Detection of tetracycline residues in broiler kidney samples in Damascus Countryside Governorate – Syria

Dr.Ahmad Quaider* **Dr.Abduulkarim Hallak****

(Received:4 April 2022,Accepted:21 July 2022)

Abstract:

A total of 24 chicken kidney samples was collected from four cities in Damascus Countryside Governorate (Duma, Maraba, Nabk and Dmer). The samples analyzed for tetracycline residues (oxytetracycline, doxycycline and chlortetracycline).

The tetracycline residues were determined by using HPLC technique (according to AOAC method No 995.09, 1995), with following conditions: C`18 column, mobile phase containing: acetonitrile: methanol: oxalic acid (30:10:60), 350 nm wave length, 1 ml\min flow rate. Results showed that 22 samples (from 24 samples) are positive for tetracycline residues (91.67%).

13 from 22 positive samples contain oxytetracycline (5 samples) and doxycycline (9 samples) residues higher than local MRL (600 µg/kg w.w), but chlortetracycline residues were lower than MRL.

The changes in the oxytetracycline residues in the kidney samples were significantly differences ($P<0.05$) for all studded cites except the changes in oxytetracycline residues in kidney samples between Duma and Maraba and between Maraba and Nabk were not significantly differences ($P>0.05$).

The changes in the doxycycline residues in the kidney samples were not significantly differences ($P>0.05$) for all studded cites except the changes in doxycycline residues in kidney samples between Duma and Maraba were significantly differences ($P<0.05$), The changes in the chlortetracycline residues in the kidney samples were not significantly differences ($P>0.05$) for all studded cites.

Keywords: doxycycline, oxytetracycline, chlortetracycline, residue, kidney, broiler

*Master candidate –Department of puplic health and preventive medicine – veterinary faculty –Hama university.

**Lecturer in Department of puplic health and preventive medicine – veterinary faculty –Hama university.

الكشف عن متبقيات التتراسيكلينات في عينات كلى الفروج في محافظة ريف دمشق – سورية

أحمد قويدر* د.عبدالكريم حلاق**

(الإيداع:4 نيسان 2022، القبول:21 تموز 2022)

الملخص:

أجريت هذه الدراسة للكشف عن متبقيات ثلاث مركبات من مجموعة التتراسيكلينات (أوكسي تتراسايكلين، دوكسي سايكلين و كلورتتراسايكلين)، حيث تم جمع 24 عينة من كلى الفروج من أربع مدن منتشرة في محافظة ريف دمشق (دوما، معربا، النبك و الضمير).

لتحليل متبقيات التتراسيكلينات استخدمت تقنية الكروماتوغرافيا السائلة عالية الأداء لتحليل مركبات التتراسيكلينات وذلك باستخدام عمود تحليل C18 و طور متحرك ناتج عن مزج ثلاث محاليل هي الأسيتونتريل والميثانول وحمض الأوكزاليك بنسبة (30، 10، 60%) و بتدفق 1مل/دقيقة وعلى طول موجة 350 نانو متر، تم استخلاص التتراسيكلينات بطريقة التحليل الرسمية (995.09) (AOAC, 1995).

دلت النتائج أن هناك 22 عينة من أصل 24 عينة كلى (91.67%) إيجابية لمتبقيات التتراسايكلينات منها 13 عينة احتوت على تراكيز لمتبقيات التتراسيكلينات أعلى من الحد المسموح به محلياً (٦٠٠ميكروغرام/ كغ) حيث كان الأوكسي تتراسايكلين (5 عينات) و للدوكسي سايكلين (9 عينات) في حين كانت جميع متبقيات الكلورتتراسايكلين في عينات الكلى أدنى من الحد المسموح به. أعلى نسبة متبقيات للتتراسيكلينات كانت للدوكسي سايكلين و أقلها كانت للكلورتتراسايكلين.

الفروقات في تراكيز متبقيات الأوكسي تتراسايكلين ما بين مناطق الدراسة الأربعة كانت معنوية (P<0.05) ما عدا الفروقات في متوسط تركيز متبقيات الأوكسي تتراسايكلين ما بين العينات التي تم جمعها من منطقة دوما و منطقة معربا و ما بين معربا و النبك فقد كانت غير معنوية (P>0.05) أما الفروقات في متوسط تركيز الدوكسي سايكلين فقد كانت غير معنوية ما بين مناطق الدراسة ما عدا الفروقات في متوسط تركيز الدوكسي سايكلين ما بين دوما ومعربا فقد كانت معنوية و بقيت الفروقات بتركيز متبقيات الأوكسي تتراسايكلين غير معنوية ما بين جميع مناطق الدراسة.

