Sudan university of Science and Technology

College of Agricultural Studies

ANTIBIOTICS RESIDUS IN THE MARKETD COW MILK – A CASE STUDY KHARTOUM STATE.

متبقى المضادات الحيوية فى لبن الابقار المسوق ـ دراسة حالة ولاية الخرطوم

A Dissertation subedited for partied fulfilling The Requirements of the Degree of Master of Science in Animal Production

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September 2016

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Thesis title: RESIDUS oF ANTIBIOTICS IN THE MARKET MILK – THE CASE STUDY – THE STATE oF KHARTOM

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PREFACE

This study has been done in the

Department of Animal Production, College of Agricultural Studies, University of

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DEDICATION

To my father to my mother to whom I am greatly thankful, to my sister and brothers

ACKNOWLEDGEMENTS

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ABSTRACT

This study was conducted to determine the presence of antibiotic residues in cattle milk in Khartoum state, as these residues may have a negative impact on human's health.

Milk samples were randomly collected from two sources; the first source was dairy farms while the second source was dairy sale centers, markets and hawkers venders.

In Omdurman city 26 samples were collected from dairy farms and 18 samples from the markets ,white while in Khartoum city 24 samples were collected from dairy farms and 23 samples were from markets .As for Khartoum North 24 samples were collected from dairy farms and 8samples were from markets.

All these samples were tested for the presence of residues of antibiotics by using the inhibitory activity and the micro-biological methods. A strain of bacteria *Bacillus subtilis* was used and cultured in agar media, while milk samples were placed on cavities of the agar.

Chi- square test was used for comparison between different localities and between farms and markets samples at 5% probability level to determine the percentage of antibiotic residues.

Among the milk samples collected from Omdurman, one sample from the dairy farms and one sample from the market were found positive, while in the samples collected form Khartoum only one sample from dairy farms was found positive. All sample of from Khartoum North were negative.

ملخص الاطروحة

اجريت هذه الدراسة لمعرفة وجود بقايا المضادات الحيوية في ألبان الأبقار في ولاية الخرطوم وذلك للأثر الصحي الذى تسببه هذه البقايا على الإنسان .

تم جمع العينات عشوائياً من مصدرين الأول مزارع الألبان والثاني الأسواق ومراكز بيع الألبان والباعة المتجولين .

في مدينة ام درمان تم جمع عدد 26 عينة من المزارع وعدد 18 عينة من الاسواق وفى مدينة الخرطوم جمع عدد 24 عينة من المزارع وعدد8 عينات من الأسواق أما مدينة الخرطوم بحرى فقد تم جمع عدد 24 عينة من المزارع وعدد23 عينة من الأسواق .

تم اختبار هذه العينات لوجود متبقيات المضادات الحيوية عن طريق استخدام النشاط التثبيطى وبالطريقة المكروبيولوجية حيث تم استخدام سلالة من البكتيريا العصوية الرقيقةحيث تم تذريعهافي وسط آجار غذائي وتم عمل تجاويف على الآجار وضعت فيها عينات اللبن .

تم استخدام اختبار مربع Chi- للمقارنة بين المحليات المختلفة، وبين ,المزارع والأسواق عند مستوى احتمالية5٪ لتحديد نسبة من بقايا المضادات الحيوية.

بين عينات الحليب التي جمعت من ام درمان وجدت عينة ايجابية واحدة في كل من المزارع والاسواق، بينما العينات التي جمعت من الخرطوم فقد وجدت عينة ايجابية واحدة في المزارع ، اما عينات الخرطوم بحري فقد كانت كلها سلبية.

CHAPTER ONE

INTRODUCTION

CHAPTER ONE

1. INTRODUCTION

Antibiotics are used for the treatment and prevention of diseases and for growth promotions .This may result in formation of antibiotic residues in milk, if withholding times are not adhered to .Previous surveys indicate that these residues are generally found at levels blow 1ppm (micrograms per gram), but even at these low concentration they may have an effect on human gut flora (Zwald et al., 2004). Most dairy farms occasionally require the use of antibiotics for treatment of sick animals (Allison, 1985). In recent survey, 95% of conventional dairy herds (n=99) reported administrating at least one dose of antibiotics during the two months that preceded the interview (Zwald et al., 2004). McEwen et al (1991) estimated usage of selected antibiotics by using mail survey of Canadian dairy herds; approximately 20%of the herds reported the use of medicated feeds (McEwen et al 1991). Mastitis was the first disease of dairy cattle to be treated with antibiotics and remains their most common disease .A study of dairy herds in the Netherlands (n=201) reported the use of 1.9 antibiotic treatment per case of clinical mastitis (Barkema et al., 1998) U.S., public health is protected by regulations (Anonymous.2001).

The presence of antimicrobial residues in the foodstuffs of animal origin is one of the most important indices for their safety. Currently, approximately 80% of the all food-producing animals receive medication for part or most of their lives (Pavlov *et al*., 2008)

Residues of veterinary medicines are defined as pharmacologically active substances (whether active principles, recipients or degradation products) and their

metabolites, which remains in foodstuffs obtained from animals that have been administered (Codex Alimentarius , 2006).

Antimicrobial classes used extensively as therapeutics in the livestock industry in Sudan especially in the treatment of mastitis include; B-lactam antibiotics, amino glycosides, tetracycline, fluroquinolones and sulphonamides and trimethoprim.

