بسم الله الرحمن الرحيم

Sudan University of Sciences and Technology
College of Graduate Studies

Monitoring of Oxytetracycline Residues in Poultry Meat in Khartoum State

رصد متبقيات الأوكسي تتراسيكلين في لحوم الدواجن في ولاية الخرطوم

A Thesis Submitted in Fulfillment of the Requirement of the

Degree of M.Sc. in Preventive Veterinary Medicine

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August 2021

Dedication

I DEDICATE THIS WORK

TO ALL OF MY FAMILY, COLLAGES, FRIENDS

WHO ENCOURAGED ME THROUGHOUT THIS WORK

I ALSO DEDICATE THIS WORK TO THE SOUL OF MY SUPERVISOR

DR. YOUSIF HUSSSIN ALMANSOURY

AND TO THE SOUL OF

DR. YAHIYA ALI SABEEL.

TASNEEM

Acknowledgement

Thanks first and last to Allah who made me the resolve and made this work possible. I want to express my deep appreciation and gratitude to my supervisor Prof. Siham Elias for her patience, guidance and encouragement.

Thanks a lot to Dr. Amna Altayb who supported and helped me a lot in the bench work of immunology. Thanks are extended to all my collages in the department of radioisotopes and immunology especially Dr.Wafaa Mohammed, Dr. Nogod Eltegani, and Dr. Khalid Mohamed Taha, the head of the department, Central Veterinary Research Laboratories. Also I would like to thank the staff of the department of bacteriology at Central Veterinary Research Laboratories for their help in preparing the media and facilitating the bench work for bacteriology, with special emphasis to Dr. Huda Osman, the head of the department and to Dr. Abeer Abass. I wish to express my sincere thanks to International Atomic Energy Agency (IAEA) which I made this research under their regional project IAEA project RAF5078 RAF5084 and the Central Veterinary Research Laboratories for providing me all necessary facilities for the research. My deepest appreciation and gratitude goes to Ahmed El Fadil Akoud for his great help and encouragement. I take this opportunity to express gratitude to my soul friends Dr. Ekhlas Ali which is help me in sample collections from the poultry abattoir and Dr. Razaz Eltayb and, Rofida Ahmed Dr. Wefag Alfoz who supported me a lot.

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Abstract

This study was designed to monitor antibiotic residues in tissues of poultry from Khartoum state. A total of 360 tissue samples, (muscle, liver and kidney) were collected randomly from the market in Omdurman, Khartoum and Khartoum North localities. The samples were taken in summer, autumn and winter seasons (each season 120 samples). All samples were qualitatively screened for antimicrobial inhibition test using *Bacillus sibitilues* ATCC-6633. To quantify the oxytetracycline positive samples were further analyzed with high performance liquid chromatography (HPLC) after screening the results revealed that 52 (14.4%) samples were positive. Muscle tissues showed high percentage (18.8%) than those liver and kidney (15.0%) each. The positive samples obtained from Omdurman, Khartoum and Khartoum North were 20 (16.6%), 18 (15.0%), 14 (11.6%) respectively. The positive samples in summer were higher (35.8%) than autumn (10.8%) and winter (6.6%).Samples were analyzed using high performance liquid chromatography (HPLC UV/VIS) at 360 nm using isocratic system with mobile phase consist of oxalic acid: acetonitrile: methanol (75:20:5) and reverse phase (C18) column (150mm×4.6, 5-µm). The result revealed that 91% of the samples contain oxytetracyclines residues ranged from 3972.78 – 34.58 ppb, with 30% over the MRL. OTC residue presents in all tested liver and kidney sample while 70% of the tested muscle showed positive result. Comparing with the MRL, liver sample showed the highest percentage 54.5% followed by muscle 42.9% and kidney 8.3%. Generally OTC was detected in a high percentage in poultry tissues emerging the need for development of practical and scientifically based monitoring system for detection and quantification of antimicrobials residue all over the country.

المستخلص

أجريت هذه الدراسة للكشف عن متبقيات الأوكسي تتراسيكلين في لحوم الدواجن في ولاية الخرطوم. تم جمع 360 عينة من العضلات والكبد والكلي عشوائيا من اسواق الثلاث محليات (امدرمان و الخرطوم وبحري) . تم أخذ العينات في فصل الصيف والخريف والشتاء بمعدل 120 عينه لكل فصل . تم مسح كل العينات لوجود متبقيات مضادات الجراثيم بالطريقة الميكروبيولوجية بإستعمال البكتريا العضوية السبتليزية (ATCC6633). وتم تحديد كمية متبقي الأوكسي تتراسيكلين في العينات الموجبة بطريقة سائل فاصل اللون عالي الكفاءة (HPLC/UV). بعد المسح أظهرت النتائج 52 عينة موجبة بنسبة (14.4 %). .أظهرت العضلات أعلي نسبة (18.8 %) من الكبد والكلي (15 %) لكل. النسبة الموجبة المتحصلة من امدرمان و الخرطوم والخرطوم شمال كانت 20(16.6%) و18 (15%) و 14 (11. %) علي التوالي .وكانت النسبة الموجبة في فصل الصيف (35.8 %) و فصل الخريف (10.8 %) و فصل الشتاء (6.6 %). اظهرت العينات بطريقة الكروماتوغرافي عالي الكفاءة (HPLC/UV) علي طول موجي 360 وطور سائل مكون من حامض اوكساليك و اسيتونيترايل و ميثاول بنسبة 75:20:5 علي التوالي وكولوم سي 18 بطول 150 ملم. أظهرت هذه الطريقة إحتواء العينات علي متبقي الأوكسي تتراسيكلين بنسبة 91% و تركيز يتراوح بين 34.58 – 3972.78 جزء من البليون جميع عينات الكبد و الكلي كانت موجبة أما 70% فقط من العضلات أظهرت بقايا دواء .30% من العينات أظهرت تراكيز أعلي من الحد المسموح به عالميا حسب المنظمات العالميه أعلي نسبة سجلت في الكبد ثم العضلات ثم الكلي بنسبة 54.5 % و 42.9 % و8.3% علي التوالي. تاتي أهمية هذه الدراسة لرفع القدرات العلميه والعمليه وخلق جهاز رقابي لكشف و تحديد كميات متبقيات المضادات الحيويه.

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Introduction

Chicken and poultry products have been distributed worldwide because of being healthier than other animal products. Poultry meat is essential part of animal food market and its production is increasing to satisfy the public demand world-wide as relatively in expensive cost if compared with other animal protein (Bryan, 1985). In Sudan, there is an increase in poultry industry. Khartoum state is considered to be number one in poultry production (meat and eggs) compared to other states. It however, produced about 23,803,806 broilers during June to December 2013 and 19,057,644 broilers from January to June 2014 for human consumption (SMAAI, 2014). The demand and consumption of poultry meat has also increased over the last few years this may attributed to the rapid production rate, availability all around the year and white meat concept. Antibiotics are used by poultry industries and poultry veterinarians as routine practice to enhanced growth, feed efficiency and reducing disease and consequent mortality (Hind *et al*., 2014).Antibiotic usage has facilitated the production of poultry, allowing the consumer to purchase, as a reasonable cost, high quality meat and egg (Karmi M, 2014) during the past 50 year antibiotic specially oxytetracycline has been used in poultry production as therapeutic agent to treat bacterial infection that decrease performance and cause disease (Roudaut *et al.,* 1987; Yoshimura *e tal.*,1991 and Omija *et al.*,1994),promote growth of birds, improve feed conversation ratio (FCR), increase weight gain and maximize economic returns from individual bird. Many of antibiotic used in poultry industrial has been used in human medicine as well as. Drugs accumulated usually in liver, kidney, site of administration and different tissues (Doyle, 2006). The residues of these substances can be present in edible tissues, milk and eggs

causing toxicity to consumers with anemia, hypersensitivity and resistance to antibiotic (Suhren *et al.*, 1996). There are international bodies that regulate the presents of residues in edible products including poultry product. World Health Organizations (WHO), Food and Agriculture Organizations (FAO) have set maximum residue limits (MRLs), acceptance daily intakes (ADIs) to assure the safety of food produce for humans consumptions as to protect human health (FAO/WHO, 1991), many regulations have been put which regulates the use of veterinary medicines with establishment of (MRL) values for products monitoring of pharmacologically active compound in products (karmi M, European commission 1990, 1996). Many sensitive methods were optimized and validated for detection and determined of different antibiotic residues in animal and poultry product such as microbial growth inhibition tests which are widely used as the primary screening approach for the detection of antibiotic residues in tissues (Pikkemaat *et al.*, 2010), thin layer chromatography (TLC), ELISA, bio-sensor, high-performance liquid chromatography (HPLC), gas Chromatography (GC), liquid chromatography-mass spectrometry (LCMS) (Karmi M, 2014). The microbiological inhibition tests were the earlier test used and are still in use because they are inexpensive and can cover entire antibiotic spectrum, but are less specific than other tests (Hind *et al.*, 2014).

Objectives

Main objective

The main objective of this study was to screen preset of antibiotic in poultry tissues in Khartoum State.

Specific objective

1- To screen antibiotic residues in muscle, liver and kidney of poultry in Khartoum state.
2- To detect and quantify oxytetracycline residues in poultry meat by using High Performance Liquid Chromatography (HPLC).
3- To establish an initiate database and to help in raising awareness amongst professional and the public authorities known problem in order to protect consumers.

Chapter One

1. Literature Review

1.1. Poultry

Poultry plays a pivotal role in bridging the protein gap of animal origin in most countries of the world (Mumtaz *et al*., 2000). Its significance is even greater in developing countries where poultry are relatively cheap and can be kept in a small enclosure, usually providing both protein and income for a family. The use of modern systems of planning, organization along with new technologies has enabled a steady growth in poultry production.

1.2. Poultry Industries in Sudan

Poultry industry in Sudan saw considerable development in the last 10 years, with production increasing from 5 million broilers in 2006 to close to 90 million in 2017. More than 85% of the broiler production is located in the Khartoum area including Khartoum North, Omdurman, and around 70% of sales are in the Khartoum states. It is however, produced about 23million broilers during June to December 2013 and 19 million broilers from January to June 2014 for human consumption (SMAAI, 2014).