الكلمات المفتاحية:

الدوكسي سايكلين، الأوكسي تتراسايكلين، الكلورتتراسايكلين، متبقيات، كلى ، فروج.

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*طالب ماجستير صحة عامة في قسم الصحة العامة والطب الوقائي – كلية الطب البيطري – جامعة حماة.

**مدرس في قسم الصحة العامة والطب الوقائي – كلية الطب البيطري – جامعة حماة.

1.Introduction :

The indiscriminate and excessive use of antibiotics in animals in general and poultry in particular, is considered one of the most dangerous factors because it causes the presence of their residues in food products (meat and organs), as these residues can bind to plasma proteins or large molecules in the cell and can be It becomes bioactive if affected by enzymes in the digestive tract (Asad, 2012).

The danger of these residues in the organs of poultry comes from the fact that they can become carcinogenic agents (such as nitrofurans and chloramphenicol) or allergens (such as penicillin and streptomycin).

In addition, human affect to high levels of antibiotic residues from animal sources may exacerbate the immune response in immunocompromised individuals negatively affecting their health (Normanno et al, 2007), and illegal use of antibiotics may lead to the development of resistant strains. of bacteria, thus reducing the efficiency of antibiotics used in the treatment of animals, which leads to the failure of the treatment of livestock, and negatively affects the health of the animal, which leads to many diseases and a lack of response to drug compounds (Laxminarayan et al, 2013).

Antibiotics are widely used in the field of poultry farming to treat or prevent bacterial diseases that spread in poultry in general and meat birds in particular. Which includes many compounds such as doxycycline, oxytetracycline and chlortetracycline (Hamweh, 2005 , Kibruyesfa and Naol, 2017).

Tetracyclines are characterized by their broad spectrum of anti gram-positive and gram-negative bacteria that cause many respiratory, urinary, and digestive diseases. It is excreted mainly by the kidneys, and also by the bile and the unabsorbed portion can be excreted through the feces, in addition to that it can be excreted through milk (Emea, 1997).

The extensive use of antibiotics in general and tetracyclines in particular in the field of broiler, in addition to the lack of correct use by poultry farmers through the use of large doses and for long periods and non-compliance with the withdrawal period had negative and dangerous effects on human and animal health (Nonga et al ,2009) ,The presence of tetracycline residues in broiler meat and organs is one of the most important and dangerous negative effects, as these drug residues can reach humans through eating meat and organs of broilers (Tajick and shohreh, 2006), which in turn leads to the growth and development of bacterial resistance

(Apata, 2009) In addition, it can have effects on internal organs such as the liver and kidneys (Laxminarayan et al, 2013).

Also, high intake of these residues in children will lead to staining of teeth and bones, intestinal disorders (an effect on the gut microflora) and serious immunological effects (Lawal et al, 2015. Keyvan et al, 2020).

In an attempt to contain the negative effects of antibiotics, the World Health Organization developed a plan of action (WHO, 2015) in which it called on each country, within its capabilities, to develop national plans in line with its procedures, to monitor the use of antibiotics as one of the strategies used to prevent the occurrence of drug resistance. Developing countries still face real challenges related to monitoring systems due to the lack of integrated technical capabilities they have (Queenan et al., 2016).

Maximum Residues Limits in food have been adopted by the World Health Organization, where Codex has been approved (CAC, 2006) and by the European Union a European standard has been established that must be adhered to to ensure safe and healthy food (EU 37/ 2010), and in the Syrian Arab Republic, the Syrian Standard Specification was developed (Syrian Standards Organization, 2011), Which includes the safe limits for the levels of antibiotics and veterinary drugs in animal products. For example, the permissible limit for tetracyclines (individually) in broiler kidneys is 600 mcg/kg wet weight according to the above-mentioned Syrian standard. Numerous studies in different countries, especially developing countries, indicate that broiler meat and organs contain residual tetracyclines, with dangerous proportions of samples containing concentrations exceeding the permissible (limits Hussein and Khalil, 2013. Salehzadeh et al, 2007. Sarker et al, 2018).

