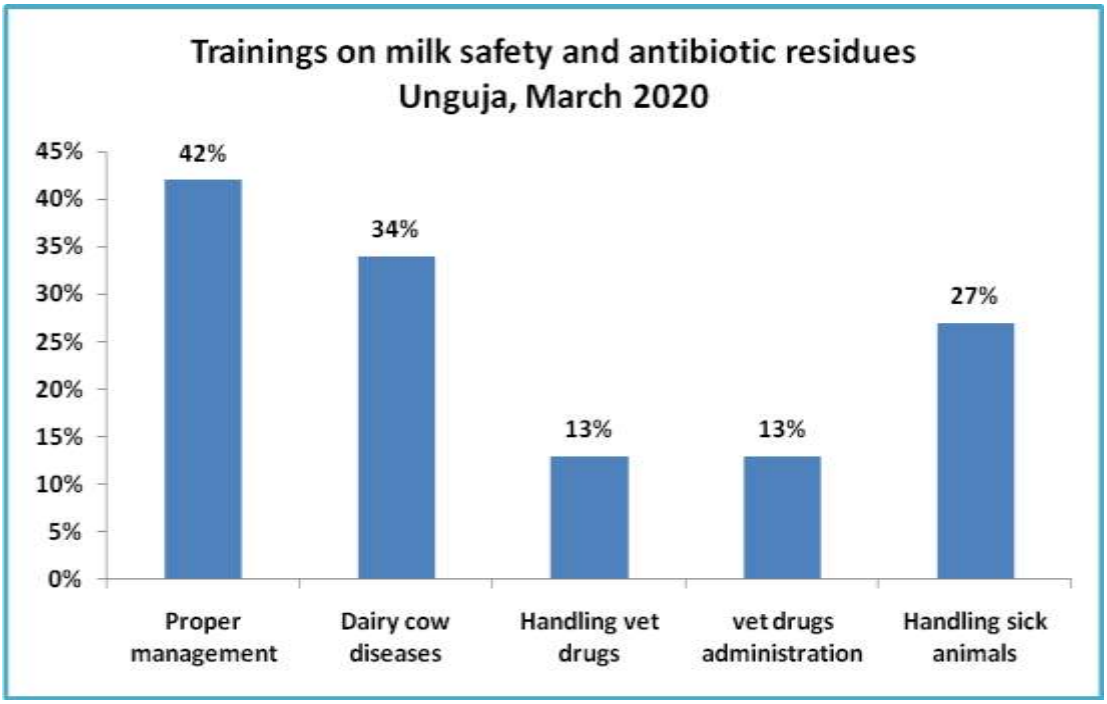

Fig 5: Proportions of farmers trained on milk safety and antibiotic residues (N=136)

4.4.2 Farmers' knowledge to raw milk safety in particular antibiotic residues

A total of 136 farmers were interviewed to determine their knowledge and understandings on milk safety and antibiotic residues contamination in milk. Farmers' responses on their knowledge on milk safety and antibiotic consumption are shown in (Fig.5).

Majority of farmers with knowledge on milk safety were from West A with the mean knowledge of 49% while farmers with least knowledge on milk safety were from North B with the mean knowledge of 19%.

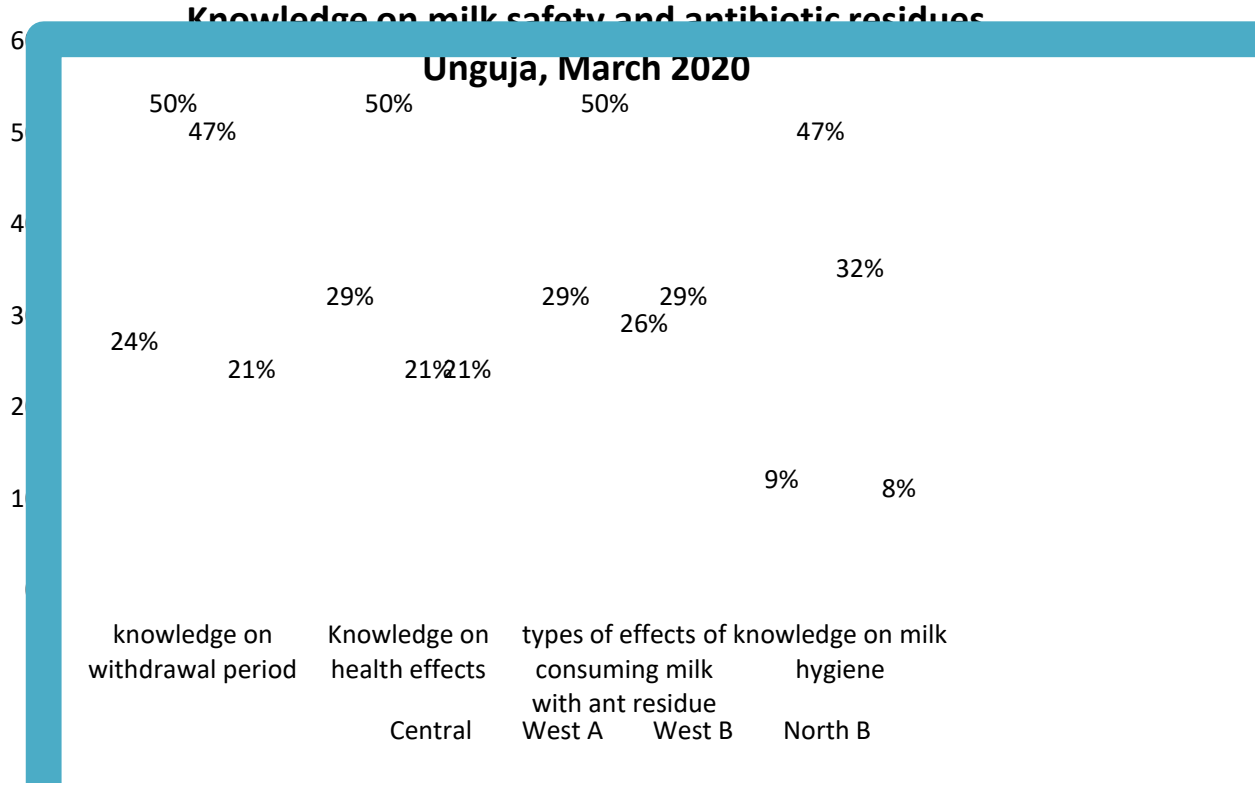

Fig. 6: Proportion of farmers with knowledge on milk safety by district (N=136)