1.2. Objectives

1.2.1. General objectives

To raise awareness about food safety and the concerns made about the presence of antimicrobial residues in food of animal origin.

1.2.2. Specific objective

To determine the level of the presence of antibiotic residues in milk supply in Khartoum state.

CHAPTER TWO

Literature review

CHAPTER TWO

Literature review

2.1. Livestock in Khartoum state

A livestock census in Khartoum state, reported about (897,687 units animals), cattle are estimated at about (295,175) head, Goats (794,107) head, sheep (609,742) head and camel (295,175) head. (Databases of the Ministry of Agriculture, Livestock and Irrigation - Sudan) (2015).

2.2. Milk

2.2.1. Definitions

Milk is defined as the physiological secretion of the mammary gland of mammals to provide nourishment for their young. Throughout history man has recognized the milk value and dairy products as food not only for the young but also for the adults (Nickerson, 1999).

Milk is the most complete food for all mammals, and this is especially true during the early period of life until weaning. It supplies the body with protein, fat, carbohydrate, minerals and vitamins in a manner to suit the nutritional requirements of the body (Omer, 2006).

The milk consumption in the state was estimated in 1999 to be about 400000 tons, although the actual production 360000 tons is produced in the state, almost 95% of which is produced from cows (Awad , M.2006).

2.3. Definition of the term Antibiotics

The term antibiotic meant any microbial product which inhibit or kill certain microorganism (Singleton, 1995).

Antibiotics may be responsible for certain allergic reaction in man , moreover repeated administration of even a small amount may lead to the development of resistant strains of bacteria and therapy well threaten people's health (Jones, 1999). Antibiotic therapy has been widely employed in the treatment of diseases in farm animals .Assessment of consumer safety of using veterinary medicines in food producing animals requires comprehensive set of data on the nature and quantity of residues in edible tissues and their basic pharmacokinetic role .this information is required to characterize the type and level of possible dietary intake of residues (Allison, 1985).

2.4. Residues

Antibiotic residues in milk are concerned in two respects, firstly may curtail proper lactic acid fermentation in cultured products and consequently spoil the product, secondly the ingestion of antibiotic contamination milk may cause sensitization or sickness to consumer, (Jones.1999).

2.5. Withdrawal time

This was described by FAO,1996 as the period of time between the last administration of a drug and the collection of edible tissue or product from a treated animal that ensured the contents of residues in food were complying with MRLVD.WHO,2000 defined as this is the time which passes between the last dose given to the animal and the time when the level of residues in tissue (muscle , liver , kidney , skin and fat) or products (milk , eggs , homey) is lower than or equal to the MRL. Unless the withdrawal period has elapsed, the animal and its products must not be used for human consumption.

2.6. Types of antibiotics

2.6.1. Penicillin

Is one of the most important antimicrobial agents. Although many other antimicrobial agents have been introduced since the discovery of Penicillin it is still widely used as a major antibiotic in as much as new derivatives of the basic nucleus are being introduced every year (Mandell and Sande, 1980).

2.6.2. Cephalosporin's

In 1948 professor Giuseppe Brotzu hypothesized that the relative sterility of sea-water coast of Sardinia was due to substance produced by certain bacteria. These substances, he thought, inhibited the growth of other organisms. In confirmation of his theory Brontzu isolated afungus, cephalosporium a cremorium, from sea water off the coast of Sardinia and found that it

inhibited the growth of a variety of gram-positive and gram-negative bacteria. Similar to Penicillin in various respects (Alexander, 1985).

2.6.3. Tetracyclines

They are broad spectrum antibiotics. There are three naturally occurring members of this group:Oxytetracycline,Chlortetracycline & Dimethyl chlortetracycline. Act by binding reversibly to bacterial 30 s ribosmes and inhibit protein synthesis generally bacteriostatic but at high concentration they become bactericidal because the organisms seem to lose the Functional

integrity of the Cytoplasm membrane. (Aiello & Mays, 1998).

2.6.4. Chloramphenicol

Is a relatively simple natural nitrobenzene derivative with a bitter taste. It is highly effective and well tolerated broad-spectrum. Chloramphenicol inhibts protein synthesis by binding to 50s sub unit of 70s ribosome and impairing

peptidyl transferase activity it is a bacteriostatic but at high concentration may be bactericidal for some species (Aiello& Mays, 1998).

2.6.5. Quinolones

These are synthetic antibiotics (Reynold, 1989)

Pharmacokinetics By 1/V, 1/M, and S/C they penetrate all tissues well and quickly. Some quinolones are eliminated un changed e.g (ofloxacin), some are partially metabolized e.g giprofloxacin and enrofloxacin and some are completely degraded. Metabolites are sometimes active. Major excretion through Renal Route, Biliary (Cipprofloxacin and Nalidix acid). Quinolones appear in milk of lactating animals often at high concentrations that persist for some time.

2.6.6. Sulfonamides

Derivatives of sulfanilamide Sulfonamides are structural analogs of Paramino Benzoic Acid (PABA) and competitively inhibit on enzymatic step. (Dihydropterate synthetase) during which PABA is corporated into the synthesis of diyhdrofolic acid (Folic acid). This result in suppression of protein synthesis impairment of metabolic processes and inhibition of growth and multiplication. They are most effective in early stages of acute infections when organisms are multiplying (Aiello & May, 1998).