1.3. Poultry Disease

Diseases are the main challenge to poultry production in Sudan. Poultry diseases are once again fully revised with addition of vital new material .it remains the stander reference work on health and disease for those involved in the poultry industries, government and veterinary education (Mark Pattison, *et al*., 2007).

1.4. Treatment of the Poultry Disease

The uses of antimicrobial or antibiotic are for many purposes in poultry and in livestock as general which include therapeutic when the animal diagnosed as ill;

recent study has identified tetracycline, chlortetracycline, oxytetracycline, sulfonamide, sulfadimethoxine, sulfamethazine, sulfamethoxazo -le, penicillin, ampicillin, arsenicals, roxarsone, enrofloxacin, erythromycin as the most used antibiotics administered orally and in water in animal husbandry in Nigeria (Lawal, J.R., *et al.*, 2015) also as metaphylaxis that treatment of batch of animals when at least one bird diagnosed as ill, prophylaxis that treat the folk to prevent the incidences of disease, there is also the use of sub-therapeutic doses that taken as feed additive or added in their drinking water as growth promoter and improve feed efficiency in broiler poultry. The use of antibiotic as growth promoter or therapeutic purposes are regulated internationally because of their hazard of some drugs that can inter the human food chain, despite rigorous withdrawal measurement and testing exists to prevent the occurrence of it (Ahmed, 2019). Other drugs may use under restricted limits there is other organization or authorities search for further restrict of the randomly uses of antibiotic in animal sector, (OIE, 2012). The use of antimicrobial drugs in poultry is powerful resources that have been applied throughout the world to insure animal health with guaranteed high production (Paschoal *et al*., 2009). The most commonly antibiotic used in treatment of animal and birds was tetracycline group, macrolides, aminoglucosids, beta-lactams and sulphonamides (Kiribs, 2007).

1.5. Antibiotics

The term antibiotic was coined from the word „antibiosis“ which literally means „against life“. In the past, antibiotics were considered to be organic compounds produced by one microorganism which are toxic to other microorganisms. As a result of this notion, an antibiotic was originally, broadly defined as a substance, and produced by one microorganism or of biological origin which at low concentrations can inhibit the growth of, or are lethal to other microorganisms.

However, this definition has been modified in modern times, to include antimicrobials that are also produced partly or wholly through synthetic means (Ebimieowei E and Ibemologi A, 2016). Whilst some antibiotics are able to completely kill other bacteria, some are only able to inhibit their growth. Those that kill bacteria are termed bactericidal while those that inhibit bacterial growth are termed bacteriostatic. Although antibiotic generally refers to antibacterial, antibiotic compounds are differentiated as antibacterial, antifungals and antivirals to reflect the group of microorganisms they antagonize (Brooks *et al*., 2004). Penicillin was the first antibiotic discovered in September 1928 by an English Bacteriologist, late Sir Alexander Fleming who accidentally obtained the antibiotic from a soil inhabiting fungus Penicillium Notatum but its discovery was first reported in 1929, and clinical trials first conducted on.

1.5.1 .Pharmacokinetics of the antibiotics

1.5.1.1. Absorption

Absorption is the process that brings a drug from the administration, e.g., tablet, capsule, and in to the systemic circulation. Absorption affects the speed and concentration at which a drug may arrive at its desired location of effects, e.g., plasma. There are many possible methods of drug administration, including but not limited to oral, intravenous, intramuscular, intra-thecal, subcutaneous, buccal, recto-vaginal, ocular, inhaled, nebulized and transdermal. Each of these methods has its own absorption characteristics, advantages, and disadvantages. The process of absorption also often includes liberation or the process by which the drug is released from its pharmaceutical dosage form this is especially important in the case of oral medication. For instance, an oral medication may be delayed in the throat or esophagus for hours after being taken, delaying the onset of effects or even causing mucosal damage. Once in the stomach, the low pH may begin to

chemically react with these drugs before they even arrive in the systemic circulation (Rosalinde M and Frans G. M. Russel, 2001).

1.5.1.2. Distribution

Distribution describes how a substance is spread throughout the body. This varies based on the biochemical properties of the drug as well as the physiology of the individual taking that medication.in its simplest sense, the distribution may be influenced by two main factors: diffusion and convection. These factors may be influenced by the polarity, size, or binding abilities of the drug, the fluid status of the patient (hydration and protein concentrations), or the body habits of the individual. The goal of the distribution is to achieve what is known as the effective drug concentration. This is the concentration of the drug at its designed receptor site. To be effective, a medication must reach its designated compartmental destination, described by the volume of distribution, and not be protein-bound in-order to be active (Rosalinde M and Frans. Russel, 2001).

1.5.1.3. Metabolism

Metabolism is the processing of the drug by the body into subsequent compounds. This is often used to convert the drug into more water-soluble substances that will progress to renal clearance or, in the case of prodrug administration such as codeine; metabolism may be required to convert the drug into active metabolites. Different strategies of metabolism may occur in multiple areas throughout the body, such as the gastrointestinal tract, skin, plasma, kidneys, or lungs, but the majority of metabolism is through phase (CYP450) and phase 11(UGT) reactions in the liver. Phase 1 reactions generally transform substances into polar metabolites by oxidation allowing conjugation reactions of phase 11 to take place. Most commonly, these processes inactivate the drug, convert it into a more hydrophilic metabolite, and allow it to be excreted in the urine or bile (Fiderico pea, 2018)

1.5.1.4. Excretion

Excretion is the process by which the drug is eliminated from the body. The kidneys most commonly conduct excretion, but, for certain drugs, it may be via the lungs, skin, or gastrointestinal tract. In the kidneys, drugs may be cleared by passive filtration in the glomerulus or secretion in the tubules, complicated by reabsorption in some compounds (Rosalinde M and Frans G. M. Russel, 2001).

1.6. Tetracycline

Tetracycline was discovered in 1945 from a soil bacterium of genus Streptomyces by Benjamin Duggar (Sanchez *et al*., 2004). The first member of this class was chlortetracycline (Aureomycin). Members of this class have four (4) hydrocarbon rings and they are known by name with the suffix – cycline". Historically, members of this class of antibiotics are grouped into different generations based on the method of synthesis. Those obtained by biosynthesis are said to be First generation. Members include Tetracycline, Chlortetecycline, Oxytetracycline and Demeclocycline. Members such as Doxycycline, Lymecycline, Meclocycline, Methacycline, Minocycline, and Rolitetracycline are considered Second generation because they are derivatives of semi-synthesis. Those obtained from total synthesis such as Tigecycline are considered to be Third generation. Tetracyclins are broad spectrum antibiotic which is used to treatment of infection of the respiratory tract, urinary tract, middle ear skin infection, intestine and gonorrhea, Withdraw time 5-28 days. Excretion via kidneys and gastrointestinal tract (Pindel *et al*., l959). Their target of antimicrobial activity in bacteria is the ribosome. They disrupt the addition of amino acids to polypeptide chains during protein synthesis in this bacterial organelle. Patients are advised to take tetracyclines at least two hours before or after meals for better absorption. All tetracyclines are recommended for patients above eight (8) years because the

drugs have shown to cause teeth discoloration among patients below this age can be used in treating malaria, elephantiasis, amoebic parasites and Rickettisia. In the past, antibiotics belonging to this class were very much the envy of numerous Clinicians owing to their wide antimicrobial spectrum but this is no longer the case because numerous bacteria are now able to resist them. (Chopra and Roberts, 2001).

1.6.1. Oxytetracycline

Oxytetracycline was the second of the broad-spectrum tetracycline group of antibiotics to be discovered. Oxytetracycline works by interfering with the ability of bacteria to produce essential proteins. Without these proteins, the bacteria cannot grow, multiply and increase in numbers.

Formula: C22H24N2O9

Molar mass: 460.434 g/moll

Melting point: 181 to 182 °C (358 to 360 °F)

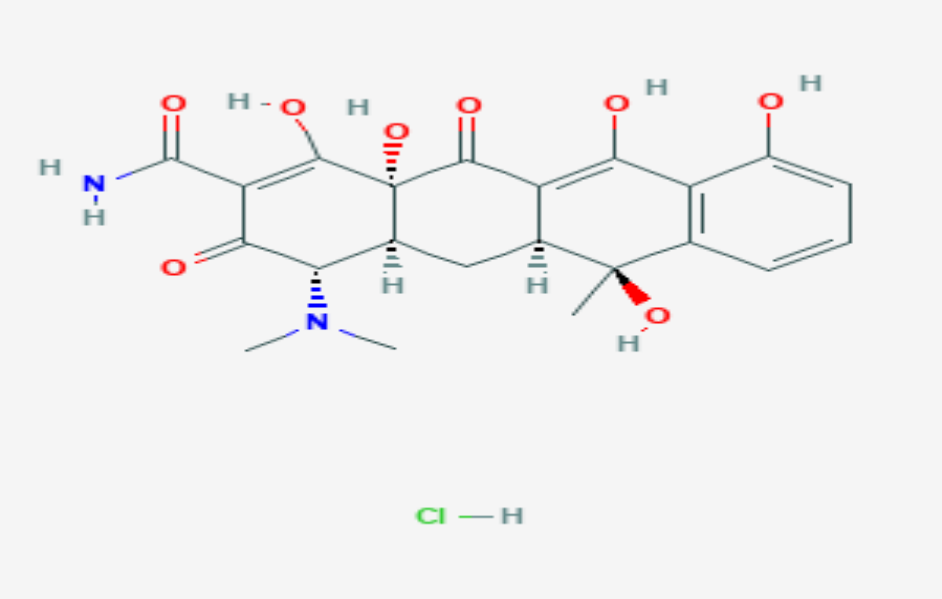

Figure 1 Oxytetracycline chemical structure

1.6.2 .Mode of Action of the Oxytetracycline

Inhibition of protein synthesis Living things including bacteria are defined by the amount and type of proteins they are composed of, and continually produce. Proteins are responsible for the structural composition metabolic and physiological processes, and response to adverse conditions, amongst other roles. However, the type and amount of proteins produced by a bacterium at any given time is dependent on information contained in yet another very important biomolecule

1.6.3. Withdrawal Period of Oxytetracycline

The Oxytetracycline residue values in marketed poultry have been found after one to five days of drug administration. It may be due to the use of tetracycline in feed as the growth promoter and treating of layer (Razia Khatun, 2018).

