بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Detection of Some Antibiotic Residues in Sheep Meat In Khartoum State

الكشف عن بقايا بعض المضادات الحيوية في لحوم الضأن بولاية الخرطوم

By

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Initiation

قال الله تعالى:

{ أَوَلَمْ يَرَوْا أَنَّا خَلَقْنَا لَهُمْ مِمَّا عَمِلَتْ أَيْدِينَا أَنْعَامًا فَهُمْ لَهَا مَالِكُونَ * وَذَلَّلْنَاهَا لَهُمْ فَمِنْهَا رَكُوبُهُمْ وَمِنْهَا يَأْكُلُونَ * وَلَهُمْ فِيهَا مَنَافِعُ وَمَشَارِبُ أَفَلَا يَشْكُرُونَ }

سورة يس (71-73)

Dedication

To:

All my family, my father, my mother, my wife, my brothers, my sisters, for their continuous support for me, especially my brother Hassan for his help and my brother Osman for his help in collecting samples .

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Abstract

In this study, we aimed to shed the light on the antibiotic residues in sheep meat in Khartoum State, hundred samples of sheep meat from the slaughterhouse (Elshaheed Nasr El-din) were collected were placed in plastic bags and kept in ice box and sent to the laboratories of Sudan University of Science and Technology for analysis. Data were collected through questionnaire, formal and informal interviews, and observations. Data and results were analyzed by (Statistical Package for Social Sciences) version 24. The result showed that 53 (53%) of the samples were positive for antibiotic residuce. The highest result in the inhibition zone test was 26.4% for the circumference of 2 millimeters and the lowest was 17.12 by the 1.9%.The results of the social study showed that the imprudent use of antibiotics on farms was 53%. The result about the frequency and percentage of residuce was 53 and 53%. Sixty presents of the interview I heard about withdrawal time. Based on the results obtained from this study we recommend the application of the accurate diagnosis for detection of antibiotic residuce, proper use of antibiotics usage only after the prescription from the veterinarian, taking into account the withdrawal period when slaughtering and increasing of awareness of the breeders and the population about the food safety.

الخلاصة

هدفت هذه الدراسة إلى إلقاء الضوء على بقايا المضادات الحيوية في لحوم الأغنام بولاية الخرطوم، حيث تم جمع مائة عينة من لحوم الأغنام من مسلخ (الشهيد نصر الدين) في أكياس بلاستيكية وحفظها في صندوق ثلج. وإرسالها إلى معامل جامعة السودان للعلوم والتكنولوجيا للتحليل. تم جمع البيانات من خلال الاستبيان والمقابلات الرسمية وغير الرسمية والملاحظات. تم تحليل البيانات والنتائج بواسطة (الحزمة الإحصائية للعلوم الاجتماعية) الإصدار 24. وأظهرت النتائج أن 53 (53٪) من العينات كانت موجبة لبقايا المضادات الحيوية. اعلى نتيجة فى اختبار منطقة التثبيط كانت 26.4٪ لمحيط 2 مليمتر واقلها 17.12٪ 1.9٪ واظهرت نتائج الدراسة الاجتماعية ان الاستخدام غير الحكيم للمضادات الحيوية في المزارع كان 53٪. كانت النتيجة حول التكرار والنسبة المئوية للمخلفات 53 و53٪. ستون عرضًا للمقابلة التي سمعتها عن وقت الانسحاب، وبناءً على النتائج التي تم الحصول عليها من هذه الدراسة نوصي بتطبيق التشخيص الدقيق للكشف عن بقايا المضادات الحيوية، والاستخدام السليم للمضادات الحيوية فقط بعد وصفة الطبيب البيطري، مع مراعاة ما يلي: فترة الانسحاب عند الذبح وزيادة وعي المربين والسكان بسلامة الغذاء.

Introduction

Sudan is a country in Northeast Africa, and it is occupies 1,886,068 square kilometers (728,215 square miles) with a population of 43 million (2018 estimate). Sudan has one of the harshest climates in the world, with one third of its area being desert and 60% of the remainder suitable for grazing and less than a quarter being potential arable land (FAO, 1997).

Based on FAO data, Sudan is the first to third among all African countries in the number of cattle, sheep, goats, and camels, third in the number of poultry and fifth in the number of donkeys (Ahmed, *et al* 2020).

The sheep population of Sudan is about 49 million over 36 % of the livestock in the country (El-Hag *et al*., 2007)

This vast area and the large animal population make the use of veterinary medicines more common in cities and in the countryside to keep the animals healthy.

This left a great challenge to control the use of veterinary drugs for fear of side effects from the miss-use or extra-label and their effect on human and animal health together.

The most commonly used treatments is antibiotics, anthelmintic, organophosphorus, anti -inflammatory and growth promoters, but the antibiotics is the most.

Antibiotics residues is causes harmful effects including bacteria resistance, allergies, toxicity, kidney failure and other side effects that lead to death (Mc Evoy 2002).

Residues occur mostly when animals are slaughtered within the withdrawal period of the drug (Guest and Paige, 1991), and particularly when treated with an extra label dose (Krainock, 1991; Riviere, 1991).

Research objectives:

1-1 The overall objectives:

To detect some of antibiotic residues in mutton

1-2 Specific objectives:

1/ To detect the presence of some antibiotics residuce in sheep meat by using one plate screening test method

2/ To point out the commonly used antibiotics and factors associated with their usage.

3/ To provide up to date information about the levels of antibiotics residues in sheep meat in Khartoum state.

Chapter One

Literature Review

1. Antibiotics:

The term antibiotic used to be derived from the idiom „antibiosis" which means „against life". In the past, antibiotics had been organic compounds produced with the aid of one microorganism which is toxic to different microorganisms (Russell, 2004). As result of this notion, an antibiotic was originally, largely defined as a substance, produced by one microorganism (Denier et al., 2004), or of biological origin (Schlegel, 2003) which at low concentrations can inhibit the increase of, or are lethal to other microorganisms (Russell, 2004).

1.2 The classification of antibiotics:

There are several ways of classifying antibiotics, but the most common classification schemes are based on their molecular structures, mode of action and spectrum of activity (Calderon and Sabundayo, 2007).

1.3 Classification of antibiotics according to their molecular structures:

1.3.1 Beta-lactams:

The most prominent representatives of the beta-lactam class include penicillins, cephalosporins, monobactams and carbapenems.

1.3.1.1 Penicillin:

Members of Penicillin class include Penicillin G, Penicillin V, Oxacillin (dicloxacillin), Methicillin, Nafcillin, Ampicillin, Amoxicillin, Carbenicilin, Piperacillin, Mezlocillin and Ticarcillin (Boundless, 2016).

1.3.1.2 Cephalosporin:

The first known member of this group of antibiotics was first isolated by Guiseppe Brotzu in 1945 from the fungus Cephalosporium acremonium. Although the drug was first isolated by Guiseppe Brotzu, it was Edward Abraham who got the credit to patent it having been able to extract the compound.

Members of this group of antibiotics are similar to penicillin in their structure and mode of action. They form part of the most commonly prescribed and administered antibiotics; more succinctly, they account for one-third of all antibiotics prescribed and administered by the National Health Scheme in the United Kingdom (Talaro and Chess, 2008).

1.3.1.3 Monobactams:

The discovery of this class of antibiotics was first reported by Skyes and co-workers. The antibiotic was obtained from the bacterium Chromobacterium violaceum. They are part of beta-lactam compounds but unlike most other beta-lactams, the beta-lactam ring of monobactams stand alone and is not fused to another ring (Bonner and Sykes, 1984).

1.3.1.4 Carbapenems:

This class of antibiotics, represented in Figure 5, was discovered out of necessity in 1976.

Prior to this time in the late 1960's the effectiveness of penicillin was greatly threatened owing to the emergence of beta-lactamase in bacteria. Bacterial beta-

lactamases conferred resistance on bacteria against penicillin (Papp-Wallace *et al*., 2011) and the carbapenems include:

1.3.1.4.1 Mipenem: a broad spectrum effective against aerobic and anaerobic pathogens, usually taken orally and active in low concentrations, with minimal allergy side effects.

1.3.1.4.2 Meropenem: a broad spectrum effective against non-fermentative Gram-negative bacilli particularly against acquired infections.

1.3.1.4.3 Ertapenem: a broad spectrum with limited activity against non-fermentative Gram-negative bacilli (Brink *et al*., 2004).

1.3.2 Macrolides:

The first antibiotic belonging to this class was first discovered and isolated in 1952 by McGuire as metabolic product of a soil inhabiting fungus Saccharopolyspora erythraea. This fungus was formerly known as Streptomyces erythraeus belonging to the genus Saccharopolyspora of actinomycete bacteria (Moore, 2015), same as Erythromycin, Azithromycin and Clarithromycin (Miller, 1973).

1.3.3 Tetracyclines:

Tetracycline was discovered in 1945 from a soil bacterium of the genus Streptomyces by Benjamin Duggar (Sanchez *et al*., 2004).