2.6.7. Macrolides Antibiotic

Have typical lactone ring in their structure (Tylosine &Erythromycin). They concentrated in the biles and milk the concentration of macrolides in milk is several times greater than in plasma especially in mastitis infected cow. (Aiello& May, 1998).

2.6.8. The polypeptide antibiotics

Polymyxin are polypeptide antibiotics produced by different strain of Bacillus polymxa including Bacitracin &Neomycin &polymyxin (Alexander, 1985).

2.7. Maximum residue limits (MRLs)

MRLs is the level of the contaminat which should not be exceeded (Harding and ditton, 1995).

FAO/WHO (2004) reported that MRLs is the maximum concentration of residue resulting from the use of veterinary drug (expressed in mg/kg) that is accepted in or on food and considered to be without appreciable toxicological hazard for human health.

2.8. History of Antibiotic Residues in milk

In Sudan used delvotest SP for the detection of antibiotics residues in 236 milk samples, he found that 21.18% gave positive results (Mona 2016).

In Khartoum state 64 milk samples the presence of neomycin and tylosin were detected in all collected samples 100% (Maha 2012)

In some countries the use of antibiotics in milk for improving keeping qualities has been suggested (Start well, 1977).

In Lisbon 2248 samples of consumer milk were examined in 1981 to 1985. Six hundred and seventy four of them 30% contained inhibitory substances (Barbosa *et al* 1991).

In Zimbabwe 73 samples of raw milk from 3 main dairy market board collection centres, were tested for the presence of microbial growth inhibitory substances. 4.4% of the samples were found to contain antibiotic residues (Chagonda and Ndiku wera, 1989)

In Estonia, Paern and kind (1995) examined 47 raw milk, samples sold in Tartu for the presence of antibiotic residues, the residues were detected in 4(8-5%) row milk samples.

In Sudan Barakat (1995) used delvotest P for the detection of antibiotics residues in 80 milk samples, he found that 8.75% gave positive results.

Mustafa A. (2001) detected 100 milk samples & he got negative results in all of them.

Raga (2002) stated that the percentage of positive samples for total samples examined was 0.8% and for the samples taken directly from the udder, it was 4.0%.

2.9. Methods of detecting antibiotic residues in milk

The test for drug residues in milk is performed by several methods, such as microbial growth inhibition assays, microbial receptor assays, receptor binding assays, immunologic assays, enzymatic assays and chromatographic analysis (Mitchell et al., 1998).

2.9.1 Microbiological methods

The Microbiological tests are sheaf, easy to perform on a large scale and they possess a wide, non specific in sensitivity (Nouns et, al., 1999) .A number of microbiological tests for detecting antibiotic residues have been developed as in 1941,th the cylinder plate assay method was first described, between 1944 and 1945 ; the filter paper disc method was introduced (Bishop et al., 1992). Moreover they mentioned that since 1950s the *Bacillus subtilis* disc assay method and modifications have been used to detect residual antibiotics in milk, and during the 1970s, the disc assay and the tube assay methods that use the *Bacillus stearothermophilus* organism gained acceptance and broad usage.

Several studies have reported that false-positive results occurred on samples containing no drug in the test using the delvotest assay; one of the microbial growth inhibition assays; which is a simple, sensitive and broadly drug-detecting test system (Andrew, 2001; Gibbone-Burgener et al., 2001).Microbial growth inhibition methods make use of a standard culture of the tested microorganismin a liquid or solid medium (Heeschen 1993). e.g.Geobacillus stearothermophilus var.

calidolactis ,*Bacillus subtilis*, Bacillus megaterium, Sarcinalutea, Escherichia coli, Bacillus cereus var. mycoides or Streptococcus thermophilus. The analysed milk sample is applied on the agar surface either directly or with a paper disc (disc assay plate methods). In the course of incubation, the diffusion of the sample into the medium takes place (the agar diffusion principle), and if the sample contains inhibitor agents, reduction or total inhibition occurs of the tested microorganism growth. Depending on the method used, the presence of inhibitor agents in the tested sample is indicated by the formation of aclear zone of inhibition around the disc (disc assay plate methods) or a change in the medium colour (Hui 1993; Mitchell et al. 1998; Botsoglou & Fletouris 2001), Microbial growth inhibition methods (wides pectral rapid tests) vary in the type of the testing organism, indicator, incubation period and temperature, spectrum and detection levels of the agents analysed. A series of these methods use asthe testing microorganism Geobacillus (Bacillus) stearothermophilus var. calidolactis: BR-test/AS/Blue Star/6/7 (Enterotox Lab., Germany), CharmBlue Yellow Test (Charm Sciences Inc., USA), Delvo test SP-NT (Gist-brocades BV, The Netherlands),CMT – Copan milk test (Copan Italia,Italy), Eclipse 50 (Zeu-Inmunotec S.L., Spain).Geobacillus stearothermophilus is an outstanding testing microorganism for its properties fromwhich the most important, according to Katzand Siewierski (1995), are: the ability of rapidgrowth at higher temperatures (64°C) and a highsensitivity to the β-lactam antibiotics .Commercially available microbial inhibitortests play an important role in the integrated detection system. At present, many commercially produced microbial inhibitor tests are applied simultaneously with selective rapid tests formilk screening in primary production, in dairy industry, and in accredited laboratories (Suhren1995;Honkanen-Buzalski & Reybroeck 1997;Honkanen-Buzalski & Suhren 1999; Botsoglou & Fletouris 2001). The advantage of these