1.7. Drug accumulation

1.7.1. Causes of the Drug Residues

Residues can result for many reasons, including poor records of treatment, failure to observe recommended label withdrawal time, prolonged drug clearance, treated bird identification problems, product not used as label direction, lack of advice on withdrawal period (Jonesm and Seymourm, 1988, Kukanich, *et al*., 2005). Antibiotic residues can result from any of the following:

improper use, extra label use or over use, lack of veterinary control and lack of adherence to withdrawal times which is a major cause of violation worldwide (Adesiyun *et al*., 2005) . Residues of veterinary drugs include compounds or metabolites in an edible tissue of poultry product that because we can found the residues in their yielding product, including residues of associated impurities of the veterinary drug concerned (FAW/WHO, 2004). There are many contraries have regulations to control the hazard of the veterinary drugs to protect the public

health but there is some problem due to the variation in the maximum residue limits in some countries and the enforcement of the residue limit that can make the persistence of risk of antimicrobial residues. There is an international body that can regulate the amount of the residues in any edible products including poultry product. World Health Organizations (WHO), and Food and Agriculture Organizations (FAO). Have set maximum residue limits (MRLs), acceptance daily intakes (ADIs) for human consumption. (FAO/WHO, 1 991).

1.7.2 .Causes of Poultry Contamination

The poultry product can be contaminated by various chemical, environmental or from poultry slaughter houses. These include residues, like antibiotic, pesticides, hormones, micro toxin and dioxin metals, and cleaning agent, disinfecting agent, sanitizer and micro toxins (Harding and Ditton, 1995). There are some points considered by WHO /FAO (2004) possible causes of the antibiotic residues including.

1-Dosage rates may be in correctly recorded.

2-No quality control of antibiotic supplies and concentration.

3-Drugs are frequently administrated by unqualified farmers.

4-Lack of veterinary advice regarding withdrawal period, and this may have combined by illiteracy, label and printed instruction for drugs of limit use.

5-Individual animal or bird identification and traceability are often impossible.

6-Treatment recorded is poorly maintained or non –existent.

7-Drugs are frequently administrated.

1.7.3. Stability of Drug Residues

The thermal stability of Tetracycline residues in animal food products was considered by many researchers. The tetracycline group is affected by direct sun light and change in heat, and it may have spilt to other metabolites other research mentioned that TCs in animal tissues were more stable under heat treatment and

were not totally inactivated by cooking conditions or heat processing (Moats, W. A, 1988, Gratacós-Cubarsí.,*et al* ., 2007). Salah H. *et al*., 2013 report the effects of various ordinary cooking procedures (boiling, roasting and microwaving) on Tetracycline residues (OxyTetracycline, Tetracycline, Chloro-Tetracycline and Doxycycline) in chicken meat and determine the cooking time required for each cooking procedure to make the cooked sample safer for consumption and found variation in stability during cooking and food processing. Most of animal product is always cook before consumption, and this will influence the level of risk posed by such residue (Ahmed, 2019). On the other hand, some studies indicated that Tetracyclins in animal tissues were more sensitive to heat treatment and were totally destroyed by treatment at 100˚C for 30 min or at higher temperature for shorter times (Gratacós-Cubarsí., *et al* 2007). On the contrary, (Al-Ghamdi *et al.*, 2000) stated that OTC increased on boiling, and (Kuhne *et al.*, 2001) found that the concentrations of TCs in heat-treated (100˚C) meat and bone meals were higher than those found before the heat treatment.

1.8. Public Health Impact of Antibiotic Residue

1.8.1. Antimicrobial Residue Effect on Human Health

Veterinary medicines especially antibiotics are among the most important component which are persistent consumption of animal products containing a high level of antibiotic residues, led to allergic reactions in some hypersensitive individuals and generally led to antibiotics resistance in humans (Liu, Y., Yang, H. *et al.,* 2013). Allergic reaction develops when individual challenged by exposure after primary sensitization as rapid as strong immunological response as compare to systemic administration (Dewdney and Edward, 1984). Development of resistant pathogen the relation between the antibiotic and the development of the resistant pathogen appear to be hypothetical. Antibiotics resistance occurs

when the human body has been exposed to prolonged bits of small dosage of antibiotics in food, water, or environment, which makes the disease-causing microorganisms to develop resistance against the antibiotics (Guidi, L.R., Santos, F.A, *et al* .,2018, N. Li, K.W.*et al*., 2017) Besides, exposure to tetracycline residues in humans has been implicated in poor fetal development, gastrointestinal disturbance, tetracycline pigmentation teeth, potential carcinogens, as well as increase occurrence of antibiotic-resistant genes (N. Li, K.W.*et al.,* 2017, Bahmani, K., Shahbazi, Y, 2020). Most recent studies have found that early life exposure to antibiotics residues could be linked to obesity and possibly diabetes in children (N. Li, K.W.*et al.,* 2017), Olumayowa Joshua, *et al*.,2021). Ingestion of antibiotic can influence gut flora that can increase the antibiotic resistance of gut micro flora (Langford *et al.*, 2003). There is an observational change in the bacterial ecology in the human gut and on the weakening of the so- called barrier effect that excreted by gastrointestinal flora prevent invasion of the bowel by microbial pathogen (Wood ward., 1998).

1.8.2. Toxicological Effects

Despite their low toxicity, the antibiotic residues may be directed may be directly toxic or cause or cause possible allergic hypersensitivity reaction in humans. The residue levels present in food are unlikely to be sufficient to cause initial sensitization, and it's more likely to occur by therapeutic use in human. This level may occasionally elicit hypersensitivity in previously sensitized patients (Ahmed, 2019).

1.8.3. Discoloration of Intestinal Flora

The concept has been focused on general modifications of bacterial ecology in the human gut and on the weakening of the so-called barrier effect (Woodward, 1998). The main adverse effect of antibiotic drugs on the human intestinal flora are selection of resistant bacteria and disruption of colonization barrier of resident

intestinal flora. Other effects such as alteration of the metabolic activity of flora may be important (Ahmed, 2019).

1.9. Some Definitions of Antibiotic Residues

1.9.1. Extra - Label Drug Use (ELU)

Extra – Label Drug Use (ELU) is refers to the use of an approved drug in a manner that is not in accordance with the approved label directions. ELU occurs when a drug only approved for human use is used in animals , when a drug approved for one species of animal is used in another , when a drug is used to treat a condition for which it was not approved , or the use of drugs at level in excess in recommended dosages .For instances, Drugs that have been approved by the U.S. food and drug Administration(FDA)for the treatment of human Psychiatric disorders are increasingly being used by veterinarians for the treatment of animal behavior problems. Such use, however, is extra label. Extra able drug use is the actual or intended use of a new animal drug in a manner that is not in accordance with the drug labeling. Using a veterinary drug labeled for treatment of one veterinary medical condition to treat a different veterinary condition (e.g. behavior problem) is one type of extra label use. Using a drug labeled for treatment of a human condition such as depression, to treat an animal problem, such as aggression, is another type (Ahmed, 2019).

1.9.2. Maximum Residue Limit

As a consequence of increased public interest and health concerns regarding food safety, the European Union (EU) and Codex Alimentarius Commission have set limits for antibiotics residues in animal tissues, as well as other products of animal origin (Guidi, L.R., Santos, F.A. *et al*., 2018). This limit is the maximum allowed concentration of specific antibiotics in animal products, which would not cause any harm to human health. A maximum residue level (MRL) is the highest

level of antibiotic residue that is legally tolerated in or on food or feed when antibiotics are applied correctly (Good veterinary Practice) which can safely be consumed. The Codex Committee on veterinary Residues (CCVR) is responsible for establishing Codex MRLs for antibiotic residues in specific food items or in groups of food (FAO/WHO, 1989). The most sensitive parameter and test species have to be used. Moreover, often safety factor of 100 to correct the intra-species variability and the inter species extrapolation is applied.

1.9.3. No-Observable-Effect-Level (NOEL)

Is defined from a Varity of toxicological and pharmacological studies and represents the lowest exposure level at which there is no significant increase in frequency or severity of an effect between the exposed animal and its appropriate control.

1.9.4. Acceptable Daily Intake for Veterinary Drugs

It defined as the amount of drug, expressed on body weight bases, can be ingested daily over life's and without appreciable health risk (WHO, 1987).

ADI is estimated as a fraction of the observed-adverse-effect-level (NOAEL) determined of standardized long-term laboratory animal toxicological studies conducting on at least two animal species the NOAEL is then divided by safe factor ranging from 10^2-10^3 depending on the nature of compounds toxicology or the strength of the data (Muhammed *et al*., 2009).

1.9.5. Withdrawal Period

The withdrawal periods are the time which passes between the last doses given to the animal and the time when the level of residues in the tissues (muscle, liver, kidney, skin/fat) or products (milk, eggs and egg) is lower than or equal to the MRL/safe level. Until the withdrawal period has elapsed, the animal or its products are not safe for human health. It is absolutely true that, the declared

withdrawal periods and safe level or Maximum Residue Limit (MRL) of any antibiotic will be stay in same point (Razia Khatun, 2018).

1.10. Evaluation of the Safe Residue Levels

The safe evaluation of veterinary drugs intended for use in food producing animals, relies heavily on the result of toxicity studies in laboratory animals supported were ever possible by any data resulting from human exposure. In addition to the toxicological effect, there is other effect such as immunological and pharmacological effects were also been taken in to account. The standard approach to assessing the safety of chemical contaminants in food stuffs intended for human consumption is the acceptable daily intake (ADI), which was first used by the joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1958, and it has been modified since then. Its value now universally uses to quantify the safety of chemical food contaminants including residue veterinary drugs (Fidal and Milary 2006). The regulation has been almost improper impact on the availability of veterinary medicine. The residues are considered as safe if they cause no adverse health effect when ingested daily by human over a lifetime. The determination of MRLs is based on the Acceptable Daily Intake (ADI) which may be determined on the basis of toxicological or pharmacological data and may be calculated with the following formula (Muhammad *et al*., 2009,FAO/WHO, 1991).