The first generation Members include Tetracycline, Chlortetecycline, Oxytetracycline and Demeclocycline, the Second generation include Doxycycline, Lymecycline, Meclo cycline, Methacycline, Minocycline, and Rolitetracycline and the third generation such as Tigecycline(Fuoco, 2012).

1.3.4 Quinolones:

This class of antibiotics was first discovered as nalidixic acid by Scientists involved in search of antimalarial drugs. Nalidixic acid was discovered as an impurity during the development of quinine in the early sixties. They are able to interfere with DNA replication and transcription in bacteria. Two major groups of compounds have been developed from the basic molecule: quinolones and naphthyridones which include cinoxacin, norfloxacin, ofloxacin, ciproxacin, temafloxacin, sparfloxacin, nalidixic acid, enoxacin etc. (Domagala, 1994).

1.3.5 Aminoglycosides:

Aminoglycosides The first drug to be discovered among members of this class of antibiotics was streptomycin, first isolated in 1943 (Mahajan and Balachandran, 2012). Streptomycin has been greatly used against Mycobacterium tuberculosis, the causal agent of tuberculosis among humans same as Streptomycin-gentamicin-neomycin-amikacin and tobramycin.

1.3.6 Sulphonamides:

Sulphonamides are reportedly, the first group of antibiotics used in therapeutic medicine, and they still play very important role in medicine and veterinary practice (Eyssen *et al*., 1971).

1.3.7 Glycopeptides:

Glycopeptide antibiotics generally abbreviated as GPAs were originally obtained as natural products, but the last 20 years witnessed the emergence of semi-

synthetic derivatives with improved activity and pharmacokinetic properties (Van Bambeke *et al*., 2004; Kahne *et al*., 2005).

1.4 Antibiotics Mode of Action:

The mechanism of antibiotic actions are as follows:

- Inhibition of cell wall synthesis
- Breakdown of cell membrane structure or function
- Inhibition of the structure and function of nucleic acids
- Inhibition of protein synthesis
- Blockage of key metabolic pathways
- (Madigan and Martinko, 2006; Talaro and Chess, 2008; Wright, 2010).
- **1.5 Antibiotics residues:**
- **1.5.1Residues:**
- Residues are defined as all active ingredients or metabolites of those ingredients that remain in meat or other foodstuffs from the animal to which the medicinal product in question has been administered (EC, 2002). Regulation No. 470/2009 of the European Parliament and of the Council defines residues as all pharmacologically active substances, whether active ingredients, excipients or degradation products and their metabolites, which remain in animal-derived food. The concept of drug residues in food was developed over the second half of the 20th Century, resulting in the definition of a 'no observed effect' level, an Acceptable daily intake (ADI) and maximum residue limit (MRL) in food (CAC, 2011).
- **1.5.2 Occurrence of antibiotic residues in foods :**
- The use of antibiotics in food producing animals may leave residues in foodstuffs of animal origin like meat, milk, and eggs. The occurrence of these

residues may be due to any one of the following: a failure to observe the withdrawal periods of each drug, extra-label dosages for animals, contamination of animal feed with the excreta of treated animals, or the use of unlicensed antibiotics (Paige, 1994). The introduction of antibiotics to the veterinary field started soon after the use of antibiotics for the treatment of bacterial diseases in humans. The main use of antibiotics in animal rearing was for the treatment and prevention of diseases. Indeed, antibiotics have been used for the treatment of mastitis, arthritis, respiratory diseases, gastrointestinal infections, and other infectious bacterial diseases (Palleschi *et al*., 2001).

- **1.5.3 Withdrawal period and maximum residue limit:**

Use of animal medicines requires observance of the withdrawal period. This is the time between the last doses given to the animal and the time when the level of residues in the tissues (muscle, liver, kidney, skin and fat) and products (milk, eggs, honey) lower than or equal to the MRL. Until the withdrawal period has elapsed, the animal or its products must not be used for human consumption (Jackson, 1990).

1.5.4 Causes for occurrence of antimicrobial residues:

- Poor treatment records, poor management
- Difficulty to identify treated animals
- Lack of guidance on withdrawal periods
- Off-label use of antimicrobial
- Failure to notice drug withdrawal period
- Accessibility of antimicrobials to laymen
- Extended usage or unnecessary dosages of antimicrobials
- Absence or lack of enforcement of restrictive legislation to use antimicrobials

- Lack of consumer awareness about the magnitude of human health hazards associated with antimicrobial residues consumption through meat and meat products are some of the primary reasons for incidence of antimicrobial residues in meat and meat products (Muhammad *et al*., 1997; Kaneene and Miller, 1997; CAC, 2001).

1.5.5 Drug resistance:

Resistant microorganism can get entrance to human, either through direct contact or indirectly via milk, meat and/or egg. As the bacteria of animal origin, they may either colonize human endogenous flora or superimpose and supplement load to the reservoir of resistance genes already present in man. The potential for animal to human transfer of resistance is existed (Beyene, 2016).

The resistance of microorganisms, arising from sub therapeutic uses of penicillin, tetracyclines, and sulfa drugs in agriculture are suggested by the World Health Organisation (WHO) to be a high priority issue (Beyene, 2016).

1.5.6 Drug hypersensitivity:

Drug hypersensitivity was defined as an immune arbitrated response to a drug agent in a sensitized patient and drug allergy is constrained to a reaction mediated by IgE. Allergic reactions to drugs may include anaphylaxis, serum sickness, and cutaneous reaction.

1.5.7 Carcinogenic effect:

The term carcinogen refers to an effect produced by a substance having carcinogenic activity. The latent hazard of carcinogenic residues was related to their collaboration or covalently binding to various intracellular components such as proteins,

deoxyribonucleic acid (DNA), ribonucleic acid (RNA), glycogen, phospholipids, and glutathione (Beyene, 2016).

1.5.8 Disruption of normal intestinal flora:

The bacteria that usually live in the intestine acts as a blockade to avert incoming pathogen and causing diseases. Antibiotics might reduce the total number of the bacteria or selectively kill some important species. (Beyene, 2016).

1.5.9 Mutagenic effect

The term mutagen was used to describe chemical or physical agents that can cause a mutation in a DNA molecule or damage the genetic component of a cell or organisms. Several chemicals, including alkalizing agents and analogous of DNA bases, have been shown to elicit mutagenic activity. There has been growing concern that drugs as well as environmental chemicals may pretence a probable threat to the human population by making of gene mutagen or chromosome breakage that might have adversely affects human fertility (Beyene, 2016).

1.5.10 Teratogenic effect

The term teratogen applies to drug or chemical agent that produces a toxic effect on the embryo or foetus during a critical phase of gestation. Consequently, a congenital malformation, which affects the structural and functional integrity of the organism, was produced (Beyene, 2016).

1.5.11 Nephrotoxicosis:

Aminoglycosides given in therapeutic dosages mainly cause ototoxicosis, but may also additionally reason nephrotoxicosis, allergy, and neuromuscular disturbances. (Lozano *et al.* 2012).

1.5.12 Anemia:

Hazards of chloramphenicol observed in association with clinical use in humans include dose-related, reversible suppression of the bone marrow, which is a circulatory collapse in children less than 30 days on excessive doses and irreversible, idiosyncratic, non-dose associated aplastic anemia (Waltner -Toews and McEwen, 1994; Maria and Mary, 2012).

1.5.13 Control and prevention of antibiotics residues:

1.5.13.1 Prohibition of the Harmful Groups:

Nitrofurans, particularly furazolidone, furaltadone, nitrofurantoin and nitrofurazone for livestock production used to be totally prohibited in the EU in 1995 due to worries about the carcinogenicity of the drug residues and their potentially hazardous effects on human health (Mccalla, 1983; Vroomen *et al*., 1990; Van KotenVermeulen,1993).

1.5.13.2 Withdrawal Periods:

The withdrawal period always printed on the label to define as the time that is required for 99% of the animals in a population (treated according to label directions) to have drug residues that are lower than accepted residue levels defined via FDA (Jones, 2014).

1.5.13.3 Increase the Awareness of the owners:

Although public awareness of the drug residue trouble in meals is high and various governmental agencies spend large quantities of time attempting to control this problem, residues in animal tissues are still a vital challenge today (Seri, 2013).

1.5.13.4 Monitoring the animal feed stuff:

Animal feedstuffs are also analyzed for antibiotic residues. Animal finishing feed which is fed to animals in the period before slaughter, should be certified free of veterinary drugs and a specific withdrawal period is set for each antibiotic. The antibiotics commonly administered through animal feeds are the macrolides and polypeptides which are used in growth promoting and the ionophoric polyether antibiotics, used to improve feed effectivity and for the treatment of coccidiosis (McGrane, 2000).

1.5.13.5 Law Force:

In the USA all animal drugs have to be accredited with the aid of FDA before they can be marketed for public use. Receiving FDA approval is a complicated and expensive process, as the drug developer should prove that the medicine is safe and fine when used at the proposed labeled dosage.

The New Animal Drug Application (NADA) must include all the possible side effects the drug can also cause and show that they can consistently manufacture the product with ingredients from safe and reliable sources. If the drug is for food animals, then withdrawal times (WDT) should be provided at the labeled dosage to ensure that the residues in meat, milk, and eggs are below ranges protected for human consumption.