methods is that they have a wide detection spectrum; they are simple to carry out, and they are not costly and can be used for the screening of a large number of samples (Mitchell et al. 1998).These methods have their drawbacks, however, that limit their use: they do not enable specific antibiotic identification, they have limited detection levels for a series of antibiotics, and they are only qualitative and require a long incubation period (2.5–3.5 h). They are highly sensitive to β-lactam antibiotics, mostly penicillin, but evidently les sensitive to other antimicrobial agents such asmacrolides, sulfonamides, tetracyclines, orchloramfenicol (Botsoglou & Fletouris 2001). Manystudies proved that natural antimicrobial agents,if present in milk in higher concentrations, canbring about false – positive results (Carlsson etal. 1989; Andrew 2001; Kang & Kondo 2001;Kang et al. 2005).Commercially produced microbial inhibitor tests are delivered in the form of ampoules (mono tests)or in the form of microplates with a high number of testing cells. Apart from water bath or incubator, they do not require special laboratory equipment. To avoid subjective ifferences in the visual interpretation and to take the readings in an automated and more objective manner, some authors performing photometric measurements use the appropriate Wavelength (590 nm) and another wavelength asreference (650 nm) in ELISA reader (Althaus et al.2003). When performing microbial inhibitor tests, it is necessary to meet the standards of good laboratory practice (protection against the contamination of the test), checking the pH value of the sample, observing carefully the correct temperature and the incubation period as specified by the producer's instructions and testing a positive as well as a negative control alongside with the sample. Out of microbial inhibitor screening methods, in frequent use are, for example: Eclipse test, Charm Cow side test, Charm AIM-96, Charm Farm test, VALIO T101, Copan Milk test, and others.

2.9.2. Enzyme linked immunosorbant assay (ELISA)

Patal and bond,1996 . stated that (ELISA) was a rabid , highly specific, and easy test that could provide better identification for antibiotic residues than microbial inhibition test but cross reaction with metabolites and compound with similar structure prevented precise identification .So confirmation test with mass spectroscopy or high performance liquid chromatography (HPLC),were needed .

2.9.3. Chemicals methods

These methods include high performance liquid chromatography (HPLC), mass spectroscopy and thin layer chromatography (TLC). They can differentiate between different antibiotics (Patal and bond, 1996).

2.9.4. Electrophoresis

High voltage electrophoresis bioautography was used for identification of sulphamethazine and penicillin in milk (Loit and Vaughan, 1985). The antibiotics are extracted using acteointrite and then electrophoresis is carried out using agar medium seeded with the microorganism.

2.10. Antibiotic residues in milk and human health

The occurrence of antibiotic residues in milk intended for human consumption is undesirable for a number of reasons(Allison, 1985).As recently as 30 years ago , the presence of antibiotic residues in milk was considered primarily a manufacturing problem related to inhibition of cheese and yogurt starters(Cogan, 1972). More recently, the presence of antibiotic in milk has been prohibited because of concerns about public health. Initially, public health officials desired to protect hypersensitive individuals from exposure to specific antibiotics. More recently, attention has shifted to the potential for antibiotic residues in milk to contribute to the development and/or transmission of antibiotic residues bacteria (Allison, 1985, Mitchell, *et al.,* 1998).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Area

This study was conducted in, three selected areas of Khartoum State (Alttibnnah in Khartoum North, Alrrdwan dairy complex in Omdurman and Alsaygh dairy complex in Khartoum), as shown in map No 1.
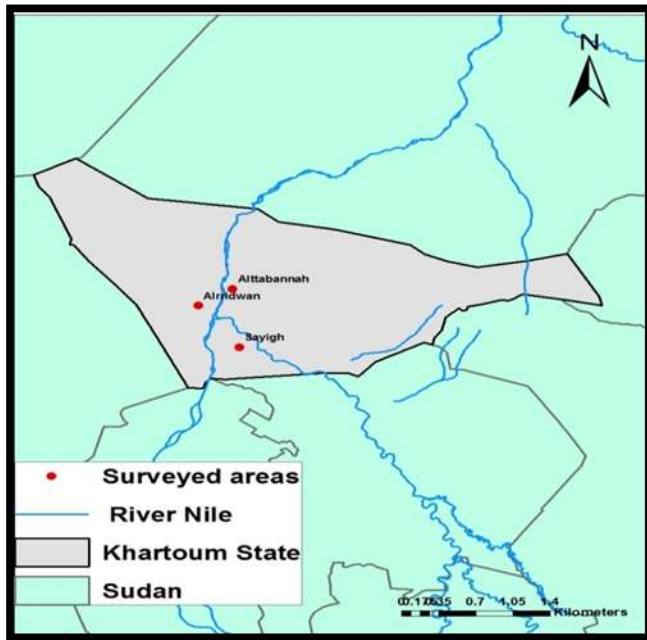
Map 1. Study area -Khartoum State

3.2 Samples collection

Raw milk samples were collected randomly in clean sterile bottles from dairy farms, milk sale points, markets and mobile venders in the study area during the period 7 - 30 December 2015 to examine the remnants of antimicrobial drugs. All samples were transported under refrigeration to the Central Veterinary Research Laboratory (CVRL) and stored under refrigeration, as shown in Plate (1):

Plate 1. Samples collection

3.3. Source of the samples

3.3.1. Khartoum

3.3.1.1. Farm samples

Twenty four milk samples were collected from Alsayigh dairy complex in Khartoum.