4.5 Farmers' practices related to raw milk safety in antibiotic residues

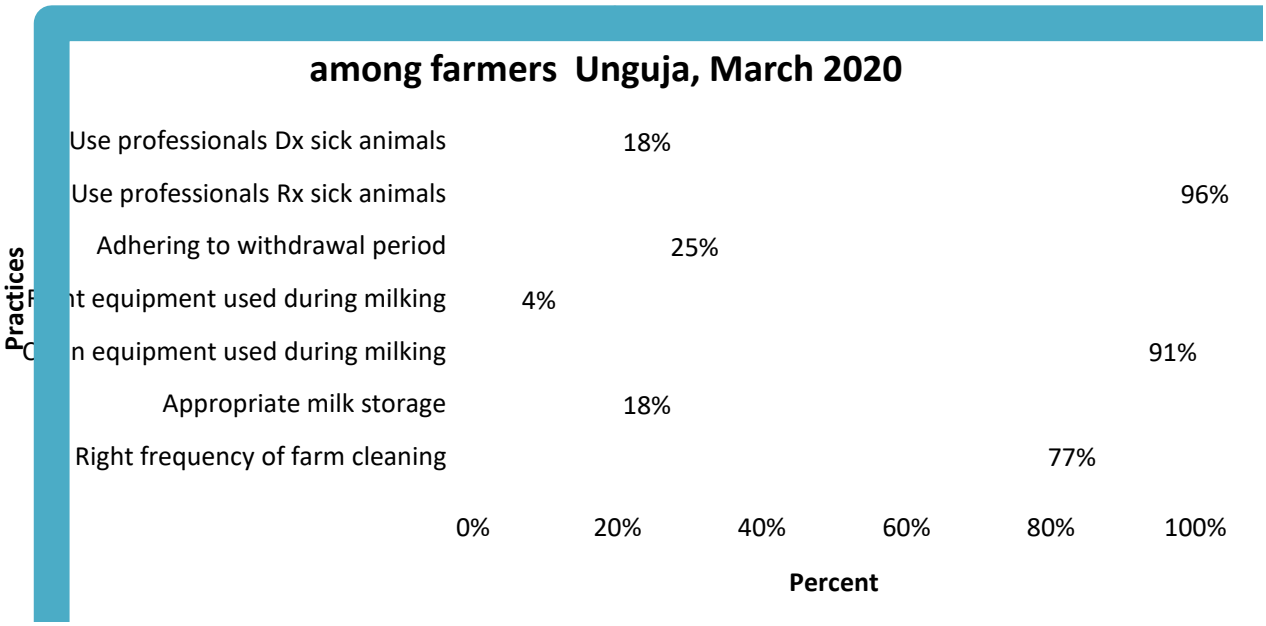

Fig. 7: Practices related to milk safety and antibiotic residues as reported by farmers (N=136)

The commonest appropriate practice among farmers was the use of professionals to treat sick dairy animals 96% (N=136), while the least implemented among appropriate practices was the use of right equipment (metallic containers) during milking 4% (N=136).

The proportion of farmers who reported to use veterinary professionals in diagnosing diseases in their sick animals was by only 18%.

Those who adhered to withdrawal period was only 25% while those used clean equipments during milking was 91%. Moreover, farmers who reported to practice cooling their milk after milking was only 18%. On the other hand, 77% of the farmers reported to clean their farms once or twice per day.

Table 2. Factors associated with antibiotic residues contamination in milk among farmers at bivariate and multivariate analysis

Variables	Antibiotic Residue		Bivariate analysis		Multivariate analysis	
	Positive	Negative	COR 95% CI	P value	AOR 95% CI	P value
Understanding milk hygiene						
Yes	63 (67.1%)	31(32.9%)	**Ref**		**Ref**	
No	33 (78.6%)	9 (21.4%)	0.55 (0.2-1.3)	0.17	1.36 (0.19-9.68)	0.753
Treatment of sick animal						
Vet professionals	89 (73%)	33 (27%)	**Ref**		Ref	
Farmer	7 (50%)	7 (50%)	2.70 (0.88-8.28)	0.07	0.97 (0.20-4.71)	0.975
Knowledge on withdrawal period						
Yes	21 (62%)	13 (38%)	**Ref**		**Ref**	
No	75 (74%)	27 (26%)	0.58 (0.26-1.32)	0.19	2.83 (0.57-13.87)	0.198
Training on drug administration						
Trained	8 (47%)	9 (53%)	**Ref**		**Ref**	
Not trained	88 (74%)	31 (26%)	0.31 (0.11-0.88)	**0.02**	2.10 (0.54-8.11)	0.278
Availability of veterinary services						
Yes	91 (72%)	36 (28%)	**Ref**			
No	5 (56%)	4 (44%)	2.02 (0.51- 7.8)	0.45	-	-
Education level						
Primary	24 (70.6%)	10 (29.4%)	Ref			
Secondary	72 (70.6%)	30 (29.4%)	1.0 (0.4-2.3)	1.00	-	-
Health effects on antibiotic consumption						
Yes	28 (62.2%)	17 (37.8%)	**Ref**		**Ref**	
No	60 (79%)	16 (21%)	0.4 (0.1-0.9)	**0.04**	0.22 (0.03-1.52)	0.127