Chapter Two

Materials and Methods

2.1 Study Area

Khartoum state is the national capital and the largest city of Sudan. It's located at the confluence of the White Nile and the Blue Nile the two Niles unite to form the river Nile. The state lies between longitudes 31.5 to 34 E and the latitude 15 to 16 N. it consists of three city Khartoum, Khartoum north and Omdurman.

2.2 Collection of samples

The target samples were 100 samples from Ovine and the samples were muscles. All samples collected in plastic bags and all of them were put in icebox to be delivered into deep freezer for further laboratory analysis.

2.3 Questionnaire survey

The questionnaire was designed and distributed in Khartoum State to have basic information about the manner of using antibiotics and it was collected from owners of animals where selected randomly that means not all the owners have the same chance for being selected and this was called Non-probability sampling methods as described by Thrusfield (2007).

2.4 One plate test (O. P. T.)

One Plate Test (O. P. T) was used as described by Koenen -Dierick *et al*. (1995) and Nada (1996). The test organism was Bacillus subtilis (strain ATCC6633). The test depends on bacterial growth inhibition. Inhibition zone appears around the filter

paper that contained tissue fluid of samples. The sample was considered positive when the inhibition zone was 2 mm and more, doubtful when it was 1 to 2 mm, and negative when it was less than 1 mm.

2.4.1 The principle of method:

The principle of the test is preparing plates seeded with sensitive bacteria (Bacillus Subtilis) at specific conditions that can presumptively indicate the presence of specific antimicrobial group residues. The samples can be applied on top of the agar layer. After over-night incubation, the presence of an antimicrobial residue becomes visible as an inhibition zone around the sample. The size of the inhibition zone depends on the type of residue and its concentration, while the sensitivity of the test is affected by many factors, such as indicator organism, pH, type of growth medium, and thickness of the agar layer (Bovee and Pikkemaat, 2009).

2.4.2 Test organism:

B. subtilis (strain ATCC6633) was used.

2.4.3 Sterilization:

2.4.3.1 Hot air oven:

This method was used for sterilization of clean glass containers, which were wrapped in paper or put in stainless steel cans, temperature was 160°C for one hour (Stainer *et al*., 1986).

2.4.3.2 Red heat:

This method was used for sterilizing wire loops, straight wire and tissue forceps (Cruick- Shank *et al*., 1975).

2.4.3.3 Autoclaving

This method was used for sterilization of culture media and for materials that could not stand the dry heat. The temperature was 115 to 121°C under 10 to 15-pound pressure for 15 to 20 min (Barrow and Feltham, 1993).

2.4.4 Samples handling:

An incision was made into the liver sample to have around 0.5 gram in 5 mm thick to be placed immediately into the Petri dish.

2.4.5 Nutrient agar preparation

Twenty-eight grams of nutrient agar powder (Oxoid, 2006) was placed in 1000 ml of distilled water. The medium was autoclaved at 121°C for 15 min. The medium was cooled at 50°C. Twenty milliliters of the medium was distributed for each Petri dish and the solidified agar was kept in refrigerator at 4°C.

2.4.5.1 Preparation of standard test organism culture

B. *subtilis* was seeded in nutrient broth. Sporulation culture medium was perpetrated in 500 ml flat slide bottles loosely closed with screw caps for adequate aeration. Each bottle contained nutrient broth culture of B. *subtilis* and incubated at 37°C for 48 h until 90% of culture was spores.

2.4.5.2 Test procedures

One milliliters of standard organism were added to 20 ml of nutrient agar in each Petri dish, then mixed and left for 10 min to solidify on a level surface bench and with clean dry and sterile forceps were picked up filter paper and tested. Plates were incubated at 37°C until growth was visible within 24 h. Zone inhibition was observed around the samples when the sample containing antibiotic was measured in millimeters by the ruler. Negative samples did not show such clear zone (Negative = less than 1 mm). Interpretation of the results was done as mentioned earlier (Nada, 1996).

2.4.5.3 Cultivation, Media and Solution:

To prepare test plates with B. *subtilis*, test agar pH 6 was used. Further, sporulation medium was used (containing, in 500 ml, proteose peptone 1.725 g (HiMedia), casein enzyme hydrolysate1.725 g (HiMedia), NaCl 2.55 g; Agar No. 1

6.5 g (Oxoid,2006); potassium dihydrogen phosphate 0.5 g (KH2PO4, Merck, Darmstadt, Germany) pH 7, sterilized at 121 °C for 15 min.

2.4.5.4 Preparation of B. subtilis spore suspension:

The suspension was prepared in accordance with the method of (Bogaerts and Wolf, 1980).

2.4.5.5 Preparation of test plates with B. *subtilis* CCM 4062:

The pH 6 test agar was heated to 55 °C and inoculated with B. *subtilis* spore suspension to approximately 104 CFU·ml-1. The agar with the test strain was pipetted at 4 ml doses to pre-heated sterile glass Petri dishes of 90 mm in diameter.

2.4.5.6 Preparation of samples:

Samples were removed from the deep-freeze and allowed to reach a temperature of about - 5 'C, before the outer (contaminated) surface was removed with a sterile scalpel. A cylindrical piece from the target organ was removed from each sample using a sterile corn borer (8 mm internal diameter), 2 mm thick, were cut from it. The B. *subtilis* plates were incubated at 30 'C for 18-24 h. A positive test result was recorded when inhibition zone not less than 1-2 mm across.

2.5 Data analysis:

The data were analyzed by using IPM SPSS (Statistical Package for Social Science) version 24 and the statistical methods used in the study are:

1. **Cranach's alpha method:**

Where reliability was calculated using Cranach's alpha equation shown below:

Reliability coefficient =

Validity =

Cranbach's alpha coefficient = (0.89), a reliability coefficient is high and it indicates the stability of the scale and the validity of the study

Validity coefficient is the square of the root, so reliability coefficient is (0.94), and this shows that there is a high sincerity of the scale and that the benefit of the study.

2. Frequency

3. Percentage

4. Chi-square test

5. Median

6. Graphic formats

7. Significant value

Chapter Three

Results

3.1 Bacterial Screening Test Result:

According to the table no (1), the frequency and percentage was 53, 53% and the Negatives was 47, 47%.

Table (1) Frequency and Percentage of the drug residence (Positive) mutton sample in Khartoum State.

Valid	Frequencies	Percentage
Positive	53	53.0%
Zero	47	47.0%
Total	100	100.0%

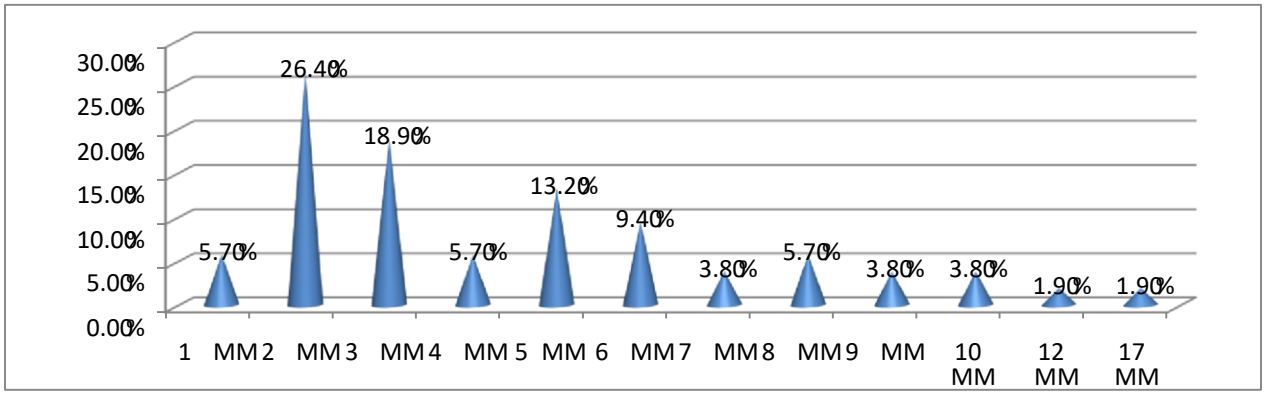


Figure (1) measurement of inhibition zone for positive sample.

Figure (1) Describing the Inhibition Zone of the distribution of the drug residence (Positive) reading sample by 1 MM by (%5.7) and 2 MM by (%26.4) and 3 MM by (%18.9) 4 MM by (%5.7) and 5 MM by (%13.2) and 6 MM by (%9.4) and 7 MM

by (%3.8) and 8 MM by (%5.7) and 9 MM by (%3.8) and 10 MM by (%3.8) and 12 MM by (%1.9) and 17 by (%1.9).

In Table (2) The views of the distribution of the drug residence (negative) sample by negative by (%57.0) and zero by (%43.0).