3.3.1.2. Market samples

Total of eight milk samples were collected from Alslama market.

3.3.2. Khartoum North

3.3.2.1. Farm samples

Twenty four milk samples were collected from Alttabannah area.

3.3.2.2. Market samples

Twenty three milk samples were collected from the Lafat elazhari and venders in Alttabannah area.

3.3.3. Omdurman

3.3.3.1. Farm samples

Twenty six milk samples were collected from Alrridwan dairy complex.

3.3.3.2. Market sample

Eighteen milk samples were collected from mobile venders and Alhara 21market in Ombada locality.

3.4. Sterilization

3.4.1. Hot air oven

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

3.4.2. Sterilization by autoclaving

This method was used for sterilization of culture media, reagents and plastic tips. The temperature of 115-121ºC under 10-15 Ib/sq inch for 15-20 minutes (Barrow and Felltham, 1993) .

3.5. Materials

3.5.1. Test medium

Standard nutrient agar in the form of dehydrated powder was used. The medium formula in liter contained:

Peptone	5 grams
Meat extract	1 grams
Sodium chloride	7 grams
Agar	13 grams

3.5.2. Solutions

3.5.2.1. Distilled water

Was obtained from Central Veterinary Research Laboratory (CVLR) .

3.5.2.2. Normal saline

Was prepared by dissolving 9g of sodium chloride in 1000 ml distilled water and sterilized at 121ºC to 15 Ib/sq inch for 15 minutes, and cooled.

3.5.3. Test organism

Bacillus subtilis used for this study was obtained from Faculty of Veterinary Medicine, University of Khartoum. As shown in Plate (2):
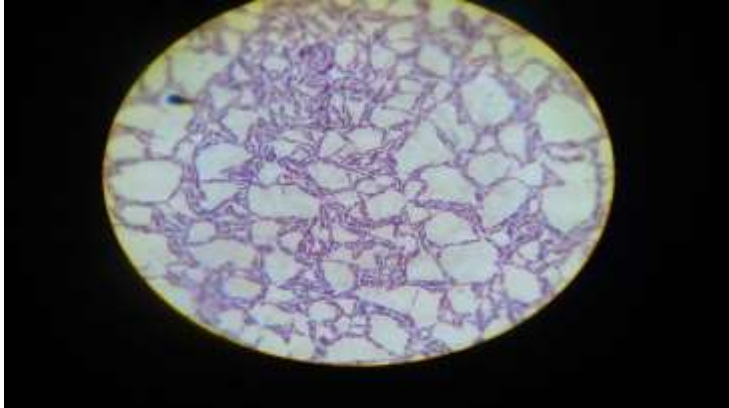
Plate 2. *Bacillus subtilis*

3.6. Preparation of test media

Colonies of *Bacillus subtilis* were transferred from Nutrient Agar plate using sterilized wire loops and 103 dilutions of the broth culture were prepared in normal saline.

3.7. Preparation of cultured media

Nutrient agar media was prepared by dissolving 28 gm of nutrient agar in 1000 ml distilled water and sterilized at 121ºC under 15 Ib/sq inch for 15 minutes. Then it was left to cool up to temp 50-55 ºC. After that add dilutions cultures (200 mm diluents isolation / liter of media), dispatched into 90 mm Petri dishes. Wells were made in the solidifying agar by the sterile tip of micropipette to be used for individual milk samples. Petri dishes were incubated at 37ºC for 24 hrs. Positive samples were manifested by formation of transparent zones around the wells (inhibition zone) and the negative samples did not show the transparent zones around the wells. As shown in Figure (4).

Plate 3. Preparation of culture media

Plate 4. Zone of inhabitation around wells containing milk sample after 24 hour of incubation.

3.8. Questionnaire

Livestock owners asked if mixing and treated milk cow's with healthy milk, and whether the veterinary supervision they have on farms.

3.9. Statistical analysis

Data on any one area was inserted into MS Excell spread sheets programme (Microft coporation) to creat a data base and transferred to Statistical Package for Socience (SPSS) version 16.0. Chi- square test was used for comparison between

different localities and between farms and markets samples at 5% probablity level to determine the percentage of antibiotic residues.

ArcGIS program (version 10.2) was used to download the study pounts and display the results in khartoum steat map .

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

4.1. Omdurman area

Among the 26 milk samples collected from Alrrdwan campus in Omdurman only one sample was found positive for antibiotics residues, in the same locality but from the milk samples collected from the market only one sample was found positive out of 18 samples collected, **Table1**.

Table1. Percentage of positive samples of milk collected from markets and farm in Khartoum area, (December 2015).

	Farm milk	Market milk	Sig. level
No of sample	26	18	
Positive + ve	1 (3.8%)	1 (5.5%)	.789
Negative -ve	25 (96.2%)	17 (94.4%)	

Source: Antibiotic residues survey in milk - Khartoum State Dec. 2015

4.2. Khartoum area

Table 2 shows that among the 24 milk samples collected from Alsaygh campus in Khartoum area only one sample was found positive for antibiotics. In the same locality but from the milk samples collected from the market all the 8 samples collected were found to be negative for antibiotics residues as shown Table 2.

Table2. Percentage of positive samples of milk collected from farms and markets in Khartoum area, (December 2015).