ADI (mg/kg BW) = NOEL (mg/kg BW/day)/ safety factor (usually 100) Where: ADI: acceptable daily intake

NOEL: no-observable-effect-level. The determination of the MRLs also takes into account the potential consumer intake of residues and bodyweight of the consumer.

The time period required for the level of active substance to decrease the MRL differs, depending on the particular animal product and the formulation of the

active substance. Withdrawal time is determined through depletion studies all substance. The withdrawal period to insures that this foodstuff does not contain residue in quantities over the MRLs for active substances laid down under regulation (EEC) no. 2377/90 (Serratosa *et al*., 2006).

1.11. Antibiotic Residues Detection Techniques

There are many methods that can detect the antibiotic residues in various animal originated products. Some of them were biological method and the other was chemical method. The biological method includes antimicrobial inhibition tests (one plate test, tow plate test, four plate test and six plate test) and enzyme linked immunosorbent assay (ELISA). Chemical method includes high performance liquid chromatography (HPLC), gas chromatography, electrophoreses, thin plate layer and mass spectrometry liquid chromatography (MS-LC/MS). Method generally divided in to screening and confirmatory methods, some laboratories add a third intermediary level based on post- screening test which gives structural or biological activity information about the residue (Stolker and Brinkman, 2005).

1.11.1. Screening Method

Screening method can be defined as methods that are used to detect the presence of substance or class of substances at level of interest (Ishraga, Ibrahim *et al*., 2016). These methods have the capacity for a high sample throughput and are used for large numbers of samples for potential non-complaint results. They are specifically designed to avoid false compliant results. Screening methods are usually inexpensive, easily to use and handle, rapid, suitable for high –throughput analysis, and have good sensitive, especially and detection capability with a probability error of $p<5$ %. (Berendsen, 2013). Several bio-based tests have been reported for the screening of antibiotic substances in different matrices. Bio-based screening methods used for detection of antibiotics in products of animal origin

have been reviewed (Poperlka *et al*., 2004, Bovee and Pikkemaat, 2009, cháfer-pericás *et al*., 2010). The most commonly applied bio-based screening techniques for antibiotics are microbiological inhibition assays, immunoassay and reporter gene assay (Bovee and Pikkemaat, 2009).

1.11.1.1. Microbiological Inhibition Tests

These methods were the most common used as screening method for detection of antibiotic residues. that can permit large number of samples rapidly. It based on the reaction between a bacteria and antibiotic present in sample (Ishraga, Ibrahim *et al*., 2016). But it not able to determine the specific antibiotic that founded in the sample screened so that the purposes of this method are qualitative detection (Heschen and Suhren, 1996). There are several tests are available to determine the organism susceptibility the specific antibiotic drug. Different inhibitory tests were developing to screen different animal products (Popelka *et al*., (2004). The tube and plate tests are the most common formats (Berendsen, 2013). The tube test consists of growth media inoculated with bacterium, supplemented with PH or redox indicator. If no specific antibiotics are present, the bacteria start to grow and produce acid, which will cause a detectable color change. If antibiotics are present that inhibit bacterial growth, no color change will occur (Hoff *et al*., 2011). The agar diffusion test or the plate test is most commonly used in small laboratories. This test involves lacing disks impregnated with different antimicrobial drugs or sample in to agar plates containing the infective organism. After specific time the plate observed for zone of inhibition. STOP and last test were commercially available. They were depending on the inhibition of the *Bacillus subtililis* spore. Some of these tests are available as kits with a high throughput. They need limited laboratory capacity to ensure reproducible conditions of application. Few of them need a more experienced laboratory that is able to produce a medium and a bacterial suspension (Ishraga, Ibrahim *et al*.,

(2016). They are widely used to perform residue control and self-control by industry (Pikkemaat, 2010). Important advantages compare to immunoassay and instrumental method is that microbiological tests can detect any antibiotic compound that shows bacterial activity (Picó and Barceló, 2008). Moreover, they have the potential to cover the entire antibiotic spectrum within one test (Pikkemaat, 2010). The most important drawbacks of the microbiological tests are their lack of selectivity, especially the tube test, relatively high detection limits and the long incubation time. As a result, microbiological inhibition assay is not suitable for detection of banned antibiotic compounds like chloramphenicol (Berendsen, 2013).

1.11.1.2. Immune- Receptor-Assay

Some screening tests are also developed using antibodies, they are most or less selective of a compound or the structure of an antimicrobial family (Gustavsson and Sternesjo, 2004).

1.11.1.3. Immunoassay

Immunoassay is based on binding reaction between a compound and antibody. The most commonly applied immunoassay in antibiotic analysis is the enzyme-linked immune sorbent assay (ELISA)(Mengi and Xi, 2011).There are different test formats for antigen quantification like the double antibody or sandwich ELISA tests (Shanker *et al* .,2010), but all tests are based upon the same principle. The sample that screened of antibiotic residue is included with antibodies, under the production of an analyte antibody binding complex.

1.11.1.4. Radioactive Labelling Techniques

The Charm I and Charm II tests are microbial receptors assay for detection of antibiotic residue (Tolder and Reig, 2006). The tetracyclines and chlroamphinicol

assays are antibody assays (Ishraga, Ibrahim *et al*., 2016).The test employs C14or H3 radio labeled antibacterial to compete for the binding sites .

1.11.1.5. Biosensors

There are different types of biosensors have been developed to screen veterinary drugs. They utilize biological molecules, such as enzymes or antibodies, Capable to recognizing specific target analytes (Ishraga, Ibrahim *et al*., 2016).

1.11.1.6. Thin layer chromatography

In the early 70sthin layer chromatography (TLC) was the method of choice for the qualitative detection of banned substances, also as antibiotic screening (Ishraga Ibrahim *et al*., 2016). (TLC)-mass spectrometry has been reported as antibiotic residue screening method. The method can be performed manually or within line detection.

1.11.2. Confirmatory Techniques

Until the last decade of the 20th century, the main instrumental techniques used for veterinary drug residues analysis were liquid chromatography (LC)using ultra violate detection(UV), diode array detection(DAD) and fluorescent detection (FLD)and gas chromatography (GC) using flame ionization detection and electron capture detection (Berendsen, 2013). in the last decade fast switching triple quadruple instruments became available. This facilitates the development of HPLC methods in which a large number of compound were detect within on run. (Stolker and Brinkman, 2005). The development of high resolution LC (HPLC), it gives higher sample throughput (Ishraga Ibrahim *et al*., 2016).

1.11.2.1. High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) with UV detector was introduced in the middle 70s, but the first instruments were expensive and not robust (Tolder and Reig, 2006). UV detector also doesn't match the specificity

and limits of detection needs for group A (substance having antibiotic affect). However, for the quantitative determination of group B substance (veterinary drugs) UV detection and post column derivatization were often used. Typical detection of multi-residues in samples is relatively simple and rapid, requires preliminary clean-up through solid –phase extraction followed by filtration before injection into reversed phase HPLC with diode array detection. This procedure has been applied to meat or detection of antibiotic residues, anabolic and corticosteroid veterinary drugs (Kribrs, 2007). The important advantages of HPLC are that, it takes short time to obtain the result, has high sensitivity and specificity depending on detector, high automatization leading to high productivity and the possible receipt of more information from spectra when using diode array detector. The disadvantages include initial investment (equipment), need of expertise, and need of sample preparation (Ishraga, Ibrahim *et al.,* 2016).

1.12. Control of Antibiotic in Poultry Product

The poultry products or meat should be free of antibiotic residues and regular testing for antibiotic is there for practiced, with the severe penalties to positive result (Harding and Ditton, 1995). We can control the antibiotic residues by correct and clear identification of the treated bird or flock and make a clear record which include identification of the treated flocks and their number of the infected houses which treated with antibiotic, person who give the antibiotic m type of treatment, dose given, and dead time, of the given antibiotic which is determined the last time by which can slaughter and sale the bird. Restricted with the duration of the withdrawal period of the treated antibiotic. Test the poultry product that slaughtered or that found in the market by using rapid screening antibiotic residues test (Harding and Ditton, 1995).

Chapter Tow

Materials and Methods

2.1. Study area

The samples were collected from local market in Omdurman, Khartoum and Khartoum north localities in Khartoum State.

2.2 .Study Design

Descriptive Cross-sectional study.

2.3. Sample Size

The sample size was estimated according to the equation below

Sample size = $[(1.96)^2 * P (1-P)] / L^2$

Were:

P: prevalence rate.

L: allowable error.

=4*0.5(1-0.5)/0.0025 = 384/3 (Localities)/3(organ)

== 42.68 samples per season.

2.4. Sample collection

A total of 360 poultry liver, muscle and kidney were taken randomly from local market of the three localities seasonally (summer, autumn and winter) during the period 2016-2017.

2.5 .Sample handling and process

The Samples were put in sterile sealed bags and stored in ice untill transported to the Central Veterinary Research Laboratory and stored at
-20°C till analyzed.

2.6 .Evaluation of the Antibiotic Residue in Poultry Meat

Microbiological inhibition test was used as a screening method. Then the positive samples were confirmed using HPLC method.

2.7 .Microbiological Inhibition Assay

Poultry tissues were screened for antibiotic residues using the microbial inhibition test described by Korean-Dierick *et al*., (1995) with some modification. The assay is based on the detection of the growth inhibition using one plate test (OPT) which depended on inhibiting *Bacillus subitiles* strain in agar media.

2.7.1 .Test Organism

Bacillus Subtilis (ATCC6633) was obtained from the Department of microbiology, Central Veterinary Research Laboratories. Bacteria were harvested in sporulation culture medium and serially diluted.

2.7.2 .Preparation of the Normal Saline

Isotonic saline was prepared by dissolving 0. 85 gram of sodium chloride in 10 ^{2}mlof distilled water then sterile by autoclaved at 121˚C for 15 minute.

2.7.3 Preparation of Bacterial Culture

The organism was used in this test *Bacillus subitiles* (ATCC6633). One colony from each fresh culture was taken by sterile loop under flame then diluted in 9ml sterile normal saline the suspension was shaken by vortex mixer to be

homogenized, then adjusted to 0.5 McFarland standard turbidity (equivalent to 3*108cell/ml).