Valid	Frequencies	Percentage
negative	57	57.0%
Zero	43	43.0%
Total	100	100.0%

Table (2) The frequency and percentage for the drug residence

3.2 Social Aspect Result:

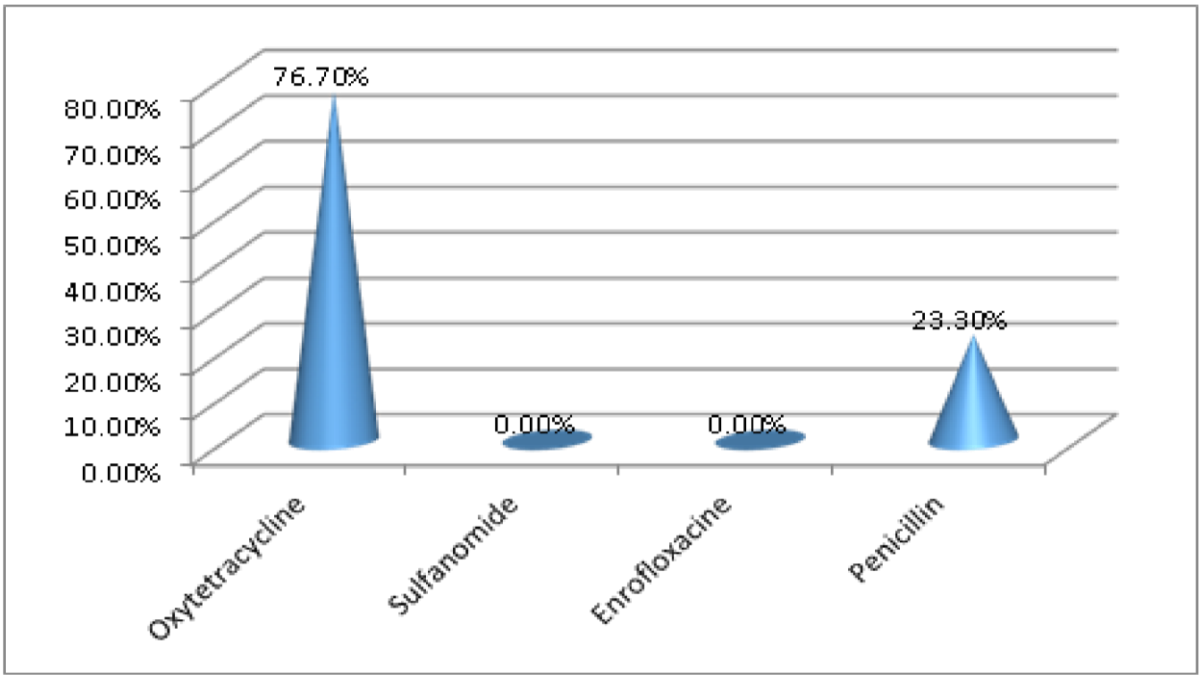


Figure (2) describe the distribution of the most effective antibiotic the owners have on their farm. The Oxytetracycline was (%76.7), Sulfonamide was (%0.0), Enrofloxacin was(0.0%) and penicillin(23.3%)

Table (3) illustrates the frequency and percentage for the you buy antibiotic depend on what? The Recommendation Of veterinarian was higher (56.7%), the Prescription was (30.0%), and according to their experience was (13.3%).

Valid	Frequencies	Percentage
Prescription	9	30.0%
Recommendation Of veterinarian	17	56.7%
Recommendation Of veterinary assistant	0	0.0%
Discerning	0	0.0%
According to my experience	4	13.3%
Total	30	100.0%

Table (4) the frequency and percentage for the Who injected antibiotic you or the veterinarian. 90% of owners inject by their self and 10% let the veterinarian do their job, depends on our study.

Valid	Frequencies	Percentage
I injected	27	90.0%
The veterinarian who injected	3	10.0%
Total	30	100.0%

Figure(3) illustrates the views of the distribution of the Do you stick to recommended dose sample? The yes was (%86.6) and no by (%6.7) and some time by (%6.7).

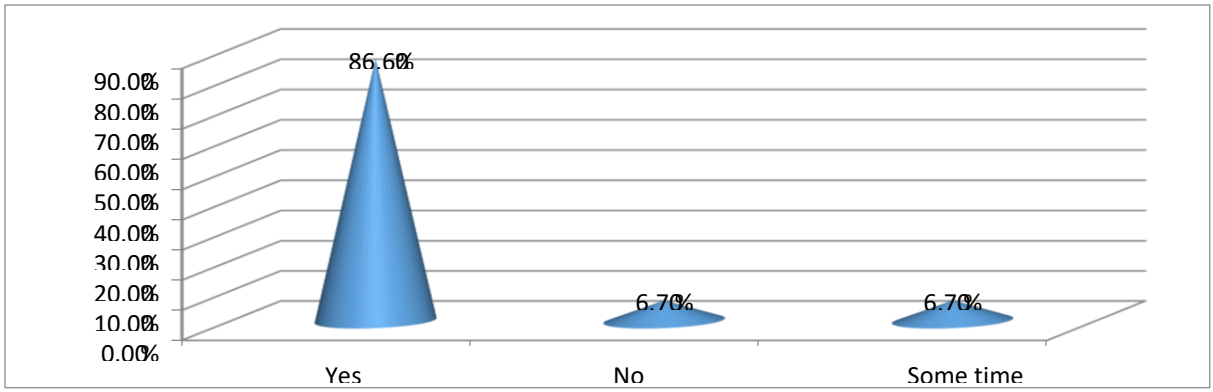


Table (5) illustrates the frequency and percentage for the Do you adhere to withdrawal periods?

The yes by (%60.0) and no by (%26.7) and some time by (%13.3).

Valid	Frequencies	Percentage
Yes	18	60.0%
No	8	26.7%
Some time	4	13.3%
Total	30	100.0%

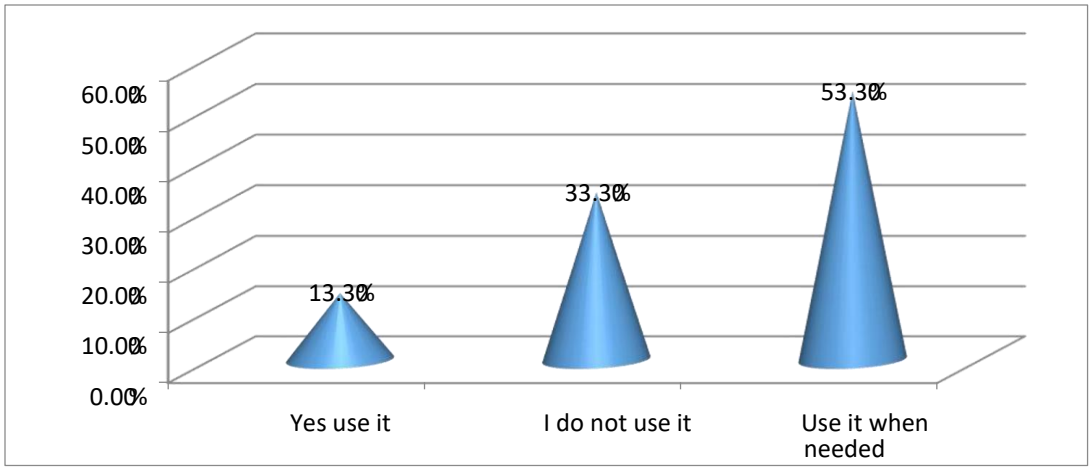
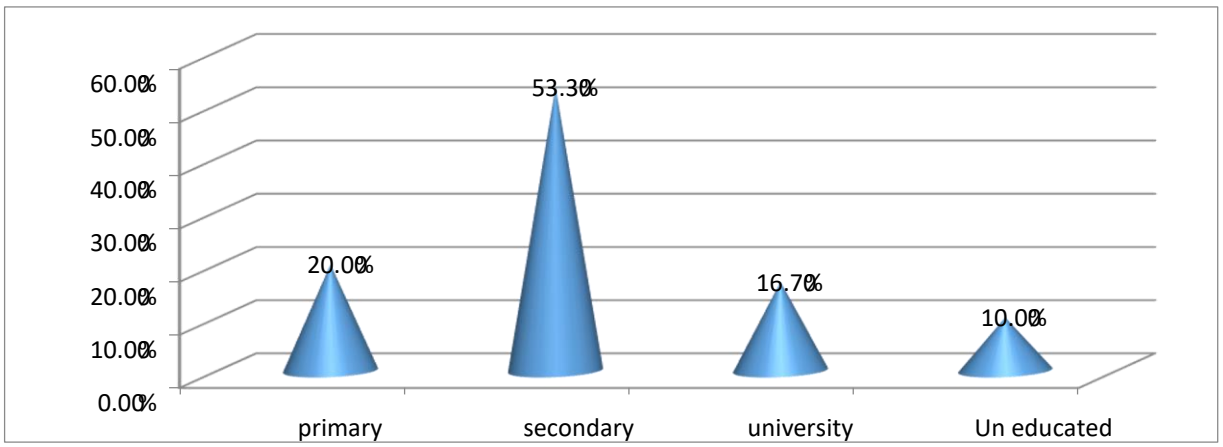


Figure NO (4)

Illustrates the views of the distribution of the Question Do you use antibiotic continuously with feed or water? the Yes I use it value was (%13.3) ,I do not use it was (%33.3) and I use it when needed was (%53.3).