	Farm milk	Market milk	Sig. level
No of sample	24	8	
Positive + ve	1 (4.2%)	0	.078
Negative -ve	23 (95.8%)	7	

Source: Antibiotic residues survey in milk - Khartoum State Dec. 2015.

4.3. Khartoum North area

Among the 24 milk samples collected from dairy farms in Khartoum North (Alttibnnah) all samples showed negative result, In the same locality but from the milk samples collected from the markets all the 23 samples were found to be negative antibiotic residues, Table 3.

Table3. Percentage of positive samples of milk collected from dairy farms and market in Khartoum North area.

	Farm milk	Market milk	Sig. level
No of sample	24	23	
Positive + ve	0	0	N.S
Negative -ve	24	23	

Source: Antibiotic residues survey in milk - Khartoum State Dec. 2015

4.4. Questionnaire result

The questionnaire results showed that 87.5% of the cattle owners mixed milk of antibiotics treated cows with the milk of healthy cows, (Figure 4).
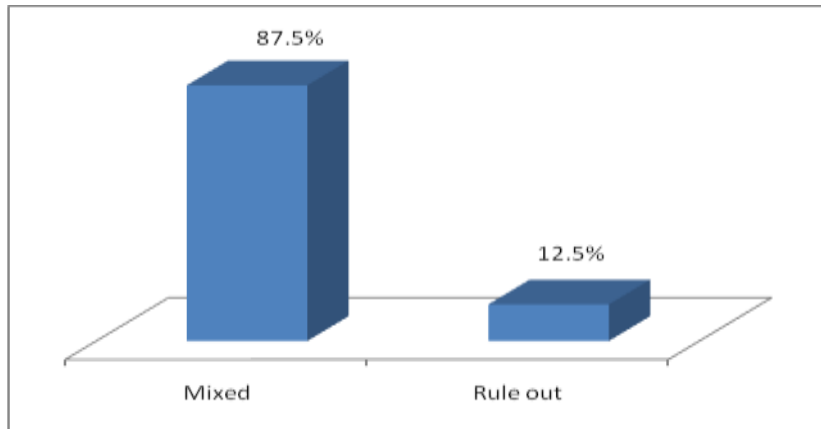
Source: Antibiotic residues survey in milk - Khartoum State Dec. 2015

Figure 1. Percentage of Cattle owners who mixed milk of treated and non-treated cows in Khartoum state, (December 2015).
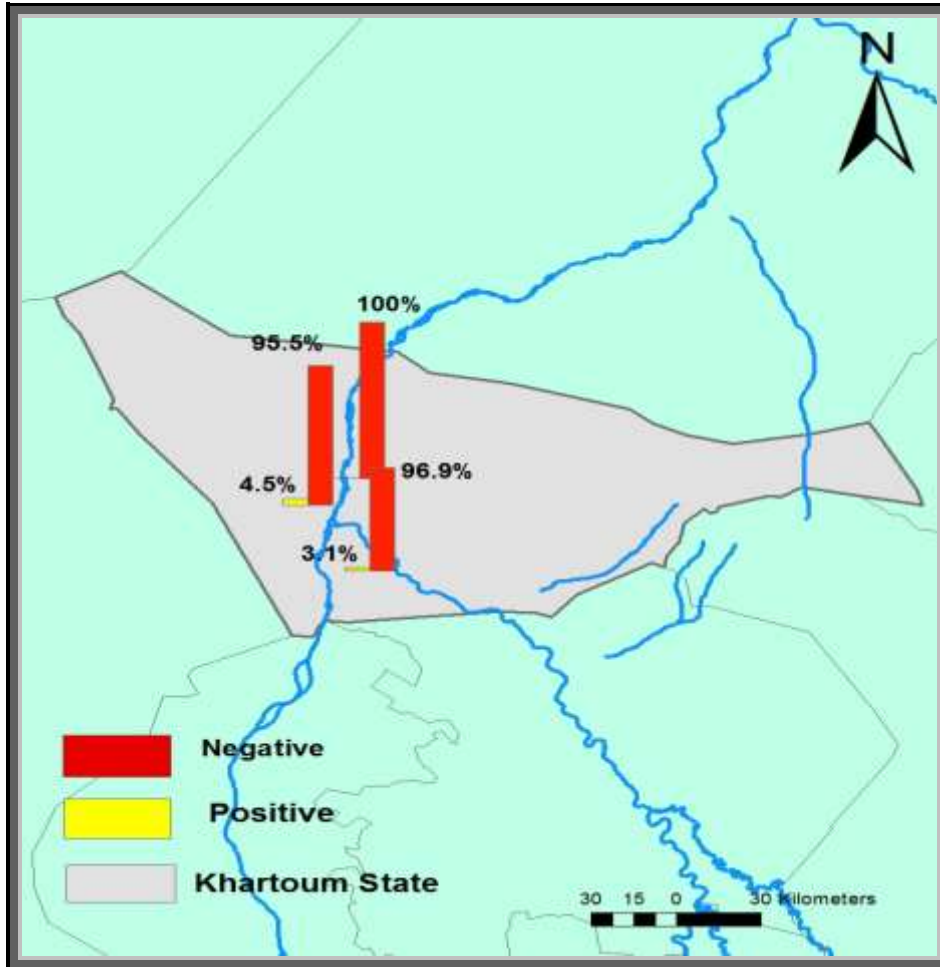
Source: Antibiotic residues survey in milk - Khartoum State Dec. 2015

Map No 2. Percentage of antibiotic residues in milk samples collected from the localities of Khartoum state (December 2015).