Source of obtaining drugs						
Private shops	34 (64%)	19 (36%)	**Ref**		**Ref**	
Vet professionals	20 (77%)	6 (23%)	1.06 (0.6-5.4)	0.25	2.81(0.50-15.87)	0.240
Training on handling sick animals						
Trained	23 (62.2%)	14 (37.8%)	**Ref**		Ref	
Not trained	73 (73.7%)	26 (26.3%)	0.58 (0.2-1.3)	0.18	1.18(0.2- 6.6)	0.865
Training on proper management of dairy cows						
Trained	36 (63.2%)	21 (36.8%)	**Ref**		Ref	
Not trained	60 (76%)	19 (24%)	0.54 (0.2-1.1)	0.10	0.68 (0.24-1.92)	0.476
Training on dairy cows' diseases						
Trained	30 (65.2%)	16 (34.8%)	**Ref**			
Not trained	66 (73.3%)	24 (26.7%)	0.68 (0.3-1.4)	0.32	-	-

4.6 Factors associated with antibiotic residues contamination in milk

Bivariate analysis

About eleven independent variables were analyzed using bivariate model. The findings in the present study showed that all eleven factors had association with the milk contamination. The two variables of training on drug administration and health effects on antibiotic consumption had protective association that was statistically significant (P- value < 0.05).

The risk of milk contamination was 0.31 less in farmers who were trained on drug administration, than in those who were not trained (OR= 0.31 95% CI= 0.11-0.88, P-value 0.02). The farmers are less likely to produce antibiotic residues contaminated milk, than those who were not trained.

For the farmers who understand that there are health effects upon consumption of milk contaminated with antibiotics, the risk of contamination was 0.4 less in farmers who understand the health effects than in those who do not (OR= 0.4 95% CI= 0.1-0.9, P-value 0.04).The farmers were less likely to produce antibiotic residues contaminated milk, than those who do not understand.

The other factors were availability of veterinary services, education level and training on dairy cows' diseases. These had association with the milk contamination but the association was not statistically significant. Others are understanding milk hygiene, knowing withdrawal period, treatment of sick animal, training on handling sick animals, training on proper management of dairy cows and training on dairy cows' diseases. Further information on the associations of the factors is as shown in the table above.

Multivariate analysis

Adjusted logistic regression model was carried out for all those variables with P- values $\leq$ 0.2 shown on the table above. At the end of the analysis we found out that understanding milk hygiene, knowing withdrawal period, training on drug administration, source of obtaining drugs, had positive associations but these associations were not statistically significant.

On the other hand, knowing that there are health effects on antibiotic consumption and having trained on proper management of dairy cows had protective associations but were also not statistically significant. There was almost no association with training on treatment of sick animals with the outcome.

The results showed that the risk of producing milk with antibiotic residues contamination was not associated with lack of understanding on milk hygiene (AOR = 1.36 CI = 0.19-9.68, P-value 0.75).

On the other hand, there was no association between treatment of sick animals by veterinary professionals and farmers in producing antibiotic contaminated milk (AOR= 0.97 CI = 0.20-4.71, P-value 0.97).

With knowledge on withdrawal period, the risk of producing milk with antibiotic residues contamination was almost three times higher in farmers who do not have knowledge on withdrawal period than in those who do have (AOR = 2.83 CI = 0.57-13.87, P-value 0.19). However there was no significant association.

Likewise, the risk of producing contaminated milk in farmers who were not trained on drug administration, was two times higher compared to those who were trained (AOR = 2.10 CI = 0.54-8.11, P-value 0.27).

On the other hand, concerning farmers who were aware that there are health effects that might be caused upon consumption of antibiotic residues in milk, they were less likely to produce contaminated milk than those who were unaware (AOR = 0.22 CI = 0.03-1.52, P-value 0.18) but the association was not statistically significant.

Moreover, depending on the source where farmers obtain veterinary drugs for treating their animals, those who obtain drugs from the private shops are at almost three times risk of producing contaminated milk than in those who obtain drugs from the vet professionals (AOR= 2.81 CI = 0.50-15.87, P-value 0.24). However there was no statistical significance.

Having trained on handling sick animals had also no association in not producing contaminated milk (AOR= 1.18 CI= 0.2 -6.6 P-value 0.86).

Lastly farmers who were trained on proper management of dairy cows had a protective association i.e. they were less likely to produce contaminated milk compared to those who were not trained but the association was also not statistically significant (AOR= 0.68 CI= 0.24-1.92, P-value 0.47).

CHAPTER FIVE

5.0 DISCUSSION

This study has revealed high proportion of farms with antibiotic residues contamination in milk with at least one antibiotic type. Degree of contamination varied among farms and districts. Five types of commonly used antibiotics were found in the tested raw milk samples. It was apparent that most farmers had limited knowledge and practices related with antibiotic residues in raw milk safety and hygiene. Understanding health effects on antibiotic residues consumption, knowledge on withdrawal period, source of obtaining veterinary drugs, training on drug administration were associated with antibiotic contamination in milk, however the association was not statistically significant ($p>0.05$).

Magnitude of milk contamination differed greatly among farms and districts. North B district had the highest proportion (85%) of farms produced contaminated milk samples while West A had the smallest proportion (56%) of farms produced contaminated milk samples. These findings are similar to those found in other studies conducted in Tanzania, Kenya, Ghana, and South Africa (11,24,28,40) that reported contamination of raw milk with different antibiotic residues.