The primary level was (%20.0), secondary (%53.3), university (%16.7) and the un educated was (%10.0).



The primary level was (%20.0), secondary (%53.3), university (%16.7) and the un educated was (%10.0).

Figure No (6) (%33.3) of the owners make the diagnosis by themselves, (%66.7) of the owners bring the veterinarian and the veterinary assistant was (%0.0).

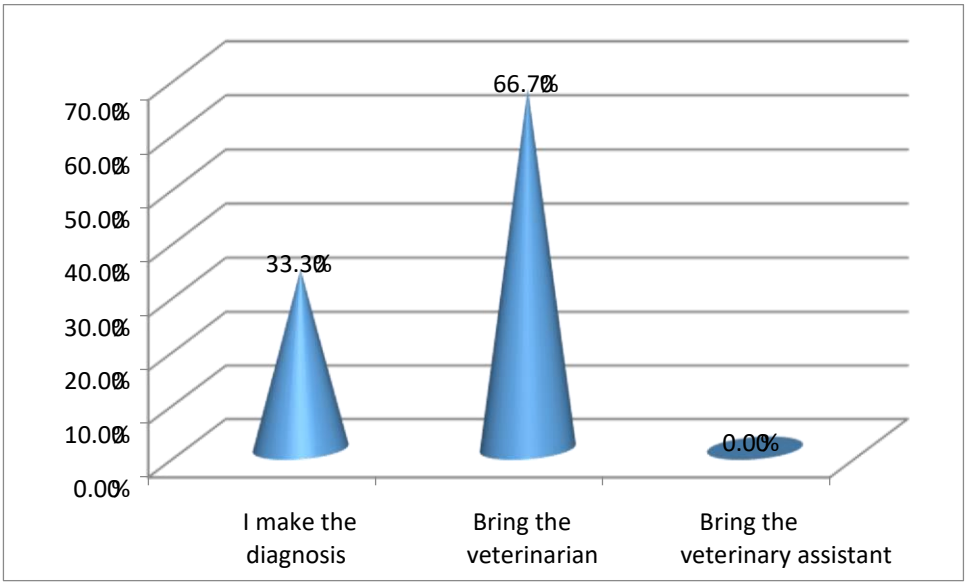


Figure No (6) (%33.3) of the owners make the diagnosis by themselves, (%66.7) of the owners bring the veterinarian and the veterinary assistant was (%0.0).

Chapter Four

Discussion

The extensive use of antimicrobials in livestock production might lead to several public health implications when contaminated meat is consumed. In the study 53% of sheep meat were found positive for antibiotics residues (**table 1**). These results are nearly the same as the results of Tajick and Shohreh, (2006).

Also the results are nearly the same as the results of (bodija abbatior) had the positive value of 56.0% of muscle sample positive for antibiotics residues-(bashorun) 50.0% of muscle samples positive (low road) 60.0% of muscle samples positive and (apata slab) 56.6 % of muscle samples positive Olatoye and Ogundipe,(2009)

Results of microbiological screening were similar to findings in the screening Er et al., (2013) who discovered that 57.7% of beef were positive for quinolone residues. However, in this study the antibiotic was identified quinolone in Ankara, turkey.

Our results are higher than results of Almashhadany, (2020) which comprised 10.4% and residues has been reported in 21% of meat samples in Ghana (Novais *et al*., 2010).

In a report, it was found that 90% reduction of the initial level of tetracycline was possible at a continuous treatment of chicken meat for 23.9, 53.2, and 106.6 min by microwaving, boiling, and roasting, respectively (Abou-Raya *et al*., 2013). According to, Hussein and Khalil (2013) mentioned studies showed that some of

the processing techniques helped to reduce different antibiotics to certain levels. However, this should not be considered as an alternative for the control of antibiotic usage during farming.

In Figure (1), the sample was considered negative when the inhibition zone was more than 2 mm and the (Positive) reading start from 2 mm it was (%26.4) as a highest reading and 3 MM was (%18.9) and 17mm was (%1.9) it was the lowest result.

This result is close to the study conducted by Hind *et al*. (2014), they found a total of screened for antibiotic residues were 27% of the samples tested positive residues and 73% were negative. Shahid *et al*., (2007) reported that, the 100 samples processed by STAF test, 29 samples had zone of inhibition ≥2 mm and were considered STAF positive, while 12 showing zone <2 mm were considered STAF negative.

Jabbar (2004) reported that incidence of antibiotic residues in kidney samples was 70%, in liver samples it was 60%, while for muscle samples it was 50%. This result is differed from the result obtained by (Fangama *et al.*, 2019) in which the inhibition zone for ovine muscle (>2 mm) was 12%.

There was a question, about the distribution of the most effective antibiotic you have on the farm? (**Table 2**). The result showed that (%76.7) for the Oxytetracycline, (%0.0) for Sulfonamide, (%0.0) for Enrofloxacin and the Penicillin was (%23.3), This result was agreed with the result reported by fangama *et al.,* (2019) who found that the oxytetracycline was the most effective antibiotic he mentioned 72% of antibiotics groups used for the animals' was tetracycline . This result is close to study conducted by (Addisalem and

Bayleyegn, 2012) and (Nonga *et al*., 2013), in Ethiopia and in Tanzania . In Saudi Arabia, 35% of the samples of broiler meat were positive for quinolone residues (Silfrany *et al.* ,2013). This result disagree with our study because we did not find quinolones or sulphonamide group used by our owners, because of the local culture most of them they are trust the uses of oxytetracycline .

There is a study conducted by Hakem *et al*., (2013), most of positive samples cases (75.8%) were found contaminated by β-lactams and/or tetracyclines .Our result showed that 23.3,

76.7% the complying for the oxytetracyclines and the penicillin were 76.7% and 23.3 % respectively.

Table (3) In this study 30% of owners buy antibiotic depend on the a prescription, 56.7% buy the antibiotics based on the recommendation of veterinarian, 0.0% based on the screening recommendation of veterinary assistant, 0.0% by discerning and 13.3% buy antibiotics done according to their experience.

33.3% of owner's performed the diagnosis in their farm done by themselves and 66.7% of them bring veterinarian. Adding to that, around 13.3% of them used the antibiotics continuously and this lead to the improper diagnosis and misuse of the veterinary drugs, which both factors cause severe damage to the human and animal health.

In the **Table (4),** the frequency and percentage for the Who injected antibiotic you or the veterinarian? the result showing that, about 90 % of the owners injecting the medicines by themselves without the supervision of the veterinarian. This finding agreed with (Fangama *et al.*, 2019) he were find 92% of antibiotics administration follow-up was done by the owners and workers and only 8% of

the veterinarian continued the treatment by themselves . This way is surly lead to misuses or extra-liable because only 20% of the owners adhere with the guidelines.

(Sawant *et al*, 2005), who reported that in 93% of the farms, the owners administere antibiotics and untrained personnel. According to Katakweba *et al*. (2012), about 70% of dairy farm owners give the drugs to their animal. Komolafe (2003) and El Zuber *et al*. (2012) reported that the antibiotic abuse is one or perhaps the most important cause of the high prevalence of drugs residues and resistance among bacteria that is mentioned by (Nisha, 2008).

Figure (2) illustrates the distribution of the question; Do you stick to recommended dose? and the answers were Yes ,No and Some Times (%86.6) , (%6.7) and (%6.7), these results have a great relationship with the results of a questionnaire that was studied on veterinary pharmacies in which the results were as follow , 90% they guiding owner to restrict dose and route of administration and 4% they did not it and 6% they do sometimes (Alla *et al.*, 201).

There was a question to the owners, Do you adhere to the guidance's, withdrawal periods? **Table (5)**

The result showed that, yes was (%60.0) and no was (%26.7) and some time was (%13.3), this result was different from the result reported by (Fangama *et al.*, 2019), who mentioned that 60% of veterinarian gives tips to the owners about the withdrawal period and40% of veterinarians they did not adhere to dosage. On the otherhand 56% of responders answered that the owners comply with guidelines sometimes.

These results are nearly the same as the results of (Fathalrhman *et al*., 2016) revealed that43.3% of the veterinarians at veterinary pharmacies practice whole sale of antibiotics to the dairy farms' owners, 60% guide them to restrict dose, 73.3% advice the owners about the routes of administration. However, only 56.7% from all interviewed veterinarians' advice the dairy farms' owners for the withdrawal period.

Alla *et al*. (2011) survey revealed that veterinarian did not restrict to the weight of animal when describing doses, which lead to over-dosing or sub-dosing, and there was no following up of cases after leaving the clinic or pharmacy (86%).

Use of antibiotics in feed and water: the results showed about 13.3% of owners use antibiotic continuously with feed or water, 33.3% do not use and 53.3% Use it when needed, in (**Figure No 4)**

This result was disagreed with the results of (Alla *et al*., 2011). 4% of owners use antibiotics with feed and water 96% of owners do not use antibiotic with feed and water and 0% of owner use it some time.