CHAPTER FIVE

DISCUSSION

Conclusion

Recommendations

CHAPTER FIVE

5. 1. DISCUSSION

There is serious international concern about the wide spread of antibiotics resistant at the global level. Among the reasons of this antibiotic fastness is the presence of antibiotics residues in animal's protein.

This study was conducted in Khartoum State, to detect antibiotics residues in milk collected from markets and farms in the different localities of the state. Showed positive detection of 0%, 4.5% and 3.1% in Khartoum North, Omdurman and Khartoum localities respectively .

Many previous surveys conducted for the detection of residual antibiotics in the milk in the Khartoum state, (Mona 2016) used delvotest SP for the detection of antibiotics residues in 236 milk samples, and she found that 21.18% gave positive results. This is by far higher than the results obtained in this study as this might be due to the sensitive techniques she used. (Maha 2012) examined 64 milk samples the presence of neomycin and tylosin were detected in all collected samples positive (100%), Barakat (1995) used delvotest P for the detection of antibiotics residues in 80 milk samples. He found that 8.75% gave positive results, Raga (2002) stated that the percentage of positive samples for total samples examined was 0.8% and for the samples taken directly from the udder, it was 4.0%, while Mustafa (2001) investigated antibiotics residues in 100 milk samples collected from different areas in Khartoum state where his results showed that all samples were negative.

In Zimbabwe 73 samples of raw milk from 3 main dairy market board collection centers, were tested for the presence of microbial growth inhibitory substances. 4.4% of the samples were found to contain antibiotic residues (Chagonda and Ndiku wera, 1989). This is similar to the results obtained in this study. In Lisbon 2248 samples of consumer milk were examined in 1981 to 1985. Six hundred and seventy four of them (30%) contained inhibitory substances (Barbosa et al 1991).

These differences might be due to effect of seasons or type of test used.

In Khartoum many factors affect the presence of antibiotics residues in milk such as mal practice of milk venders who add antibiotics to milk to avoid the effect of bacteria, when there are delays in milk marketing.

Also the milkers don't comply with the many antibiotics withdrawal time when they treat their animals as some any milk these animals in the same day of treatment.

In Khartoum North, the 0% percentage may be due to the availability of consumers near milk production units.

The overall positive percentage in this study was low and this might be due to the fact that collection of samples wear done in the cool season when the average temperature in the December 2015, varied between 15.6 C°-   28.5 C° (Metrological authority weather climate data,2015) therefore the milk venders did not add antibiotics to milk to keep it for long periods, the method used for examination of antibiotics residues in this study may also be a factor contributing to this should   have used more modern methods sensitive for the detection of antibiotics residues in milk.

5.2. Conclusion

Farmers use antibiotics to prevent, treat and control diseases of their animals increase their productivity. The results showed that 2.4 % of milk samples tested in Khartoum state was positive for antibiotic. However, there is a concern that routine antibiotics use in livestock management may have negative impact on human and animal health.

5.3. Recommendations

The following suggestions and recommendations are suggested for better control of milk production in dairy farms and milk venders:

1. Education programs for farmer and milkers about use the proper of antibiotics and observing the withdrawal period.

2. Use pasteurization Methods, boiling and cooling to keep milk marketable.

3. Enforce strict regulation to forbid venders and farmers from adding antibiotics and chemicals to preserve milk.

4. Milk collection centers should be increased.

5. Regular checks for the residues of antibiotic in milk by veterinary authorities are qualified laboratories.

6. Better effective techniques should be used authentic results authentic (reliable) result.

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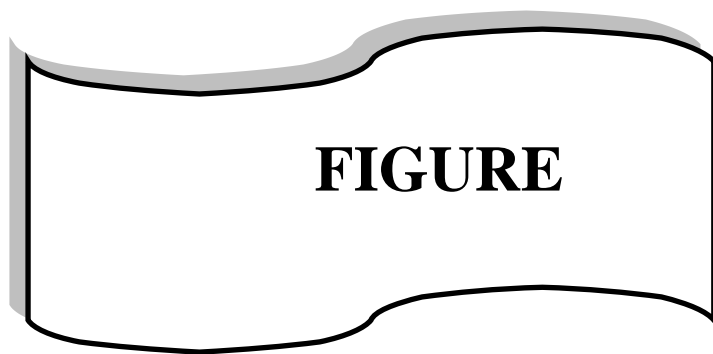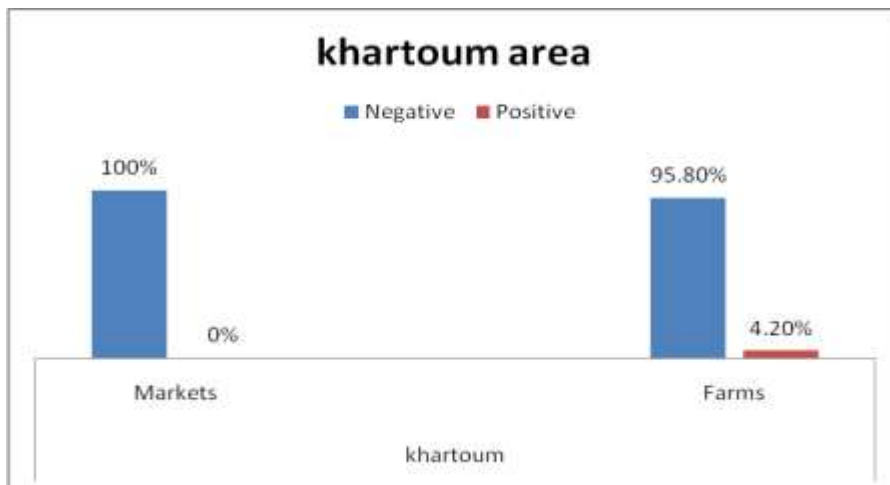
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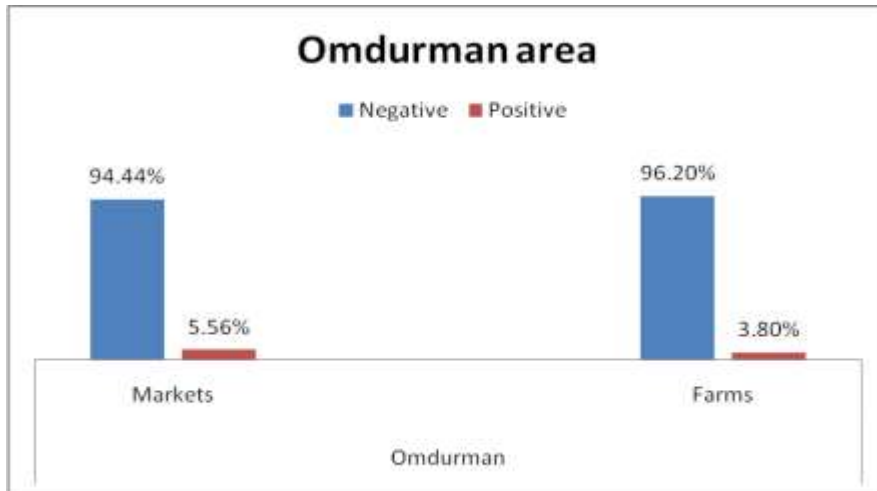
Omdurman area:

Source: Antibiotic residues survey in milk - Khartoum State Dec. 2015

Figure2. Percentage of positive samples of milk collected from dairy farms and markets in Omdurman area, (December 2015)
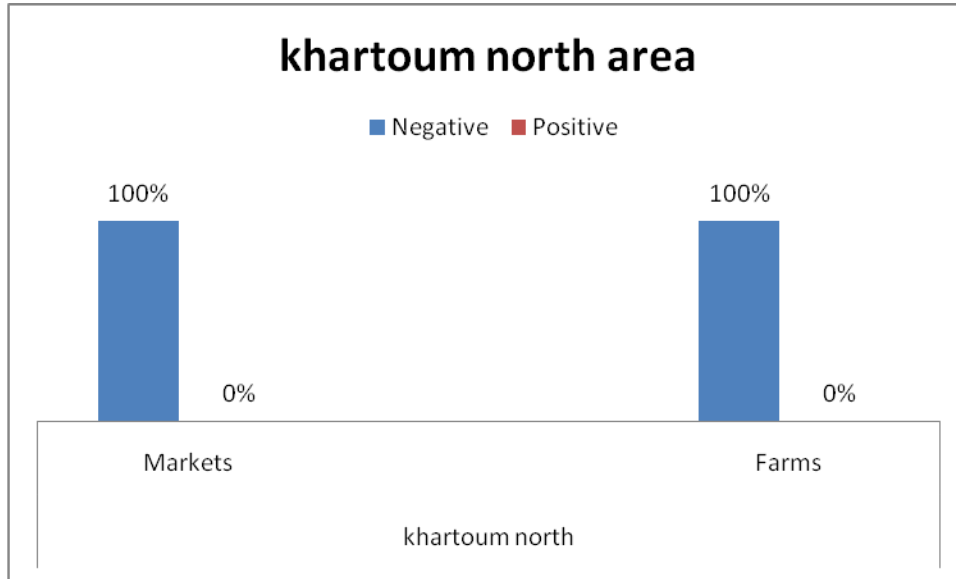
Khartoum area:

Source: Antibiotic residues survey in milk - Khartoum State Dec. 2015

Figure3. Percentage of positive samples of milk collected from dairy farms and markets in Khartoum area, (December 2015).

Khartoum North area

Figure4. Percentage of positive samples of milk collected from dairy farms and markets in Khartoum North area.

APPENDICES

Appendix.1. Metrological authority weather climate data, average temperature in the December 2015.

MINISTRY OF ENVIROMENT, FORESTRY
AND PHYSICAL DEVELOPMENT
METEOROLOGICAL AUTHORITY
WEATHER – CLIMATE DATA

Station: - KHARTOUM   Fax: - 771693
Period: - 2015   Tel: - 772992

ELEMENT Month	Mean Temperature °C							
	MAX.	MAX.						
January								
February								
March								
April								
May								
June								
July								
August								
September								
October								
November								
December	28.5	15.6						
Total/annual								

Note: -   Max = Maximum
                Min = Minimum

Appendix. 2. Sudan university of Science and Technology, College of Agricultural Studies.

Questionnaire

Details of information

Name of area:

Name of farmer:

Date of interview

1- Milk from treated animals?

    A-Mixed with healthy animal's milk   p- Excluded

2-Is there veterinary supervision at the farm?

    A-Yes   p- No