Contamination of raw milk with antibiotic residues is mainly caused by irrational use of antibiotics in agriculture and livestock such as growth promotion, inappropriate farming practices such as non-compliance to withdrawal periods and prescriptions of antibiotics by non-professionals, and limited knowledge among farmers on the proper livestock farming practices (11,12,22,39). The variation in the proportion of farms that produced contaminated raw milk among districts in Unguja can be probably due to uneven distribution of extension officers who provide professional services and knowledge on appropriate farming practices to farmers.

In this study we tested availability of five types of antibiotic residues in the raw milk samples namely, betalactams, tetracycline, streptomycin, sulphonamides and gentamycin. Out of 272 samples, betalactam was the leading type of antibiotic found in the raw milk samples,

followed by tetracyclines, streptomycin, sulphonamides. No sample was detected with gentamycin.

These results are in concurrence with other studies done in Tanzania, other African countries and Canada, that the commonest types of antibiotic residues in raw milk are betalactams and tetracyclines (11,31,45,46). In this study we were not able to quantify levels of contamination for each antibiotic due to technological factors.

Among four districts where the study was conducted the proportion of betalactam contamination varied significantly. North B district was leading (59%) followed by West B (44%), then West A and Central districts which had the same proportion of 31% each. These huge variations may be due to the same reason that there is inadequate and uneven distribution of extension officers in Unguja which in turn lead to limited knowledge and poor farming practices among farmers and hence contamination of raw milk with antibiotic residues.

The study has revealed lower awareness of farmers on health effects on antibiotic consumption (30.2%) and lower awareness on withdrawal period (37.5%).Similar findings were reported in Arusha, Kenya and Sudan (2,26,41). This differs from studies of Bagamoyo and Arusha (25,26). Furthermore, several other studies have reported lack of knowledge on effects of antibiotics consumption in farmers (38,47,48).

Awareness of farmers who reported to understand milk hygiene was lower (69%) compared to that reported by Ridhiwani at Bagamoyo and Bukuku in Arusha (25,26). Different trainings to increase farmers' awareness were reported in this study. Farmers' trainings to increase awareness has proved changes and improvement to farmers in other areas as no antimicrobial residues were detected in Tanga farmers following training programmes at Tanga dairy co-operative union (36). On the other hand, a study conducted in Malawi reported that all of the study participants having trained on various aspects of dairy farming indicating a wide range of knowledge (49).

Furthermore, our study reported low level of adherence to withdrawal period (25%). On the contrary, higher level of withdrawal adherence was reported at Bagamoyo and at Arusha city and Meru district council where 100% of the samples were found free of the antibiotic residues (25,26). Likely several other studies have reported failure of the farmers in observing withdrawal period, Arusha, Morogoro municipality and Baghladesh (47,48,50).

Other studies suggest that veterinarians should transform themselves from reactive and curative antimicrobial prescribers, towards a more proactive task as animal health advisors to farmers, rather than depending on prescribing antimicrobials (51) This probably contributes more in the proportion of the residues in the lactating dairy cows as in most cases; no laboratory confirmation is conducted to prove the disease presence. According to Lhermie, disease diagnosis depends on two factors; farmers' knowledge and availability of diagnostic tools (51).

In the multivariate analysis, the results showed that understanding milk hygiene had no association in not producing antibiotic contaminated milk. This is different from the protective association that was reported by Ondieki et al (15).

Surprisingly, the use of veterinary professionals in the treatment of sick animals and the farmers' self practices of treating their sick animals showed no significant changes or differences in the production of antibiotic contaminated milk. This shows that some veterinarians are also involved in the irrational use of antibiotics. According to Hisham, implementations of World Trade Organization (WTO) regulations demand that veterinarians working in food animal medicine, should learn how to avoid drug/chemical residues in food animals and disseminate this information to the farmers to safeguard the health of general public (52).

Concerning the withdrawal period, the risk of producing milk with antibiotic residues contamination was almost three times higher in farmers who were not aware with withdrawal period compared to those who do. This is probably due to farmers' limited knowledge in milk

hygiene, health effects of antibiotic residues, disease control and prevention in their dairy animals.

Furthermore, the risk of producing contaminated milk in farmers who were not trained on drug administration was two times higher compared to those who were trained, however it was not statistically significant. Similar positive association was reported by Ridhiwani (25).

On the other hand, depending on the source where farmers obtain drugs for treating their animals, those who obtain drugs from the private shops are at almost three times risk of producing contaminated milk than in those who obtain drugs from the vet professionals. Similar result of positive association was reported from untrained farmers who usually rely on veterinary drug sellers for instructions (25) This is probably due to the fact that most of the drug sellers do not really give proper instructions for drugs uses.

Moreover, concerning farmers who were aware that there are health effects that might be caused upon consumption of antibiotic residues in milk, they were less likely to produce contaminated milk than those who were unaware, This agrees with the study carried out at Lamu which also found out that the association on awareness of respondents on health effects of consuming antibiotic residues was protective i.e. they are less likely to produce antibiotic contaminated milk (15).

The study has also revealed that, having trained on handling sick animals had no changes on farmers in the production of uncontaminated milk. On the other hand, farmers who were trained on proper handling of dairy animals were less likely to produce antibiotic contaminated milk. This is probably because knowing proper management practices helps farmers make proper decision on when to treat the lactating animal as well as on proper disease control practices.

5.1 Study limitations

The test used was a qualitative test used for screening purposes. It indicates presence or absence of a certain antibiotic group in the milk sample. Also, this study could not determine significant factors associated with contamination of milk with antibiotic residues in Unguja. This may be due to inadequate sample of farmers included in the study.

There might have been bias which is common in many questionnaire based studies.

5.2 Suggestion for further studies

More studies with robust methodologies and sophisticated and confirmatory technologies such as high-performance liquid chromatography are needed to determine quantities of antibiotic residues in raw milk and its associated factors. This may help to develop appropriate strategies to mitigate the problem.