Despite the intense use of tetracyclines in the local market, there are few studies on the residues of this group in the internal organs of broilers, as there was a study by the researcher (Sherif et al., 2020) on the study of doxycycline residues in broiler liver samples in Latakia city, and a doctoral thesis for the same The researcher on the residues of doxycycline and a number of other antibiotics and anti-coccidiosis in the meat and organs of broilers (Sherif, 2021) and a master's research on tetracycline residues in the meat and organs of broilers in the city of Homs (Bilal, 2018). There are relatively high levels of tetracycline residues in broiler kidneys in Lattakia and Homs governorates.

Therefore, we turned in this research with the aim of intensifying the surveys on the detection of tetracycline residues in meat birds in four relatively distant cities in the governorate of Damascus countryside.

2. Objective of the study:

1. Detection of tetracycline residues (doxycycline, oxytetracycline and chlortetracycline) in broiler kidney samples prepared for sale for human consumption in Damascus Countryside Governorate.

2. Determining the viability of broiler kidneys for human consumption by comparing the concentrations of tetracycline residues with the locally permissible limits.

3.Evaluation of the intensity of the use of tetracyclines and the extent of adherence to the time of drug withdrawal before marketing.

3. Materials and methods used:

Glasses and tools used:

different glassware of different sizes (tubes, beakers, pipettes, standard balloon (flask)), 50ml plastic dosing tubes, 12ml plastic tubes, nylon bags with closing mechanism, cork cases (double-walled containers for preserving samples collected from the market).

Chemicals:

Laboratory chemicals for HPLC grade analysis were used:

acetonitrile, methanol, oxalic acid, citric acid, aqueous disodium hydrogen phosphate (Na2HPO412H2o), EDTA-2Na, deionized water for liquid chromatography. It has almost zero transportability from international companies, Standard materials made by Sigma for each oxytetracycline standard concentration_100% ,Chlortetracycline standard concentration 100% , Doxycycline Standardized Concentration 100%.

Instruments:

Microbalance HF-400, Ultrasonic LC 60-H, KUBOTA 5400 scintillation, Acidity Meter HM-60G, vortex shaker (NX-10, Starmix Sample Mill, SUPELCO Analytical High Performance Liquid Chromatograph (LC-10) by Shimadzu Japan, C18 Analyzer Column (25 cm x 4.6 mm, 5 µm) SUPELCO Analytical, Purification Cartridges C18.

Samples collection:

Broiler kidneys samples were collected from four cities in the Damascus countryside governorate (Duma, Maaraba, Al-Dmer and Al-Nabk), the number of samples collected was 6 kidney samples from each of the four regions so that each sample is a mixture of Three samples, for a total of 24 samples from the four regions, so that the three tetracycline compounds are analyzed in each sample. After collecting the samples, they were placed in nylon bags supported by a closing mechanism and kept in a refrigerated container and then sent to the laboratory to be kept in all three at -8°C.

Preparation of samples:

Samples were extracted from the refrigerator, then each sample was ground to a sample grinding device to be well homogenized, then a weight of 5 g was taken from each sample to complete the extraction process.

Extraction of tetracyclines:

The official method of tetracyclines No. 995.09 (AOAC, 1995) was adopted to extract and analyze tetracyclines with some modifications, which are summarized in the following stages:

Preparation of solutions:

1. Extraction solution (McIlvaine buffer-EDTA) is ***prepared in the following order:***

First: Prepare MCIlvaine Buffer (pH = 4)

- Prepare a solution (0.2 M) of sodium phosphate: Weigh 28.4 g of Na2HPO4 and dissolve it in 500 mL of ionized water, then complete the volume to 1000 mL of ionized water.
- Prepare a solution (0.1 M) of citric acid: Weigh 21 g of citric acid and dissolve in 500 ml of ionized water, then complete the volume to 1000 ml of ionized water.
- A solution of citric acid (1 liter) is mixed with 625 mL of Na2HPO4 solution in a two liter container to get an amount (1625 mL).
- The pH (4) was titrated with HCl solution (8.5 mL/L water) or NaOH solution (4 g/L water).