2.6.4. Preparation of Medium Plate

Nutrient agar (28g) were prepared (Oxoid, 2006) by suspending in one liter distilled water and dissolving completely in boiling water bath then sterilized by autoclave at 120bar for 15 minutes and then cooled to 47ºC.

2.7.5 .Microbiological Inhibition Test

One drop of the bacterial suspension prepared put in the petri dishes and distributed in the petri dishes by using sterile loop under flame, and then the tissues were cut into small portion (dimension) and bled and then incubated overnight. The tested samples were read by inhibition zone of their diameter 2mm or more. The positive samples from this test were further be analyzed by HPLC to detect, identify and quantify antibiotic residues in poultry meat.

2.8 . HPLC Assay:

The present of oxytetracyclin residues, in positive samples resulted from the microbiological inhibition test was confirmed using chromatographic technique, HPLC method as described by (hamide. S , *et al.*, 2000), with some modifications.

2.8.1. Laboratory Materials and Chemicals

- Precision scale ,Micropipette, 10-100 and 100-1000 µl (eppendorf),Vortex, Stainless steel knife, Blender ,centrifuge tube 50 ml, 15 ml,Volumetric flask 10ml, 100ml, 250ml, 1000ml,0.45µm Nylon filters obtained from Waters Assoc, syringe 5 cc, 10 cc, Standard Oxytetracycline HCl was obtained from Clear Inspiring Chemistry INC, Clearsyth Labs LTD, and Mumbai, India, Methanol,

acetonitrile and nitric acid were obtained from Carl Roth Gmbh,0.45 µm Nylon filters were obtained from Luer syringe filter, USA and Other chemicals were of analytical or of HPLC grade.

2.8.2. Reagent Preparation

2.8.2.1. Stock Solutions and Working Standards

Stock solution of 1mg/ml of oxytetracycline stander was prepared by dissolving 0.1g of oxytetracycline in 100 ml methanol into volumetric flask.

The working solution of 0.5, 2, 4, 6, 8, 10 µl/ ml were prepared by taking the appropriate volume from the oxtetracycline stock solution in to 1 ml vial and completed with methanol.

2.8.2.2. Preparation of nitric acid 30%

Nitric acid 30% was prepared by dissolving 30 ml nitric acid in70 ml de-ionized water in 100 ml volumetric flask.

2.8.2. 3. Preparation of Oxalic acid

Oxalic acid dehydrate 0.1m was prepared by dissolving 1.261g in HPLC water in 1000 ml volumetric flask and complete to the mark with HPLC water.

2.8.2.4. Preparation of Mobile phase

Oxalic acid dilutes 1.261g (75%) in 1 liter, methanol (5%), acetonitril (20%).

2.9. HPLC instrument:

The system consisted of the following components:

- A Cecilce CE 4100 including ,7725 rheodyne injector (20 µl loop),HP UV-Vis detector, Vacuum degasser, GradientpumpCE4100 ,Module ,Column compartment oven, Hypersil BDS C18 (5 mm, 150×4 mm) column supplied by HP (Germany).

2.10. Chromatographic Condition

Isocratic separation was achieved using the mobile phase as mention above.

- Flow rate of 1ml/min, Wave length 360 nm ,Column oven temperature 30◦C and injected volume 20 µl.

2.11 Sample Preparation

The samples were homogenized in a blender for further analysis.

2.12. Sample Preparation for Recovery

2 ± 0.01g were weight in 50 ml falcon tube (ten sample) from blank sample which are known as negative and 50, 100 and 200 µl from the working standard were added to the sample (three sample each and the last one left as blank). Then the sample stand for an hour before starting the extraction procedure.

2.13 .Extraction Procedure

The following steps were:

1- 2 ± 0.01g cured meat product (muscle, liver and kidney)
2- 0.1 g citric acid was added.
3- 1ml nitric acid (30%).
4- 4ml methanol
5- 1ml de-ionized water
6- Vortex for good mixing.
7- Keep in an ultrasonic bath for 15 min
8- Centrifuged for 10 min at 5300 rpm.
9- Filtering through a 0.45 µm nylon filter.
10- 20 µl of solution was injected into HPLC for analysis.

2.14. Some Method Validation Parameters

2.14.1 Recovery

Recovery was calculated by subtracting the amount of oxytetracyclin found in the blank sample from the spiked one according to the following equation:

Percent recovery= (C.cal – C. blank /C exp)*100

Where: C.cal is the mean of the result of the spiked sample.

C. bla is the mean of the result of the unspiked sample (blank).

C exp is the concentration add.

2.14.2 Limit of Detection (LOD)

Detection Limit (DL) is defined as the concentration of OTC that produces an analytical signal equal to three the standard deviation of the background signal. The detection limit was calculated according to the following equation:

LOD = 3*Sd/slop

Where the standard with 0.5 ppm concentration was injected 6 times and the mean and standard deviation were calculated.

2.14.3 Limit of Quantification LOQ

Limit of quantification is defined as the concentration of OTC that produces an analytical signal equal to ten the standard deviation of the background signal.

LOQ = 10* STD/ slop

STD: is the standard deviation of the standard 0.5 ppm.

2.15. Statistical Analysis

Data collected was analyzed using SPSS statistical software (Statistical Package for the Social Sciences) version 20. Descriptive methods, frequency and one- way ANOVA were done (p-value of ≤ 0.05).

Chapter Three

Results

3.1. Microbiological inhibition assay

Poultry samples collected from three localities at Khartoum state were tested for the presence of antibiotic residue using inhibition test (one plate test) as screening method our result revealed that 52 (14.4%) out of 360 tested samples were positive to antibiotic residue and the negative were 308 (85.6%) as shown in table 1 . Concerning the difference between organs muscle tissues revealed high percentage (18.0%) clear inhibition zone than liver and kidney (15.0%)(Figure 4). Our results showed that the positive samples in Omdurman were 20 (16.6%), Khartoum was 18 (15.0%) and Khartoum North were 14 (11.6%) (Figure 5). The organs collected from Omdurman locality were contained high concentration of antibiotics than other two localities. Also in this result the number of positive sample to antibiotic were 31 (25.8%) in summer while in autumn were 13 (10.8%) and in winter were 8 (6.6%) as shown in (figure 6). The figures from 7-12 showed the differences in the presence of inhibition zones concerning the different organs in the three localities among the three season, the result showed that the usage of antibiotic was increased in hot seasons than cold seasons.

Table 1: Percentage of positive liver, muscle and kidney samples for antibiotic residue in Khartoum state (n= 360)

Samples	Percent%	Frequency
Positive	14.4	52
Negative	85.6	308
Total	100.0	360

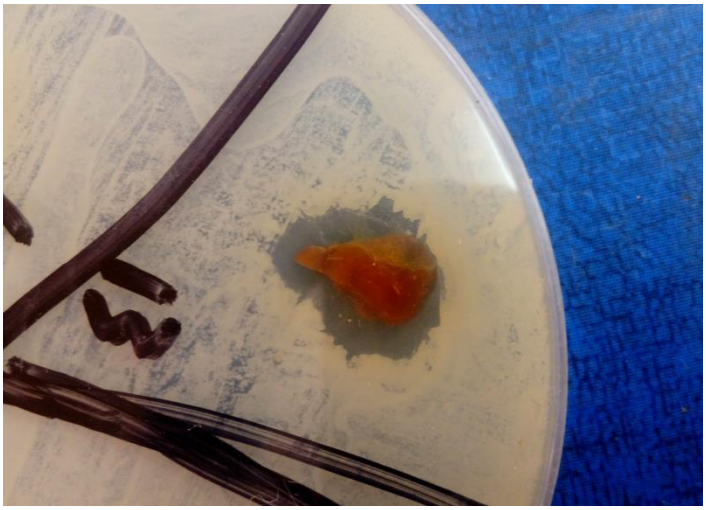

Figure 2 Plate 1: Positive sample showed inhibition zone (kidney).

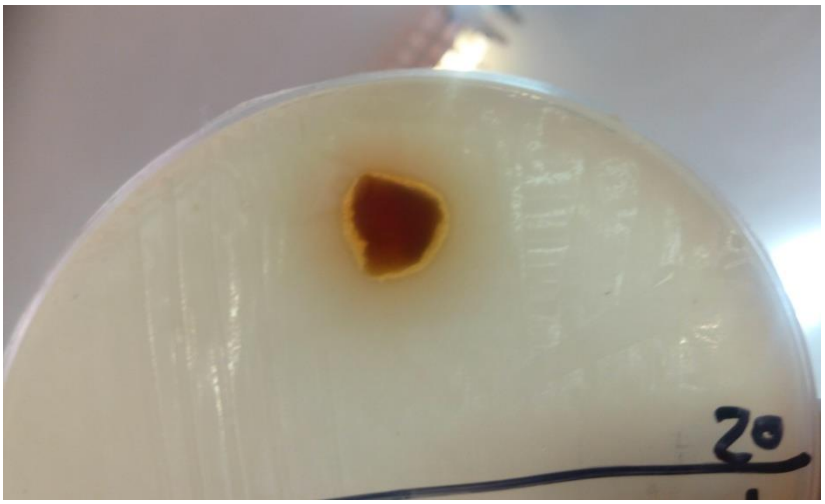

Figure3 Plate 2: Positive sample showed inhibition zone (muscle).