5.3 Conclusion

This study found more than half of farms in Unguja produced milk that contains antibiotic residues. Furthermore, the farmers' knowledge and practices on effects of antibiotic residues consumption in milk and other animals' products is very low. Farmers' understanding particularly on milk safety and hygiene, withdrawal period, good management practices and their motivation towards quality production is low. Most of the farmers were still using plastic containers which are not ideal for milking and milk handling as it is difficult to ensure their cleanliness.

5.4 Recommendations

- The Ministry of Agriculture, Natural Resources, Livestock and Fisheries (MANRLF) should direct its efforts at the farm level in order to increase farmers' awareness on effects of antibiotic residues in human and animals.

- MANRLF should provide trainings to farmers in order to improve their understandings on milk safety& hygiene and motivate them on good management practices and better quality production.

- Zanzibar Food and Drugs Authority (ZFDA) should strengthen its surveillances and monitoring systems, rules and regulations on milk safety in the country as well as motivating farmers on adhering to quality standards.

- MANRLF together with Zanzibar Livestock Research Agency (ZALIRA) should work together and carry out further studies using quantitative tests and larger sample sizes to determine the actual amount of antibiotic residues present in farm milk and to identify farmers' problems and constraints in achieving milk production with minimal antibiotic residues contamination.

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APPENDICES

Appendix 1: Questionnaire for data collection

TITLE: PREVALENCE OF ANTIBIOTIC RESIDUES IN RAW MILK AND ITS ASSOCIATED FACTORS IN COMMERCIAL FARMS UNGUJA ZANZIBAR.

Village..... Date of interview…….

District..... Shehia ..... Farm number……

Farmers'education level Primary……. Secondary…… Tertiary…… None……..

Sex..... Age.....

1) Number of Lactating cows present on a day of visit.....

2)Number of cows under antibiotic treatment/has recently received on the day of visit……

3) Do you understand proper management practices of dairy animals?

Yes.....No.....If YES mention i) Proper feeding..... ii) Proper hygiene…….

iii) Tick control…….. iv) Deworming…… v) Vaccination……

vi) Others …….

4) Have you ever got training on proper management of dairy animals? Yes..... No.....

If YES, what were you trained on……

Source of training.....

5) Do you know what milk hygiene is? Yes..... No.....

If Yes state it……

……
……..

6) What are main hygienic milking procedures?

i) Washing hands with soap &water before milking ……ii) Washing udder with warm water and clean cloth before milking……

.iii) Using milking salve..... iv) Cleaning udder with dry cloth…….

v) Squeezing not pulling the teats…….. vi) Others specify ……

.....

…….

7) What are the main precautions to take?

i) During milking.....

ii) During milk handling……

8) How do you store the milk after
milking?.....

.....

9) What type of equipments do you use during milking?..... ……..

……..... ……

Can I see them please? Status i) Clean …… ii) Dirty……..

10) What source/sources of water do you use for your farm activities?

i) Tape water…… ii) Well……. .. iii) Water trough ……

iv) Others specify……

…….

11) How often do you clean your farm? i) Once per day…… ii) Twice per day……..

iii) Thrice per day…… Others specify……

12) What do you normally use during cleaning ? i) Water……. ii) Soap…….

iii) Disinfectant…… Others specify……

13) Do you use anything else in cleaning apart from water? Yes..... No.....

If Yes what is it?.....

14) What are the common diseases affecting your animals?

i) ECF……ii) Anaplasmosis…… iii) Mastitis……. iv) Helminths……..

v) Lumpy skin disease…… vi) Brucellosis……..

Others specify ……..

……

15) Have you ever got any training on common diseases of dairy cows?

Yes..... No……..

If No, how do you diagnose different diseases in your farm? ……

……..

16) What are the common types of drugs do you use for treatment?

i) Penicillin …ii) Cloxacillin……iii) Cephalosporins……iv) Streptomycin…….

v) Gentamycin ..... vi) Kanamycin…… vii) Neomycin

viii) Oxytetracycline,…… ix) Chlortetacycline……. x) Tetracycline……...

xi) Erythromycin……xi) Sulphonamides…… …….....

xii) Others specify……..

17) Can I see the empty bottles/ boxes/ leaflets of the drugs you are using? Available ones

i) Penicillin …… ii) Cloxacillin……. iii) Cephalosporins……
iv)Streptomycin…….
v)Gentamycin……vi)Kanamycin……vii)Neomycin……..

viii)Oxytetracycline,…… ix)Chlortetacycline……. x)Tetracycline……..
xi) Erythromycin……..xii)Sulphonamides……xiii)……Others……

……..

18) Where do you normally obtain drugs for treating animals?

i) Markets……ii) Private shops…… iii) Government veterinary clinic……
iv) Others Specify……

……..

19) Who treats your animals when they get sick? i) Vet officer……ii) CAHW's…….

iii) Myself……... iv) Others specify…….

……
……

20) If own treatment how do you understand the instructions for administration?

i) Reading……. ii) Asking neighbours/friends etc……. Others specify…….

……..

21) **If own treatment**, how do you quantify the amount to administer?

i) Reading……. ii) Asking neighbours/friends etc……. iii) Estimation……

v) Others specify…….....

22) **If own treatment**, how frequently do you use drugs per day?.....

23) **If own treatment**, what are the key issues/aspects that you consider before drug administration? i) Dose…… ii) Age/ body weight…… iii) Animal's condition

iv)Withdrawal period…….. v) Others…specify……

…….

24) At what period /time do you treat your animal? i) When sick…… ii) At any time…….

iii) Before sick as prevention…… iv) When production drops down…… …….

v) Soon after calving…… vi) Others specify…….