The education level is very important because most of owners need to understand how to deal with the veterinarians directions, In **table no (6)**, most of farms owners their education level is secondary school (%53.3), and the non-educated owners they was (10%), the primary level was (%20.0) and the university (%16.7).

The perfect diagnosis lead to perfect treatment and great result, only specialist could do the right diagnosis and give the right treatment in our result in

Figure No (5) (%33.3) of the owners make the diagnosis by their selves , (%66.7) of the owners bring the veterinarian and the veterinary assistant was (%0.0).

Chapter Five

Conclusion and Recommendation

Conclusion:

From the research result, the screening test of meat showed detectable levels of antibiotic residues and that happened by the reason of the widespread of the antibiotic misuses in the farms and this indicate the level of danger to human and animal health.

This risk should be treated by the serious way through the law, education and spread the extension culture to the farm's owners and the well training to the veterinarians, and the antibiotics should be controlled and used under the supervising of licensed veterinarian

Applying of the microbiological methods for screening the antibiotics residuce because it's useful and less coast products especially for the large herds in developing countries and the legal authority should be responsible for this program.

Recommendations:

From the study result, we recommend the following:

- Do not use antibiotics without a prescription or prescription from a veterinarian.
- Raising the awareness of animal owners about the uses of the antibiotics.

- The veterinarian must ensure that the animal owner understands well the meaning of the drug withdrawal period and adheres to it when slaughtering from the veterinarian and the owner of the animal together.
- Do not use antibiotics unless necessary.
- The government must protect the public health by issuing the suitable decisions, circular to prevent the drug residuce.

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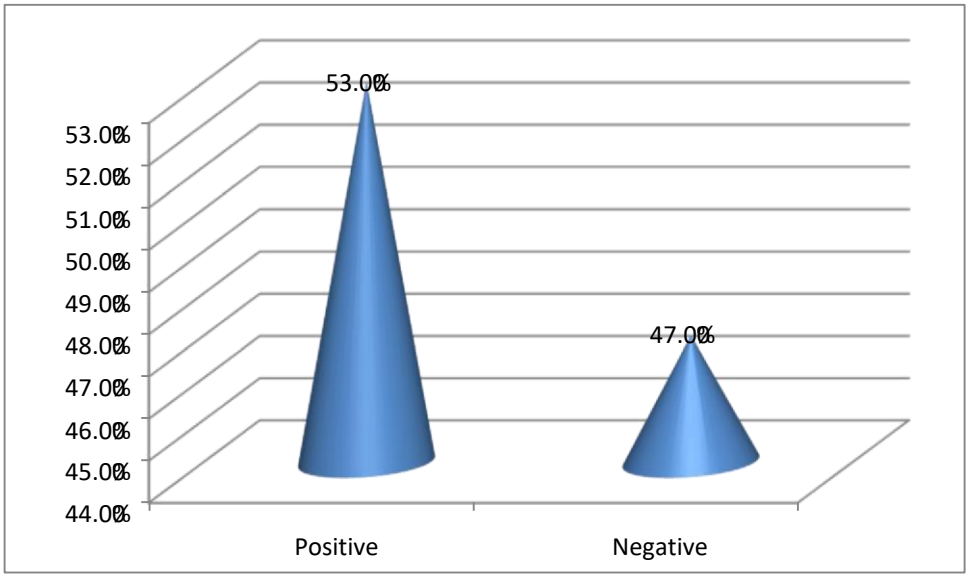
Appendix

Bacterial Study Result:



Distribution of the drug residence (Positive) sample were (%53.0) and the Negative were (%47.0).

The frequency and percentage for the Are you diagnosing on the farm or is this done by a veterinarian.

Valid	Frequencies	Percentage
I make the diagnosis	10	33.3%
Bring the veterinarian	20	66.7%
Bring the veterinary assistant	0	0.0%
Total	30	100.0%

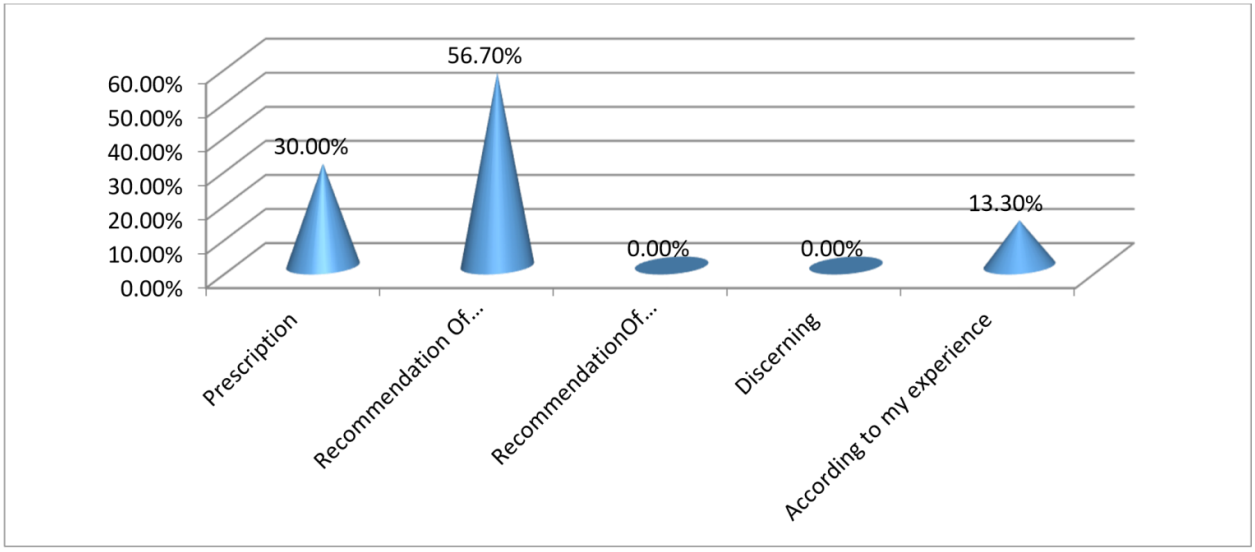
The frequency and the percentage of the inhibition zone for the drug residues. The highest percentage and frequencies was 26.4%, 14 for the 2 MM.

Valid	Frequencies	Percentage
1 MM	3	5.7%
2 MM	14	26.4%
3 MM	10	18.9%
4 MM	3	5.7%
5MM	7	13.2%
6 MM	5	9.4%
7 MM	2	3.8%
8 MM	3	5.7%
9 MM	2	3.8%
10 MM	2	3.8%
12 MM	1	1.9%
17 MM	1	1.9%
Total	53	100.0%

Field study:

Most of owners education level is secondary school by 53.3%, the uneducated was 10% and the primary school was 20% and the university level was 16.7 %.

Valid	Frequencies	Percentage
primary	6	20.0%
secondary	16	53.3%
university	5	16.7%
Un educated	3	10.0%
Total	30	100.0%



Source: excel 2016

Table (3) illustrates the views of the distribution of the You buy antibiotic depend on what sample by Prescription by (%30.0) and Recommendation Of veterinarian by (%56.7) and Recommendation Of veterinary assistant by (%0.0) and Discerning by (%0.0) and According to my experience by (%13.3).

The frequency and percentage for the Do you use antibiotic continuously with feed or water?

Valid	**Frequencies**	**Percentage**
Yes use it	4	13.3%
I do not use it	10	33.3%
Use it when needed	16	53.3%
Total	30	100.0%

Source: IPM SPSS 24 package

The frequency and percentage for the Do you stick to recommended dose?

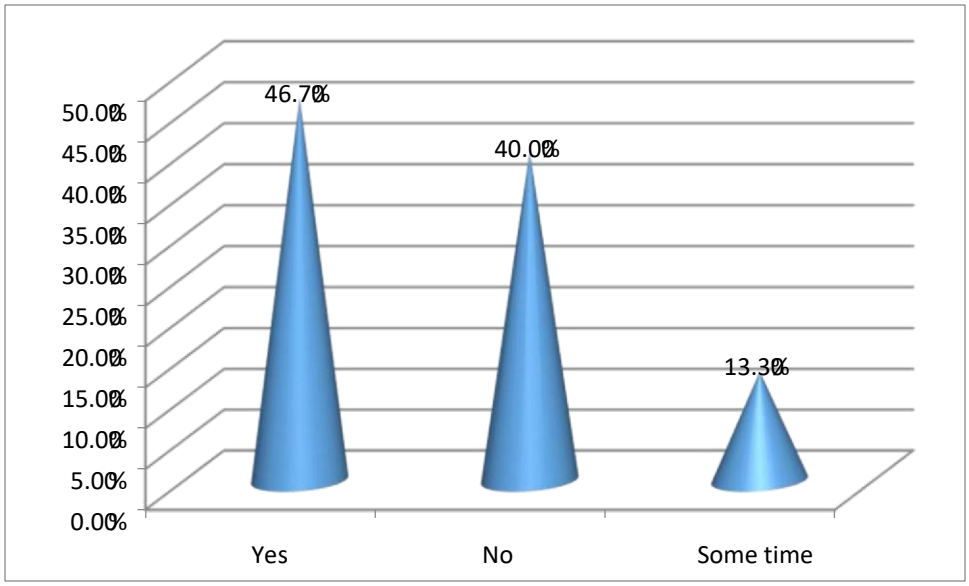
Valid	Frequencies	Percentage
Yes	26	86.6%
No	2	6.7%
Some time	2	6.7%
Total	30	100.0%