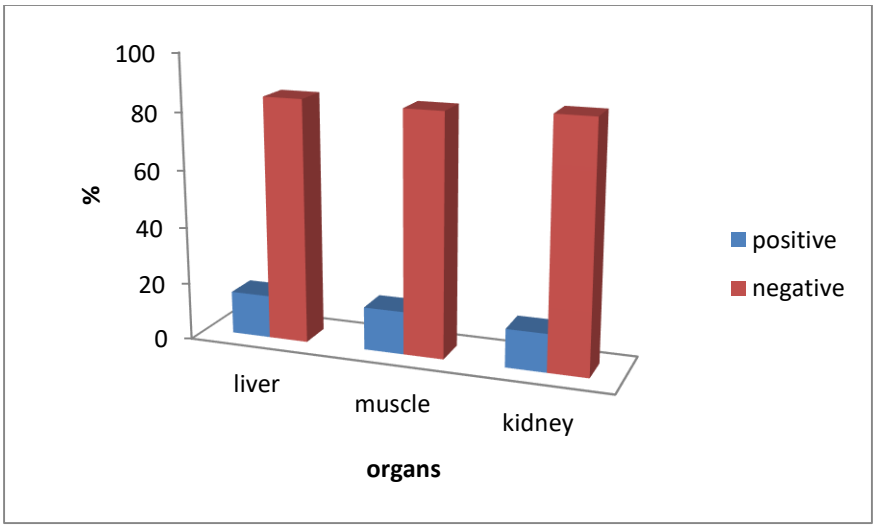

Figure 4: The percentage of positive and negative samples among organs in poultry samples collected from Khartoum State using inhibition test.

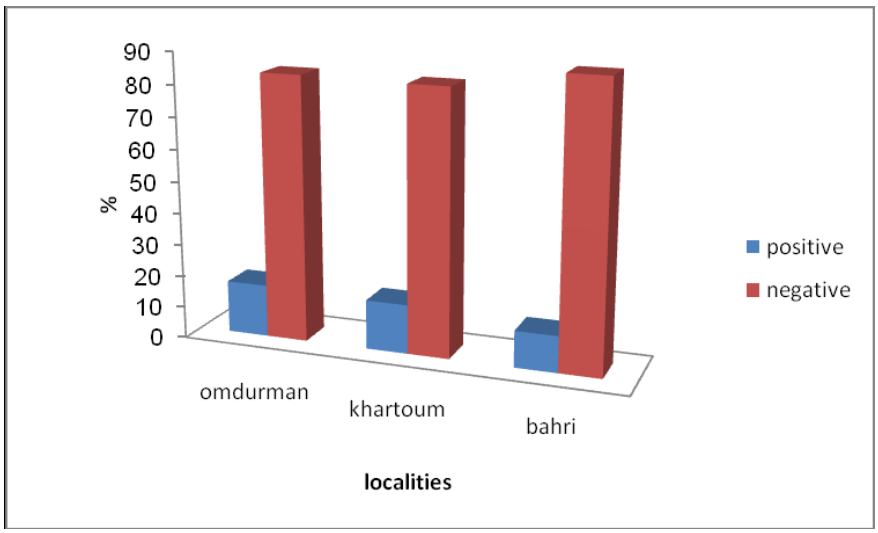

Figure 5: The percentage of positive and negative poultry samples in different localities in Khartoum State using inhibition test

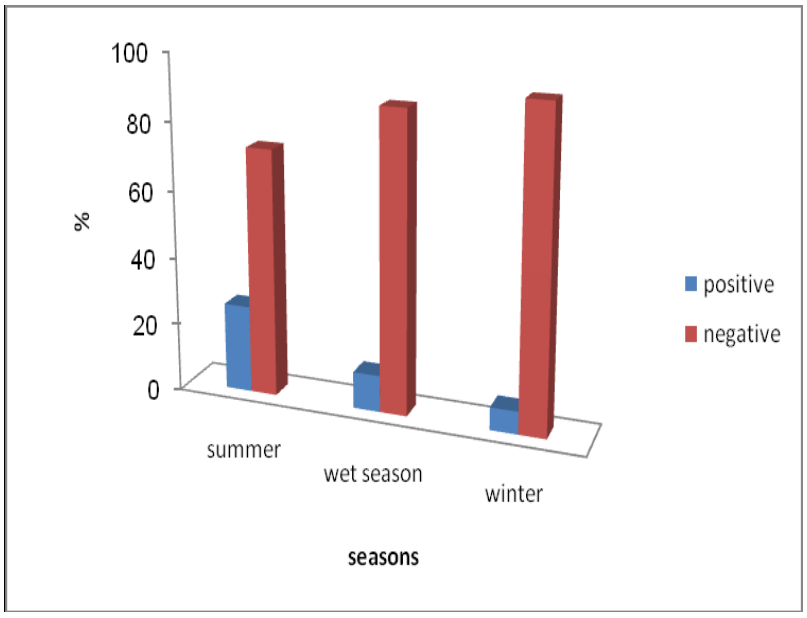

Figure 6: The percentage of positive and negative samples among seasons in poultry samples collected from Khartoum State using inhibition test.

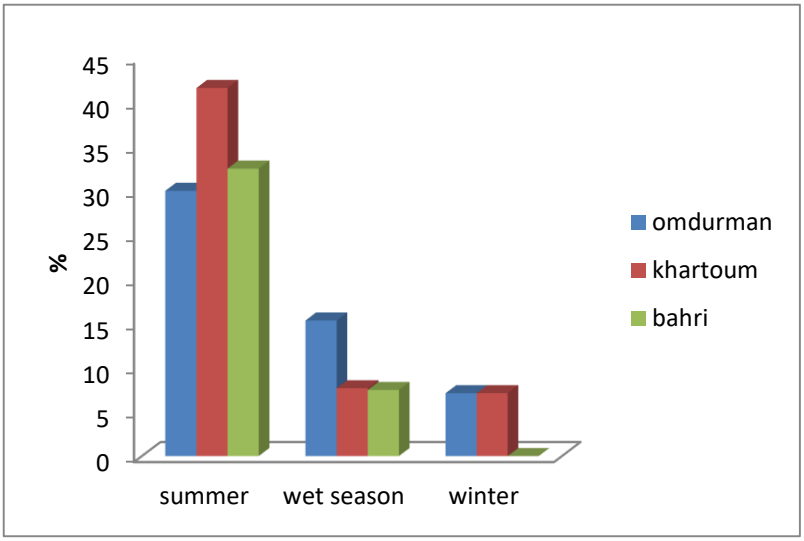

Figure7: The percentage of positive and negative liver samples collected from Khartoum state in different localities using inhibition test among seasons.

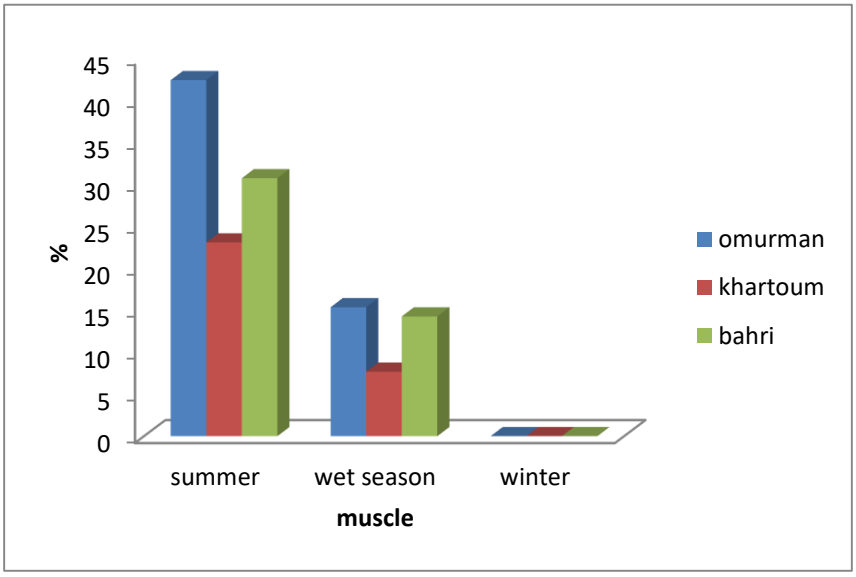

Figure 8: The percentage of positive and negative muscle samples collected from Khartoum State in different localities using inhibition test among seasons.

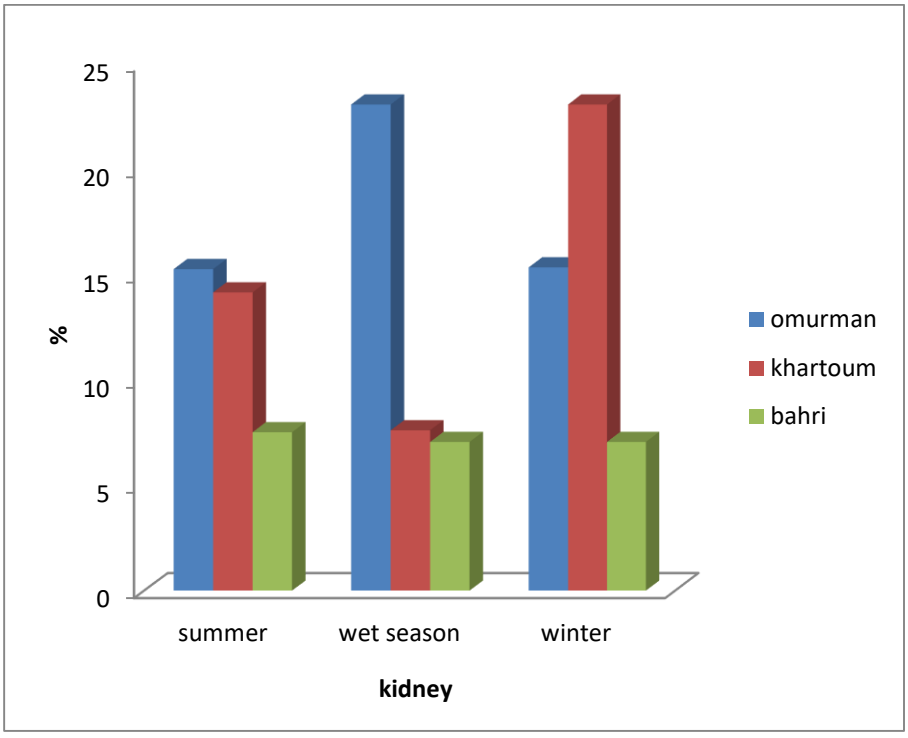

Figure 9: The percentage of positive and negative kidney samples collected from Khartoum State in different localities using inhibition test among seasons.