……

25) If a lactating cow is under treatment? When do you milk it for sell or consumption?

i) At any time …… ii) At the beginning of treatment …… ……

iii) Immediately after the last treatment……

iv) After the last dose of treatment and the drug withdrawal period has passed

26) **If the answer is 25 iv)** Why don't you sell or use the milk for consumption

At any time, at the beginning or immediately after the last dose of treatment?

……..

27) How long do you start milking an animal that has been treated with antibiotic/antimicrobials?

…….

.....

28) Do you know the meaning of drug withdrawal period? Yes..... No.....

29) **If the answer is Yes in question 28** do you follow it? Yes……
No……

30) **If No**, Why don't you follow it?

……
…

……
…

31) Are there any health effects if a person consumes milk with antibiotic drug residues?

Yes..... No……

32) **If Yes in question 31**, list down the health effects you know which may be caused by antibiotic drug residues consumption in milk.....

.....
..

33) Have you ever got training on proper management of dairy animals?

Yes…… No……..

If Yes, state what it means……

……..

34) Have you received any training on the following:

i) How to handle veterinary drugs? Yes…….. No……..

If Yes, source of training……

ii) How to administer drugs? Yes……. No…….

If Yes, source of training……

iii) How to handle sick animals? Yes…… No…….

If Yes source of training……

35) Are the veterinary services available at any time needed? Yes ..... No.....

If No, where do you get services in case you are in need?.....

……..

36) How do you store drugs used to treat your animals? i) Farm store…… iii) Cow shed……

iii) At home…….. iv) Others specify…….....

37) Where do you store drugs for ticks/pests control &farm cleanliness in general?

……

38) Is there any other way in which drugs used to treat animals are used? Yes..... No.....

If Yes, state them.....

39) Is there any problem associated with the use of drugs in animals?

Yes..... No……. I don't know……..

If Yes, give
examples……

40) Do you have any comment/suggestion in regard to antimicrobial usage? ……

…….

……
……

* **THANK YOU FOR YOUR CO-OPERATION** *

Appendix 2: Fomu Ya Kukusanyia Taarifa

UWEPO WA MABAKI YA ANTIBIOTIKI KATIKA MAZIWA NA VISABABISHI VYAKE KATIKA MASHAMBA

YA NGO'MBE UNGUJA, ZANZIBAR 2019.

Kijiji..... Tarehe ……..

Wilaya..... Fomu namba…… Shehia .....

Kiwango cha elimu ..... Jinsia..... Umri.....

1) Idadi ya ngo'mbe wanaokamuliwa kwa sasa

……..

2) Idadi ya ngo'mbe wanaotibiwa kwa sasa ……

3) Jee unajua huduma bora za utunzaji ngo'mbe wa maziwa? Ndio…… Hapana……

Iwapo Ndio, ni zipi? Lishe bora…… Usafi bora…….. kinga dhidi ya kupe……

Kinga dhidi ya minyoo……. Chanjo……... Mengineyo…….

…….

…….

4) Jee umeshawahi kupata mafunzo yoyote ya utunzaji wa ngo'mbe wa maziwa?

Ndio..... Hapana.....

Kama Ndio, taja aina

.....

.

Chanzo/Vyanzo vya elimu

…….....

.....

5) Jee unajua maana ya usafi wa maziwa? Ndio..... Hapana.....

Kama Ndio

eleza……

6) Jee unafahamu njia bora za ukamuaji wa maziwa? Ndio ……. Hapana……..

Kama Ndio taja i) Kuosha mikono kwa maji na sabuni kabla ukamuaji ……ii) kuosha kiwele na maji ya uvuguvugu kwa kitambaa safi……iii) Kutumia milking salve.....

iv) Kushafisha kiwele kwa kitambaa safi kikavu ……v) Kubinya chuchu wakati wa ukamuaji…… v) Mengineyo

……..

……..……

7) Taja mambo muhimu ya kutahadhari wakati :

i) Ukamuaji maziwa? …….....

……

ii) Uhifadhi maziwa? ……

……..

8) Vipi unahifadhi maziwa baada ya kukamua?.....

.....

9) Aina gani ya vifaa unavyotumia kwa kukamulia/ kuhifadhia maziwa?.....

Naweza kuviona tafadhali? Gradi i) Safi sana…… ii) Safi…….

iii) Chafu…… iv) Chafu sana…….

10) Jee ni chanzo/vyanzo gani vya maji unatumia kwa shughuli zako za ufugaji?

i) Maji ya bomba…… ii) Kisima…… iii) Hodhi……..

iv) Vyenginevyo…….

……

11) Jee ni mara ngapi unasafisha banda lako la ngo'mbe?

……..…….....
..

12) Jee ni njia gani unazotumia katika wa kufanya usafi?

i) Maji……. ii) Sabuni……. iii) Dawa maalum za usafi……

iv)
Nyenginezo…….....

……..

13) Jee unatumia chochote baada ya maji katika kufanya usafi? Ndio..... Hapana.....

Kama Ndio ni kitu gani?.....

14) Ni aina gani kuu za magonjwa yanayosumbua mifugo yako?

i) ECF…….. ii)Anaplasmosis……..… iii) Ugonjwa wa kiwele …….. iv) Minyoo……

v) Ugonjwa wa ngozi……vi) Kuharibu mimba…….

vii) Mengineyo

……..

…….

15) Umeshawaji kupata mafunzo yoyote juu ya maradhi makuu ya ng'ombe wa maziwa?
Ndio..... Hapana……..

Iwapo Hapana , ni vipi unatambua ugonjwa katika shamba lako?..…….

……..

……

16) Ni aina gani kuu za dawa unazotumia shambani kwa kutibia?

i) Penicillin …ii) Cloxacillin……iii) Cephalosporins……iv) Streptomycin……
v)Gentamycin ..... vi) Kanamycin…… vii) Neomycin
viii)Oxytetracycline,…… ix) Chlortetacycline……. x)Tetracycline…….....

xi) Erythromycin……xii) Sulphonamides…km…… …….....