Second: Preparation of MCILvaine Buffer-EDTA

- Weigh 60.5 g of EDTA and dissolve in MCILvaine Buffer (1625 ml) so that the extraction solution is ready.

2) Preparation of the rinse solution (Methanolic oxalic acid):

1.26 g of oxalic acid is weighed and dissolved in 300 ml methanol and then the volume is completed to 1000 ml methanol.

Extraction method:

5 g of kidney sample is placed in a centrifuging tube, 20 ml of extraction solution is added to it, the tube is closed with a stopper, and then placed on the shaker for 10 minutes, after that the sample is sedimented at a speed of 2500 rpm for 10 minutes, and the floating part is taken and placed In another 50 mL centrifuge tube.

The process is repeated on the remaining part by adding 20 ml of the extraction solution and placed on the shaker for 5 minutes, then the sample is sedimented at a speed of 2500 revolutions per minute, and the floating part is taken and added to the previous part. Finally,

the same process is repeated by adding 10 ml of the extraction solution and after centrifugation The supernatant is added to the previous two parts.

The resulting extraction quantity is filtered through a filter after wetting it with the extraction solution to get rid of any organic parts in the sample solution.

Purification of the sample:

The solution the extracted sample is purified by solid phase extraction (SPE), which is an abbreviation of the following words (Solid Phase Extraction), as this process is applied using plastic columns or plastic cartridges (Cartridge) containing the purifier (silica-C18) with a load of 5 The purification cartridge (C18) is activated by passing 20 mL laboratory methanol and then 20 mL ionized water respectively and it must not dry out, after that it is applied The sample solution is slowly passed through (1-2 ml/min), then the tube is washed with 2 ml of extraction solution and passed through the cartridge to ensure that no trace remains in the tube, then the funnel placed on top of the cartridge is washed with 2 ml of extract solution and passed into the cartridge.

Air is passed through the cartridge until it dries, then the rinse solution, which is methanolic oxalic acid, which is the substance that will extract the tetracyclines trapped in the cartridge, if any, is passed, where 6 ml of this solution is passed at a flow rate of 1 ml / min, and this solution is collected from the end of The cartridge is in a 10ml balloon, then the volume is completed to 10ml with ionized water and thus the sample extract is ready for analysis and detection of tetracyclines by liquid chromatography device, where oxytetracycline, doxycycline and chlortetracycline will be analyzed in each sample.

Method of analysis:

The official method of analysis No. 995.09 (AOAC, 1995) was followed with some modifications, where a C18 column was used to separate tetracyclines and using a mobile phase resulting from mixing three solutions of oxalic acid, acetonitrile and methanol in mixing proportions (60/30/10)% respectively, with a flow of 1 ml/min, at a temperature of 40 °C and at a wavelength of 350 nm.

Preparation of oxalic acid:

Weigh 1.26 g of oxalic acid in 50 mL of ionized water and dilate the solution to 1000 mL with ionized water.

Preparation of fixation samples to calculate the recovery rate:

100 µg of tetracycline mixture was added to three kidney samples taken from cultures that did not use any of the tetracyclines in treatment or prevention, and then the extraction process was applied in its entirety.

Preparation of tetracycline standard solutions:

A weight of 25 mg of oxytetracycline, chlorine tetracycline and doxycycline was taken separately and dissolved in a 25 ml balloon from the mobile phase, then 1 ml of each standard dissolved substance was taken and placed in a 20 ml balloon And mixed, and then the volume is completed in the mobile phase to 20 ml, so the final concentration becomes 50 µg/ml. Then a solution of a mixture of the three previous antibiotics is prepared by taking 1 ml of each previously prepared standard solution and placed in a standard 20 ml balloon and dilated in the mobile phase.

4. Statistical data processing:

Microsoft Excel was used in calculating averages, standard deviations and graphs, and Orign Pro 7 was used in calculating the degree of significance at P = 0.05.

5. Results:

After the liquid chromatography device was prepared for work and the mobile phase was applied, the device was left for a while to settle on the analytical conditions of tetracyclines, then the three tetracycline compounds were injected separately to know the retention time of each of them, and then the mixture of tetracyclines was injected several times. The average area of each peak was calculated

(Fig. 1).