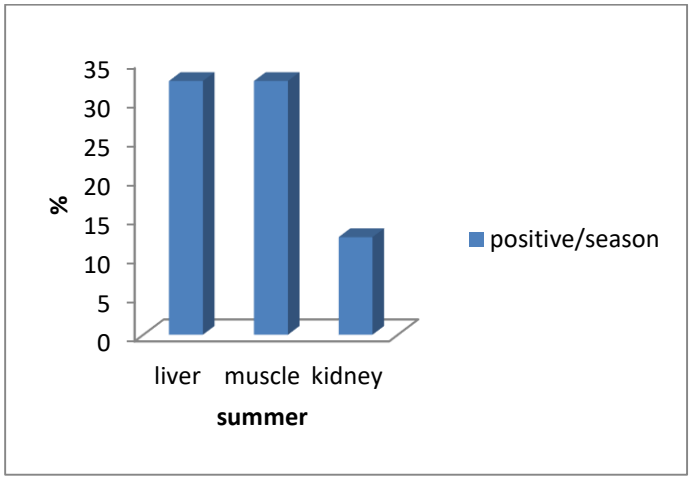

Figure 10: The percentage of positive and negative samples from different organ collected from Khartoum State using inhibition test in summer

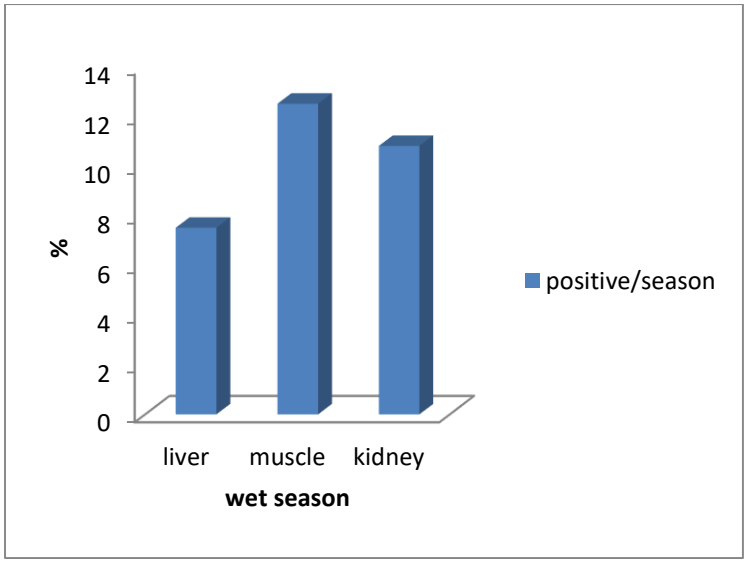

Figure 11: The percentage of positive and negative samples from different organ collected from Khartoum State using inhibition test in wet season.

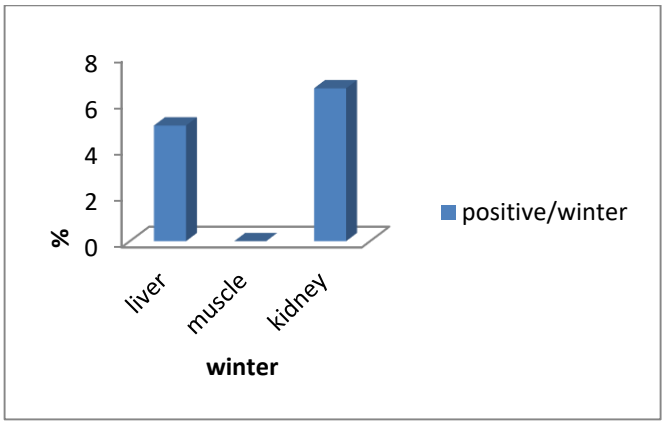

Figure12: The percentage of positive and negative samples from different organ collected from Khartoum State using inhibition test in winter.

Result

The oxytetracycline (OTC) residues were detected using high performance liquid chromatography (HPLC).

The curve of OTC standard and the spike sample showed good linearity over the range of the concentrations examined (0.5-10 PPM and 6.25- 25 PPM) respectively with correlation coefficient of R^2=0.9973 and R^2=0.9932 respectively (Figure13 and figure 2).

The percentages of recoveries for the spiked samples and the LOD and LOQ of the method were shown in table 1. The chromatograms of 0.5 ppm OTC standard, positive and negative samples were shown in the figures 3, 4, 5 respectively.

The result obtained for the spiked sample showed a recovery ranged from 61.6-88.6% and the LOD and LOQ determined were 0.100 and 0.336 ppm, respectively. However, the sample revealed responses ranged from ND to 3.97 ppm.

Concerning organs; OTC concentration was detected in muscle in 5 out of 11 samples ranged between 0.11-2.99 ppm, liver samples showed residue in 10 out of 11 analyzed samples ranged between 0.30-3.97 ppm while all tested kidney showed OTC residue ranged between 0.34-3.34 ppm.

The mean concentrations of OTC in poultry meat in different organ are shown in table 2 with no significant difference ($P \geq 0.05$) between the three organs examined.

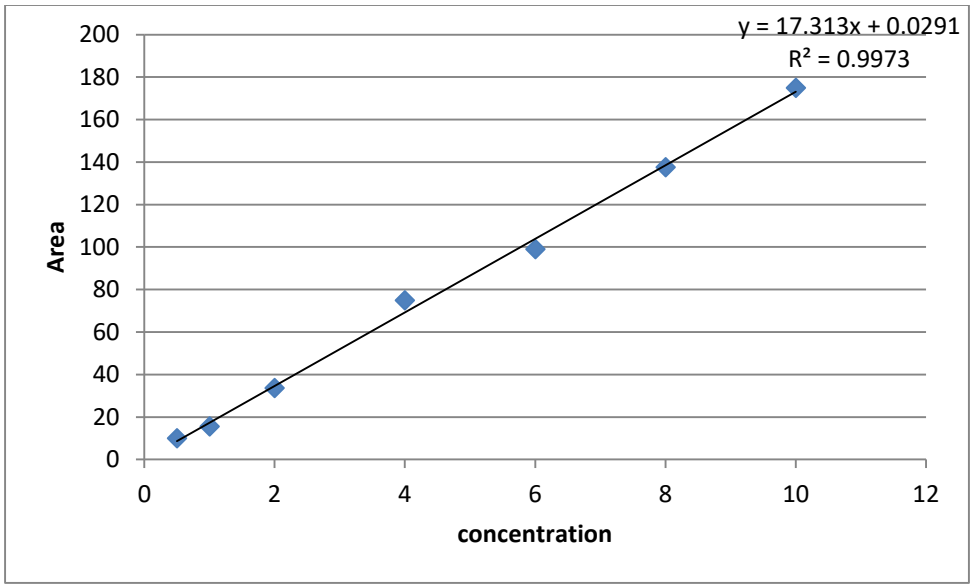

Figure 13: Standard Curve of oxytetracycline standards.

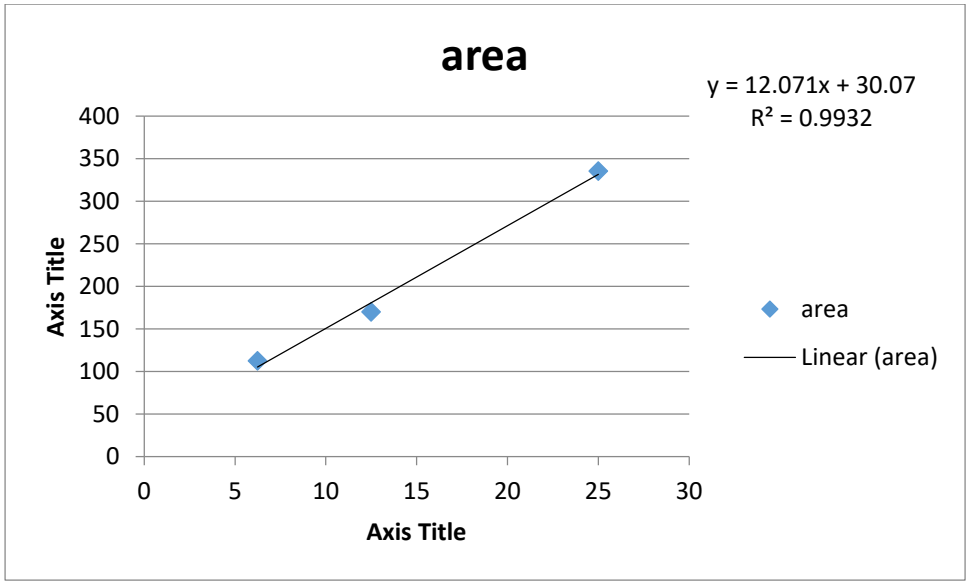

Figure 14: Standard Curve of oxytetracycline spiked sample.

Table 2: Method performance of oxytetracycline in spiked muscle sample at different concentration levels.

Spiking level (ppm)	Recovery ± SD (%)	RSD (%)	Linear range (ppm)	*R2*	LOD (ppm)	LOQ (ppm)
6.25	73.02± 13.98	19.15	0.5-25	0.9969 ± 0.0015	0.10	0.33
12.5	63.07 ± 2.41	3.82				
25	69.72 ± 3.57	5.11				

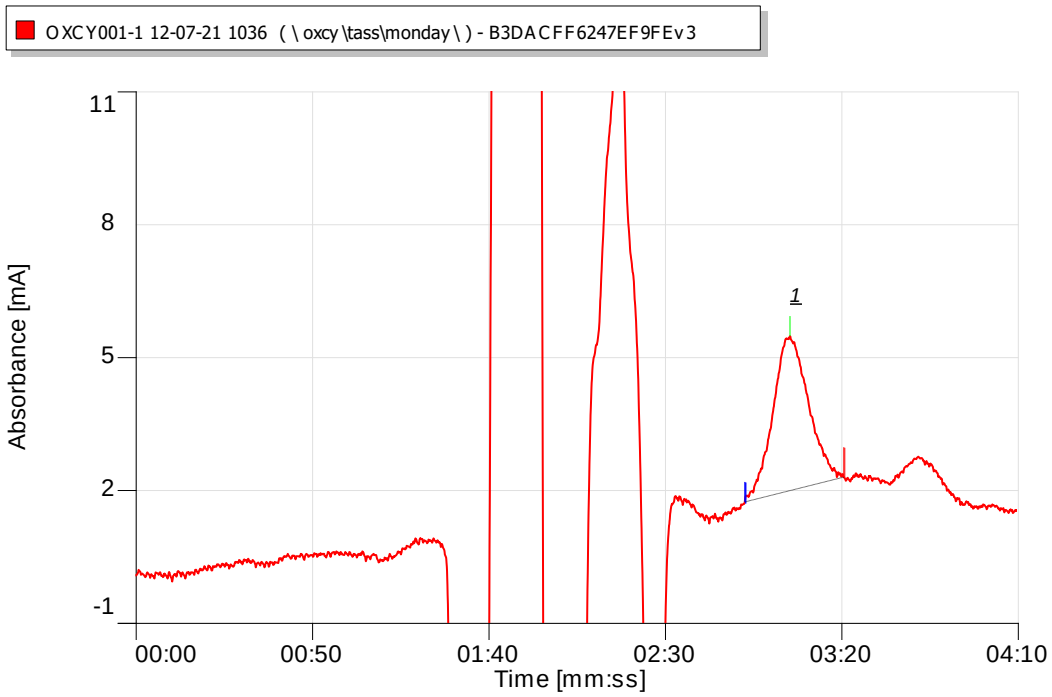

Figure 15: Chromatogram showing the peak of OTC standard 2 ppm.