…….

xiii) Nyenginezo taja…….

……

……
…17) Jee naweza kuona chupa tupu/ kiboksi/kipeperushi cha dawa hizo? Aina zilizopo

i) Penicillin …ii) Cloxacillin……iii) Cephalosporins……iv) Streptomycin…….
v)Gentamycin ..... vi) Kanamycin…… vii) Neomycin
viii)Oxytetracycline,…… ix)Chlortetacycline……. x)Tetracycline…….. …….

xi) Erthromycin……. xii) Sulphonamides…km…….

…….

xiii) Nyenginezo taja…….

……
……

…….

18) Kwa kawaida unapataje dawa za kutibia wanyama wako?

i) Sokoni……ii) Maduka ya dawa binafsi…… iii) Kliniki ya Idara ya Mifugo ……

iv) Kwengineko taja…….

……..
19) Ni nani anaekutibia wanyama wako wanapoumwa?

. i) Afisa mifugo……ii) CAHW's……. iii) Mwenyewe…….....

iv)Wengineo taja……

……..

20) Iwapo unatibu mwenyewe,ni vipi unafahamu kumpiga mnyama dawa ?

i) Kwa kusoma……. ii) Kuuliza majirani/ rafiki nk…….

iii) Vyenginevyo taja……..

…….

21) Iwapo unatibu mwenyewe, ni vipi unakisia kiwango kinachohitajika kwa matumizi?

i) Kwa kusoma……. ii) Kuuliza majirani/rafiki nk……. iii) Kukisia tu……

iv) Vyenginevyo taja…….....

……

…….

22) Iwapo unatibu mwenyewe, ni mara ngapi unatumia dawa hizo kwa siku?.....

23) Kama unatibu mwenyewe ni mambo gani muhimu ya kuzingatia kabla ya kutumia dawa?

i) Dozi…… ii) Umri/ uzito wa mwili…… iii) Hali ya afya ya mnyama……..

iv)Withdrawal period……. v) Mengineyo taja…….

……

24) Je ni wakati gani unatibu wanyama wako?

i)Anapoumwa…… ii) Wakati wowote…….

iii) Kabla kuumwa kama kinga…… iv) Uzalishaji unapopungua…… …….

v) Mara tu baada ya kuzaa…… vi) Others specify…….

…….

……..

25) Iwapo ng'ombe anaekamuliwa yupo kwenye tiba, ni wakati gani anakamuliwa maziwa kwa ajili ya kunywa au kuuzwa ?

i) Wakati wowote …… ii) Mwanzoni …… ……..

iii) Mara tu baada ya tiba/dozi ya mwisho…….

iv) Baada ya dozi ya mwisho na muda maalum wa dawa kuisha mwilini ……

26) Kama jibu ni **25 iv) Ni kwa nini hukamuwi maziwa kwa kuuza au kunywa**

wakati wowote ule, mwanzoni au mara tu baada ya tiba ya mwisho?

.....

……

27) Ni muda gani unaofaa kuanza kukamua mnyama ambae alikuwa kwenye matibabu ya antibiotiki/antimikrobial?

.....

28) Jee unafaham kile kipindi mnyama anapomaliza tiba ya mwisho hadi kukamuliwa tena maana yake ni nini? Ndio..... Hapana …….

29) Iwapo jibu ni Ndio katika swali namba **28** , jee unafuata muda huo?

Ndio …… Hapana…….

30) Iwapo ni Hapana kwanini hufuati muda huo?

…….

……

31) Jee kuna athari zozote za kiafya iwapo binaadamu atakunywa maziwa yenye mabaki ya madawa/ antibiotiki? Ndio…… Hapana …… Sijui……

32)Iwapo jibu ni Ndio swali namba **31,** taja athari ambazo binaadamu anaweza kuzipata iwapo atakunywa maziwa yenye mabaki ya antibiotic/madawa?.....

.....

33) Jee umewahi kupata mafunzo juu ya

i) Kuhifadhi madawa ya mifugo? Ndio..... Hapana.....

Kama ndiyo taja chanzo cha mafunzo……..

ii) Kumpiga mnyama dawa? Ndio…….. Hapana …….

Kama ndiyo taja chanzo…….

iii) Kumuhudumia mnyama anaeumwa? Ndio……. Hapana ……

Kama ndiyo taja chanzo cha mafunzo……

34) Jee huduma za tiba ya mifugo kutoka kwa wataalamu ni rahisi kupatikana pale zinapohitajika?

Ndio…….. Hapana ……..

Kama Hapana vipi unapata huduma hiyo pale unapoihitaji?.....

……..

…….

35) Jee ni vipi unatunza/ wapi unapoweka madawa yanayotumika kutibia/wanyama wako?

i) Farm store…… …… ii) Cowshed…… .. ……. iii) At home……

iv) Others specify…….

……

36) Jee vipi unatunza/wapi unaweka madawa ya kuogeshea na/kusafishia shamba lako?

……

…….

37) Jee kuna matumizi mengine ya madawa haya ya wanyama kutumika?

Ndio..... Hapana.....

Iwapo Ndio, taja matumizi hayo

……..

……

38) Jee kuna matatizo yoyote yanayohusiana na matumizi ya madawa kwa wanyama?

Ndio..... Hapana……Sijui……

Iwapo Ndio, taja
unayoyajua…….

…….....

39) Jee ni mara ngapi unakunywa maziwa katika familia yako?.....

41) Jee una mawazo/maoni yoyote kuhusiana na matumizi ya
madawa(antibiotiki/antimicrobial)?

…….

……

……..