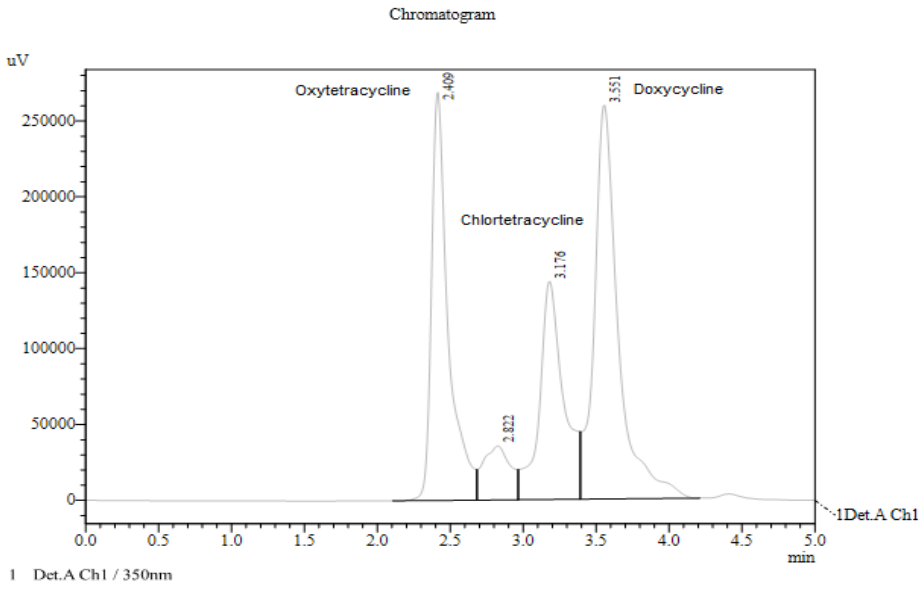

Figure No. (1). Chromatographic scheme of the mixture of standard tetracyclines.

After that, the recovery samples were applied several times to calculate the recovery rate of each compound in order to evaluate the extraction method that was applied with the

conditions available in the laboratory by applying the following relationship: Recovery rate = calculated concentration / added concentration multiplied by 100.

The results we obtained showed that the recovery rate was for doxycycline, oxytetracycline and chlortetracycline (96.1,97.8,95.8%) respectively, and therefore the extraction method used in this research can be credible and can be adopted to analyze the residual tetracyclines in the kidney tissue of birds, as according to the researcher (Abu-Raya et all, 2013) and the researcher Sharif et al. (2020) it is possible to accept the proportions of Retrieve antibiotics from animal tissues if they are in the field 60-115%.

Analysis of tetracycline residues in broiler kidney samples:

We note kidney samples collected from the study areas in Damascus countryside contained high percentages of tetracycline compounds residues (Table No. 1), where doxycycline residues had the largest percentage, followed by oxytetracycline residues and Relatively less were the chlortetracycline residues with a difference in their distribution according to the studded areas.

The concentration of oxytetracycline residues in broiler kidney samples: It is noted from the results that we obtained (Table No. 1) that all broiler kidney samples collected from the Dmer and An-Nabk regions were positive for the presence of oxytetracycline residues, while there were four positive samples. In the Duma area and only three samples in the Maaraba area. The concentration of oxytetracycline residues in kidney samples collected from Duma region ranged between 4.77 and 103.15 µg/kg wet weight, while in Ma'riba region, the concentration of oxytetracycline residues was relatively higher than in Duma region, where it ranged between 21.39 and 364.73 µg/kg wet weight.

The concentration of oxytetracycline residues in broiler kidney samples in the Nabk region ranged between 64.64 and 693.53 µg/kg wet weight, although the highest concentration was recorded for oxytetracycline residues It was in the Nabk region (693.53 µg/kg), but the rest of the samples had relatively low concentrations compared to the residual oxytetracycline in the kidney samples collected from the Dmer region, as the lowest concentration recorded (270.76 µg/kg) was relatively high compared to Residues of this compound in samples of Nabk region and samples of other regions, and the highest concentration of oxytetracycline residues in samples of this region (Al Nabk) reached 612.72 µg/kg wet weight.