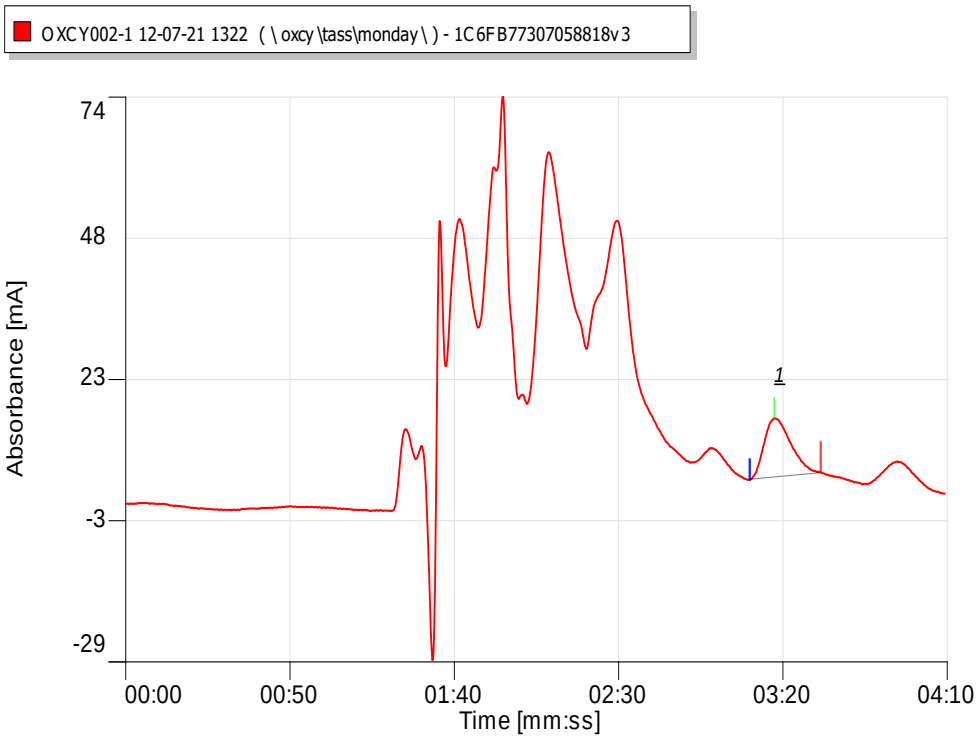

Figure 16: Chromatogram showing the peak one of the positive samples.

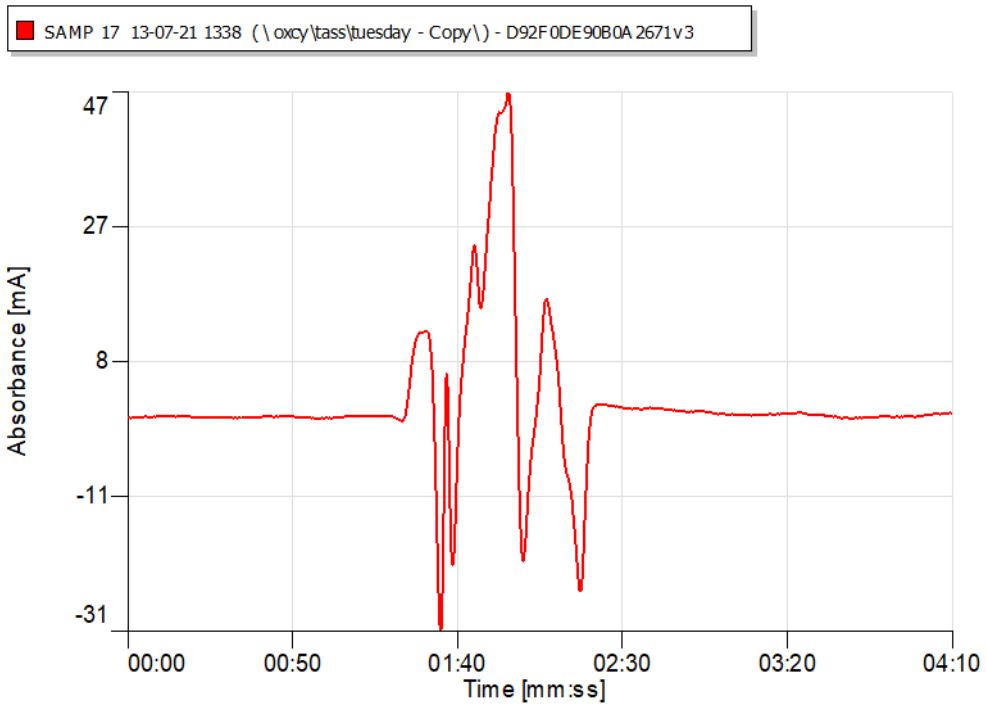

Figure 16: Chromatogram showing one of the negative samples**.**

Table 3: The mean concentration, std, min and max (ppm)ofoxcytetracycline residue in poultry sample (kidney, Liver and muscle) collected from Khartoum state.

	Liver (n=11)	**Kidney (n=12)**	**Muscles (n=10)**	**Total**	P value
Mean	1.27	0.89	0.52	1.13	0.35
Std	1.23	0.78	0.98	1.3	
Min	ND	0.34	ND	ND	
Max	3.97	3.34	2.99	2.99	

Chapter Four

Discussion

A little work concerning residue monitoring has been done in Sudan. Our study examined oxytetracycline (OTC) in chicken samples obtained from some parts of Khartoum state and revealed residual OTC in some of the samples examined. The level of (OTC) obtained in our study ranged between ND - 3972.78 µg/kg from which 30% were more than the MRL set by the codex. This was similar to that reported by Ahmed El Rayah *et al*., (2015) in his study in sheep found that 28.6% had detectable levels above MRL of OTC residues which indicated a widespread misuse of veterinary drugs by food animal producers However, Hala (2006) reported a low percentage (16.87%) of antibiotics residues in poultry in Khartoum State. Our results were higher than that obtained by Olumayowa Joshua Onipede (2021) who reported range from ND to 0.031µg/g in chicken in Nigeria and in other studies in Thailand (Chiaochan *et al*., 2010), Canada (Khaled, A *et al*., 2019) oxytetracycline concentration was found to be ND in chicken samples.

Concerning the organ tested kidney, liver and muscle there was no significant difference obtained between the three organs, however, Hussein *et al*., (2015) reported significantly higher prevalence of OTC residues in sheep muscle samples as compared with liver. While Muhammad Danish (2017) reported a

deposition of oxytetracycline and chloramphenicol residues in substantially higher amounts within the liver and kidney samples of broilers.

In poultry industries the producers used a variety of products to control diseases and increase yields. Many researchers investigated deferent antibiotic residue and reported that the highest concentrations of antibiotic residue found in tissues were tetracycline (8%) followed by ampicilin (4%), streptomycine (2%) and aminoglycosides (1%) as compared to other antibiotics (Abdul Sajid *et al*., 2016)

The risks of residues in foodstuffs from animal origin could be reflected into several forms, carcinogens, allergies toxicity alteration of the intestinal flora, bacteria resistant (Wageh *et al*., 2013; Mohamed *et al*., 2019).emphasis should be placed on the importance of food safety (Isam, 2016) antibiotic abuse is most important cause of high prevalence of residue and large number of resistant bacteria. The risk of violative drug residues is minimized when treatment protocols are carefully followed. Therefore, poultry industries have started to enhance the production of meat using enriched feed for disease prevention (Mehdi. *et al*., 2018).

Conclusion

OTC residues are detected in poultry meat (kidney, liver and muscle) with different levels in Khartoum state although it is more than the MRL that we need further investigations covering other antibiotics, other farms and other organs throughout the country are recommended. Developing monitoring system for screening residues in food is an important issue now days to produce healthy food and to meet the international standard to enhance international trade.

This data may not suggest an immediate threat to the health of the consumers, but, could suggest the need for the federal government to enforcement agents to ensure stricter compliance to adequate withdrawal period in poultry industries in Sudan. This study was summarized from the results of the IAEA project RAF5078 RAF5084: “Strength Food Contamination Monitoring and Control System and Enhancing Competitiveness of Agricultural Exports Using Nuclear and Isotopic Techniques”

Recommendation

The deposition and subsequent detection of residual amounts of antimicrobial substances exceeding MRLs in poultry products is really a matter of concern. Therefor we recommend:

1- Strict legislations and control measures at national and local levels should be established for production, sale, and use of veterinary drugs in treatment or as feed supplements.

2- Withdrawal periods between administration of drugs and slaughtering must be instigated and enforced by the respective food and drug administration, other regulatory authorities, and professional veterinarians

3- Regular monitoring and assessments for the presence of drug residues in edible tissues of poultry to ensure the consumers safety

4- Increase the awareness of food safety around the policy maker and the consumer.

5- Analyze other important drugs used in poultry industry and/or other animal production line.

6- Introduction of advanced technology for residue detection.

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