* **AHSANTE KWA USHIRIKIANO WAKO ***

Appendix 3: Informed Consent (English Version)

Consent to participate in this study

Fatma W. Suleiman is a student **Masters of Science in Applied Epidemiology and Laboratory Management at Muhimbili University of Health and Allied Sciences (MUHAS).** I am aiming at conducting a study with titled

Prevalence of antibiotic residues and its associated factors in raw milk in commercial farms Unguja, Zanzibar.

Introduction:

This study is conducted for fulfillment the requirement of MSc in Applied Epidemiology which is provided by Muhimbili University of Health and Allied Sciences (MUHAS)

Purpose and Benefits: The purpose of the study is to determine the types of antibiotic residues, farmers' knowledge and practices and factors that contribute to antibiotic residues in dairy milk in Unguja. A questionnaire of about 42 questions will be used that will take about 42 minutes to be completed. Once you have completed the questionnaire, you will end up your role in this study.

Your rights and Confidentiality: The information you share is confidential. We will not record your name, phone number, or address that could identify you. Your answers will be kept in a private place that only people on the study can see. We will not include your name on any reports from this study. If you agree to participate, we will ask for your signature or right thumb mark, as indication of your consent

Risks, Stress, and Discomfort

There will be no harm to you or to your animal. Your response to the questions will be highly appreciated. Participation will be voluntary and you have the right to feel free to end at any time if you want to do so. Results obtained after the study will be confidential and will only be shared to the authorities for the purpose of helping the farmers.

Compensation

There will be no compensation of time spent during completing the questionnaire

If you decide not to take part in the study, you have the right to do so. We will give you a copy of this form to take with you. If you ever have any questions about this study or you want to understand more about the study, you may contact Madam Fatma W. Suleiman, Msc Student at MUHAS**,** mobile +255773051132 email: fatwasu@gmail.com, Professor M.I.N Matee supervisor MUHAS, Mobile: +255713081162, email mateemecky@gmail.com, Dr. Amir Juya supervisor TFETP mobile +255755696867, email amirjuya@gmail.com or Persons to contact or

Director of Research and Publication Dr. Bruno Sunguya (MUHAS), P. O. Box 65001, Dar es Salaam, Tel. no 2150302-6.

Participant agree ☐ Participant disagree ☐

Signature of Participant _____

Date consent signed _____

Appendix 4: Fomu Ya Kuombea Ridhaa (Swahili Version)

Fatma W. Suleiman ni mwanafunzi wa ngazi ya shahada ya uzamili katika chuo kikuu cha Muhimbili fani ya Epidemilogia na usimamizi wa maabara**.** Tuna madhumuni ya kufanya utafiti wenye kichwa cha habari kinachoeleza **Utambuzi juu ya uwepo wa mabaki ya madawa katika maziwa, ufahamu wa wakulima, mbinu wanazotumia na mambo yanayosabibisha uwepo wa mabaki ya madawa katika maziwa kisiwani Unguja Zanzibar.**

Utangulizi: Utafiti huu unafanyika kwa ajili ya kutimiza matakwa ya mahitaji ya kumaliza chuo kikuu katika fani ya epidemiolojia ngazi ya shahada ya uzamili katika chuo cha sayansi ya afya Muhimbili

Madhumini na faida: Madhumuni ya utafiti huu ni kugundua aina ya madawa katika maziwa, elimu za wakulima na vitendo na mambo yanayosabibisha uwepo wa madawa katika maziwa kisiwani Unguja Zanzibar.Tutakua na mahojiano ya maswali 42 yatayo chukua wastani wa dakika 42. Baada ya kumaliza kuhojiwa na kupata sampuli ya maziwa itakua ndio mwisho wa ushiriki wako katika utafiti huu.

Haki zako na usiri: Taarifa utakazo tupatia ni siri, hatuto andika jina lako mahala popote wala namba yako ya simu na chochote ambacho kitakacho kutambulisha wewe. Majibu yako yatawekwa sehemu pa siri ambapo watu ambao wanashiriki kwenye utafiti huu. Ikiwa utaamua kushiriki katika utafiti huu, tutakuomba sahihi yako au sahihi ya kidole ili kuonesha utayari wa ushiriki wako.

Hatari na madhara: Hakutakua na madhara kwako na wanyama wako, tutakushuru sana endapo utatujibia maswali yetu, vile vile ushirki wako ni kwa kujitolea, una haki ya kukataa kushiriki katika utafiti au kusitisha kuhojiwa muda wowote utakapojiskia kufanya hivyo. Majibu yatakayo patikana katika utafiti huu yatakua siri na yatapelekekwa kwa mamlaka husika ya mifugo kwa ajili ya kuboresha huduma za wanyama na wafugaji kwa ujumla.

Malipo: Hakutokua na malipo ya aina yoyote kwa muda utaotumika kwa mahojiano.

Ikiwa utaanua kutokushiriki katika utafiti huu una haki ya kufanya hivyo, pia tutakupatia kopi ya karatasi hii kwa ajili yako.Ikiwa una swali lolote kuhusu utafiti huu au unataka kuelewa zaidi kuhusu utafiti huu unahaki ya kuwasiliana na Fatma W. Suleiman, mwanafunzi chuo kikuu cha Muhimbili simu namba +255773051132 barua pepe: fatwasu@gmail.com, Professa M.I.N Matee misimamizi chuo kikuu Muhimbili simu namba: +255713081162, barua pepe mateemecky@gmail.com, Dokta. Amir Juya msimamizi TFETP simu namba +255755696867, barua pepe: amirjuya@gmail.com au

Mkurugenzi tafiti na uchapishaji Dokta. Bruno Sunguya Chuo kikuu Muhimbili, S L P 65001, Dar es Salaam, simu namba. no 2150302-6.

Weka alama ya vyema kwenye kiboksi ikiwa umekubali au umekataa

Nimekubali ☐ Nimekataa ☐

Saini ya mshiriki _____ Date consent signed _____