Table No. (1). Oxytetracycline residue concentration (µg/kg wet weight) in broiler kidney samples :

	Duma A	Maraba B	Dmer C	Nabk D
1	0	21,39	612,96	384,72
2	0	232,51	606,09	601,25
3	4,77	0	467,72	133,42
4	9,79	364,76	270,76	92,31
5	39,62	0	360,89	64,64
6	103,15	0	407,37	693,53
Average positive samples	45,24 ±39,33	173,19 ±206,22	136,39 ± 454,30	273,59 ± 328,31
overall average	24,77± 26,22 ac	123,68 ± 103,76 Bc	136,39 ± 454,30	273,59 ± 328,31 ad

Note: The presence of two letters is evidence of significant differences between the groups that represent them.

In comparison with the locally permitted upper limit for oxytetracycline residues in the kidneys (600 µg/kg wet weight), we note that all kidney samples collected from Duma and Maraba regions were hygienically safe as the concentration of oxytetracycline residues in them did not exceed the locally permitted limit. While there were two kidney samples in both Al- Nabk and Al-Dmer area, the concentration of oxytetracycline residues exceeded the locally permissible limit (Table 1).

By comparing the average concentration of oxytetracycline residues in broiler kidney samples according to each region, we note, as shown in Table No. (10) and Figure No. (2), that the highest average was for kidney samples collected from Al-Dmer region (454.3 µg/kg wet weight) and that As the general mean and the mean of the positive samples (all samples are positive), the overall mean and the mean of the positive samples were for the residuals of this compound in the broiler kidney samples collected from the Nabk area (328.31 µg/kg wet weight).

As for the average concentration of oxytetracycline in Duma and Maaraba regions, it was relatively low compared to its concentration in Nabk and Dumeir samples. The lowest general and average mean of residual positive samples in kidney samples was in Duma area, where the general and average positive samples reached 26.22 and 39.33 µg/kg wet weight,

respectively, while it was in broiler kidney samples in Maaraba area 103.76 and 206.22 µg/kg weight. straight wet.

As is clear from Figure (1), we note that all averages of oxytetracycline residues in broiler kidney samples collected from all areas of Damascus countryside did not exceed the locally permitted maximum (600 µg/kg wet weight) despite the presence of samples in my areas AdDmer and Nabk leftovers exceeded the locally allowed limit.

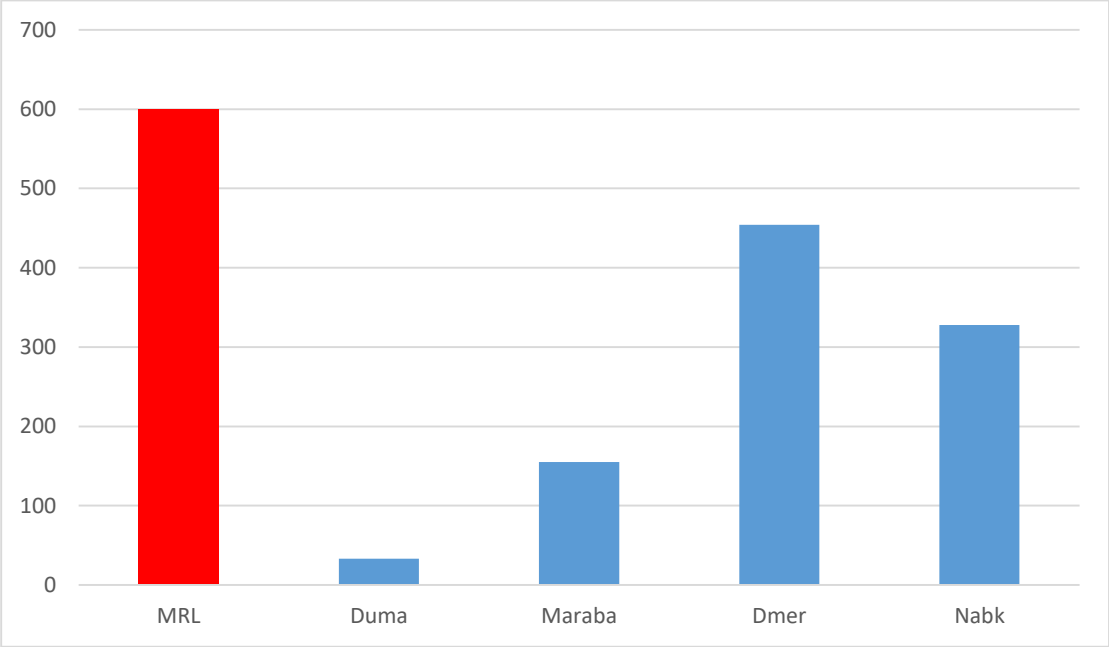

Figure 2. Average oxytetracycline residues in broiler kidney samples.