Source: IPM SPSS 24 package

The frequency and percentage for the Do you have sufficient knowledge of withdrawal time for antibiotics you use

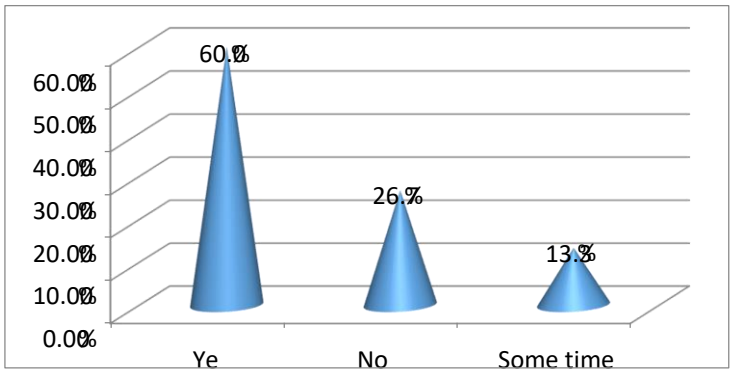
Valid	Frequencies	Percentage
Yes	14	46.7%
No	12	40.0%
Some time	4	13.3%
Total	30	100.0%

Source: IPM SPSS 24 package



Source: excel 2016

Illustrates the views of the distribution of the Do you have sufficient knowledge of withdrawal time for antibiotics you use sample by yes by (%46.7) and no by (%40.0) and some time by (%13.3).



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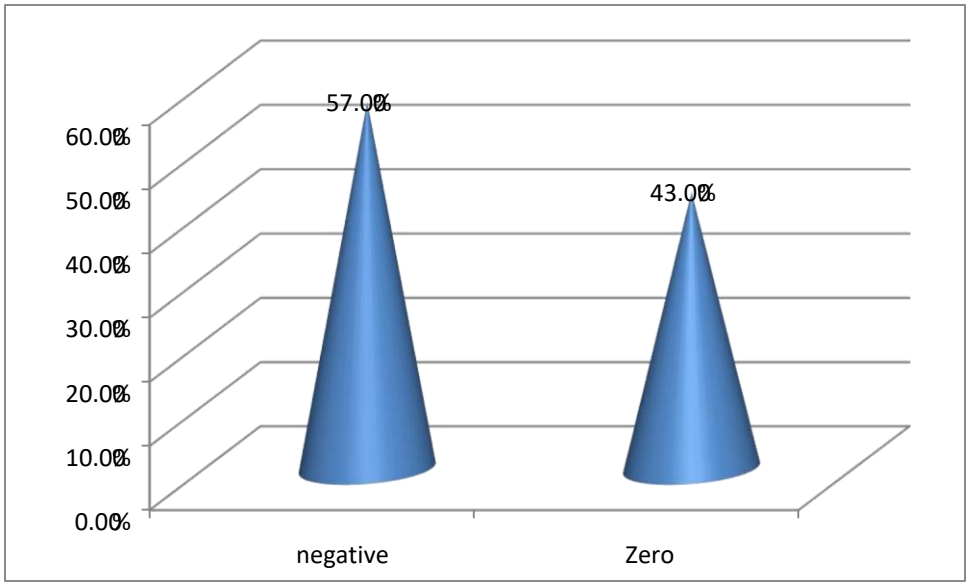
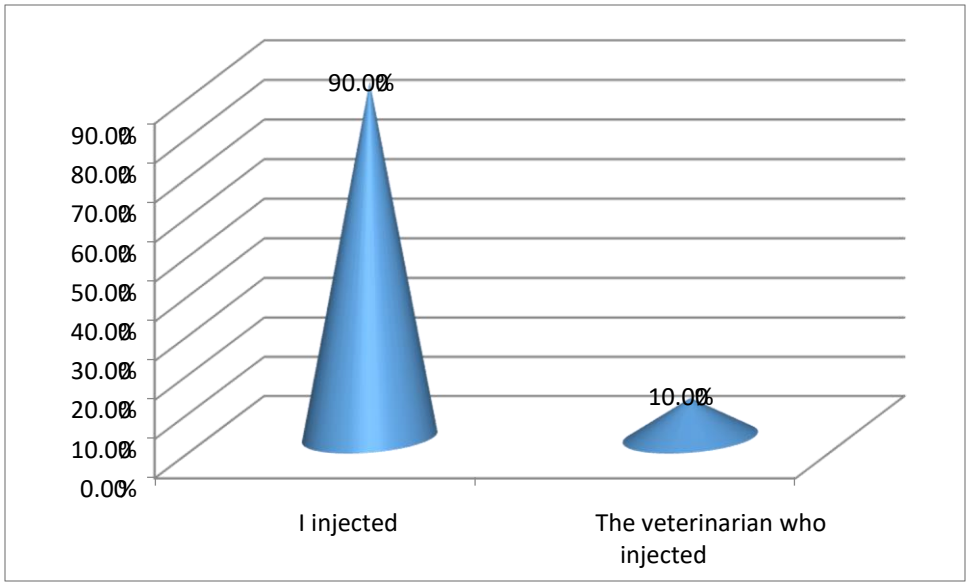


illustrates the views of the distribution of the drug residence

The views of the distribution of the drug residence (negative) sample by negative by (%57.0) and zero by (%43.0).

The frequency and percentage for what is the most effective antibiotic you have on the farm.

Valid	Frequencies	Percentage
Oxytetracycline	23	76.7%
Sulfanomide	0	0.0%
Enrofloxacine	0	0.0%
Penicillin	7	23.3%
Total	30	100.0%



Source: excel 2016

illustrates the views of the distribution of the Who injected antibiotic you or the veterinarian sample by I injected by (%90.0) and the veterinarian who injected by (%10.0).

Illustrates chi-square teat results for Quality and System Standards.

Results of table (10) are interpreted as follows:

1- The value of chi – square calculated to signify the differences between the What is the most effective antibiotic you have on the farm was (18.53) with P-value (0.000) which is lower than the level of significant value (5%) These refer to the existence of differences statistically.
2- The value of chi – square calculated to signify the differences between the You buy antibiotic depend on what was (18.60) with P-value (0.000) which is lower than the level of significant value (5%) These refer to the existence of differences statistically.

3. The value of chi – square calculated to signify the differences between the Are you diagnosing on the farm or is this done by a veterinarian was (13.33) with P-value (0.000) which is lower than the level of significant value (5%) These refer to the existence of differences statistically.

4. The value of chi – square calculated to signify the differences between the Who injected antibiotic you or the veterinarian was (19.20) with P-value (0.000) which is lower than the level of significant value (5%) These refer to the existence of differences statistically.

5. The value of chi – square calculated to signify the differences between the Do you use antibiotic continuously with feed or water was (17.20) with P value (0.000) which is lower than the level of significant value (5%) These refer to the existence of differences statistically.

6. The value of chi – square calculated to signify the differences between the Do you stick to recommended dose was (38.40) with P-value (0.000) which is lower than the level of significant value (5%) These refer to the existence of differences statistically.

7. The value of chi – square calculated to signify the differences between the Do you have sufficient knowledge of withdrawal time for antibiotics you use was (15.60) with P-value (0.000) which is lower than the level of significant value (5%) These refer to the existence of differences statistically.

8. The value of chi – square calculated to signify the differences between the Do you adhere to withdrawal periods was (10.40) with P-value (0.000) which is lower

than the level of significant value (5%) These refer to the existence of differences statistically.

No	Phrases	Chi-square value	df	Sig.	Median	Interpretation
1	What is the most effective antibiotic you have on the farm	18.53	1	0.000	4.0	Oxytetracycline
2	You buy antibiotic depend on what	18.60	2	0.000	4.0	Recommendation Of veterinarian
3	Are you diagnosing on the farm or is this done by a veterinarian	13.33	1	0.000	3.0	I make the diagnosis
4	Who injected antibiotic you or the veterinarian	19.20	1	0.000	2.0	The veterinarian who injected
5	Do you use antibiotic continuously with feed or water	17.20	2	0.000	1.0	Use it when needed
6	Do you stick to recommended dose	38.40	2	0.000	3.0	Yes
7	Do you have sufficient knowledge of withdrawal time for antibiotics you use	15.60	2	0.000	2.0	No

8	Do you adhere to withdrawal periods	10.40	2	0.000	3.0	Yes

Figure (1) Measuring the inhibition zone

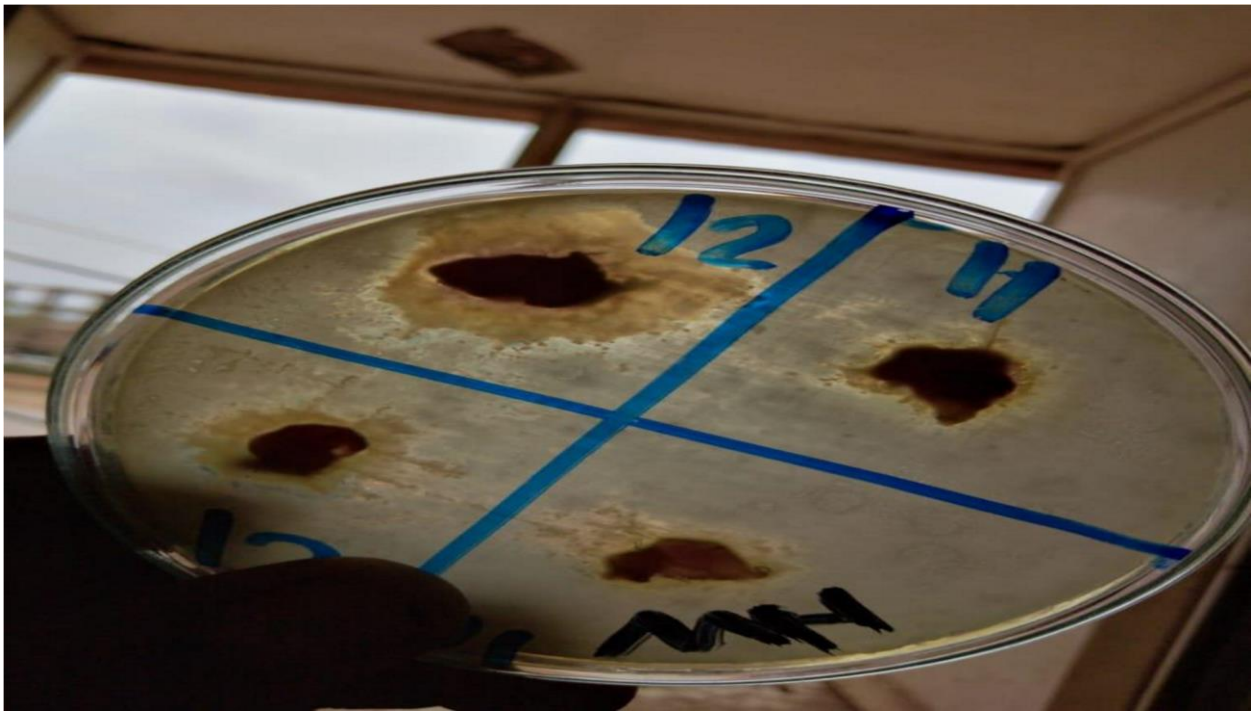


Figure (2) the inhibition zone

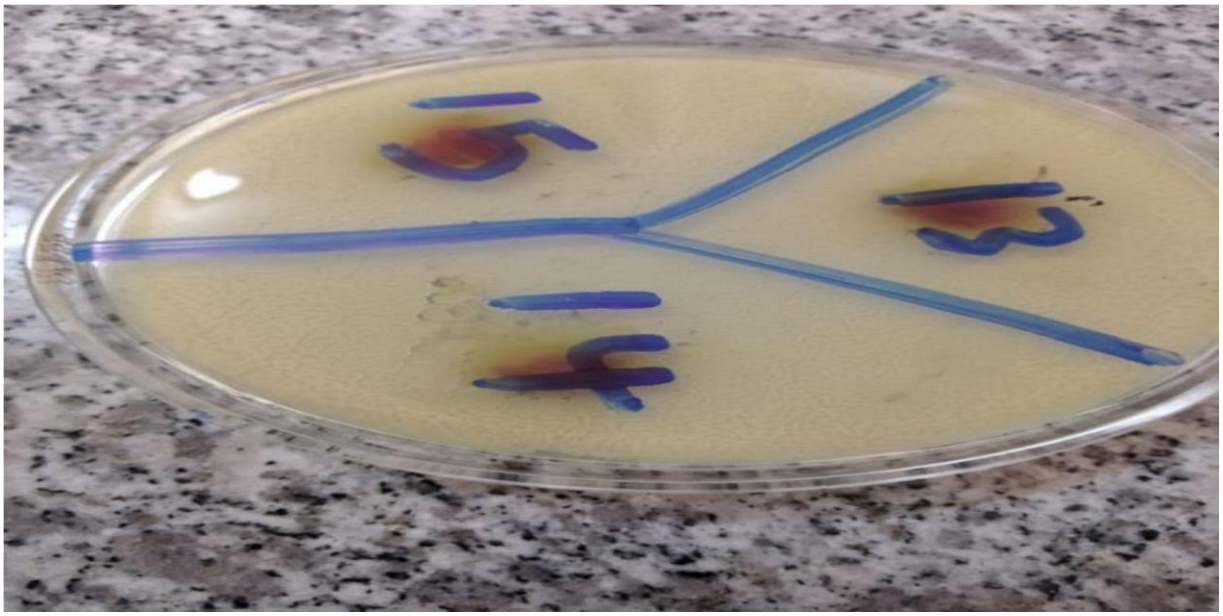


Figure (3) Putting the meat sample

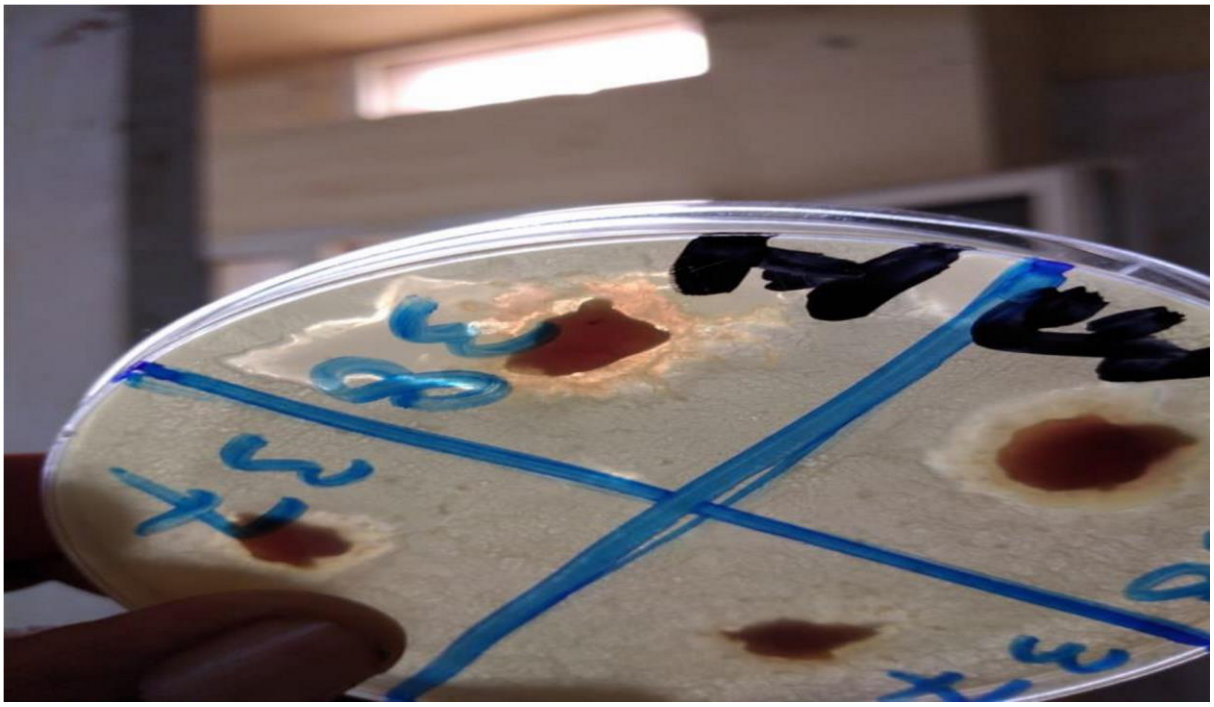


Figure (4): Numbering and Dividing the inhibition zone field

استبيان لنيل درجة الماجستير

الطب الوقائى والصحة العامة

Question No:1

Owner educational level	primary	secondary	university	Un educated
	6	**16**	**5**	**3**

Question No:2

What is the most effective antibiotic you have on the farm.	Oxytetracycline	Sulfonamide	Enrofloxacin	Penicillin
	23	0	0	7

Question No:3

You buy antibiotic depend on	Prescription	Recommendation 0f veterinarian	Recommendation Of veterinary assistant	Discerni ng	According to my experience
	9	17	0	0	4

Question No:4

Are you diagnosing in the farm or it is done by a veterinarian?	I make the diagnosis	Bring the veterinarian	Bring the veterinary assistant
	10	20	0

Question No:5

Who injected antibiotic you or the veterinarian?	I injected	The veterinarian who injected
	27	3

Question No:6

Do you use antibiotic continuously with feed or water?	Yes use it	I do not use it	Use it when needed
	4	10	16

Question No:7

Do you stick to recommended dose	yes	no	Some time
	26	2	2

Question No:8

Do you have sufficient knowledge of withdrawal time for antibiotics you use?	yes	no	Some of them
	14	12	4

Question No:9

Do you adhere to withdrawal periods?	yes	no	sometime
	18	**8**	**4**