The results of the statistical analysis indicate that all the differences in the mean concentration of oxytetracycline residues in the kidney samples were significant ($P<0.05$) between Duma and Al-Dmer and between Duma and Al-Nabk, and it was also significant between Maaraba and Al-Dmer regions, while the differences were Between Maaraba, Nabk, AdDmer and Nabk is not significant ($P>0.05$).

Doxycycline residue concentration in broiler kidney samples:

Table No. (2) shows the results of analysis of doxycycline residue in broiler kidney samples collected from the four Damascus countryside regions.

We note through the results that we obtained that all samples of broiler kidneys collected from the Al-Dmer and Al-Nabk regions were positive for the presence of residual doxycycline, as the concentration of the residues of this compound in the kidney samples collected from the Al-Dmer region ranged between 100.41 and 726.83 µg/kg weight. While

in Nabk area, the concentration of residues ranged between 99.93 and 608.36 µg/kg wet weight.

The lowest concentration of doxycycline residues recorded in broiler kidney samples was in Duma area, where there were four positive samples and only two negative samples. The concentration in positive samples ranged between 72.42 and 498.10 µg/kg wet weight. In Maaraba region, there was only one negative sample that did not show the presence of residual doxycycline, and five positive samples with a concentration ranging between 87.91 and 726.67 µg/kg wet weight.

Table No. (2). Doxycycline residue concentration (µg/kg wet weight) in broiler kidney samples:

	Duma A	Maraba B	Dmer C	Nabk D
1	0	582,54	100,41	135,74
2	0	87,91	663,08	99,93
3	383,15	726,67	718,11	608,36
4	498,10	0	656,92	402,92
5	119,50	719,83	389,76	606,86
6	72,42	1118,62	726,83	166,84
Average positive samples	205,35 ± 268,29	424,21 ± 647,11	249,49 ±542,52	235,14 ± 336,78
overall average	210,94 ±178,86 ac	371,08 ±539,26	249,49 ±542,52	235,14 ± 336,78

Note: The presence of two letters is evidence of significant differences between the groups that represent them.

Looking at the results presented in Table No. (2), we note that there are 9 kidney samples (42.87%) The concentration of doxycycline residues in it exceeded the locally permissible limit (600 µg/kg wet weight), as these samples were distributed by three samples in Maaraba area, four samples in Al-Dmer area and two samples in Al-Nabk area, while all the doxycycline residues were in the broiler kidney samples. Which were collected from the Duma area is safe from a health point of view, as the concentration of residuals in it did not exceed the permissible limit locally. Through the results, we note that most of the positive broiler kidney samples that were analyzed were associated with residuals of oxytetracycline or with residuals of chlortetracycline, but some samples were only positive for residuals of doxycycline, which indicates the intensity of the use of this compound compared to the rest

of the tetracycline compounds and that the highest concentration of residues Doxycycline in broiler kidneys was in Maaraba area (1118.62 mcg/kg live weight) (Fig. 4).

By comparing the average concentration of doxycycline residues in broiler kidney samples, whether positive samples or the general average (Fig. 3), we note that the lowest general and average positive samples were in the Duma region, which reached 178.86 and 268.29 µg/kg wet weight, respectively.

And the highest general and average mean of the positive samples of residual doxycycline in the kidneys was in Maaraba region, where the average concentration of residuals reached 647.11 µg/kg wet weight for the positive samples and 539.26 µg/kg wet weight as a general average, while the general average of the residuals of doxycycline in the kidney samples was Broilers in Al-Nabk region (336.78 µg/kg wet weight) are lower than the general average and the average of the positive samples of broiler kidney samples in Al-Dmer area (542.52 µg/kg wet weight) although all broiler kidney samples in Al-Nabk and Al-Dmer region were positive, while There was only one negative kidney sample in Ma'raba area, but positive kidney samples in Ma'raba area contained relatively high concentrations.

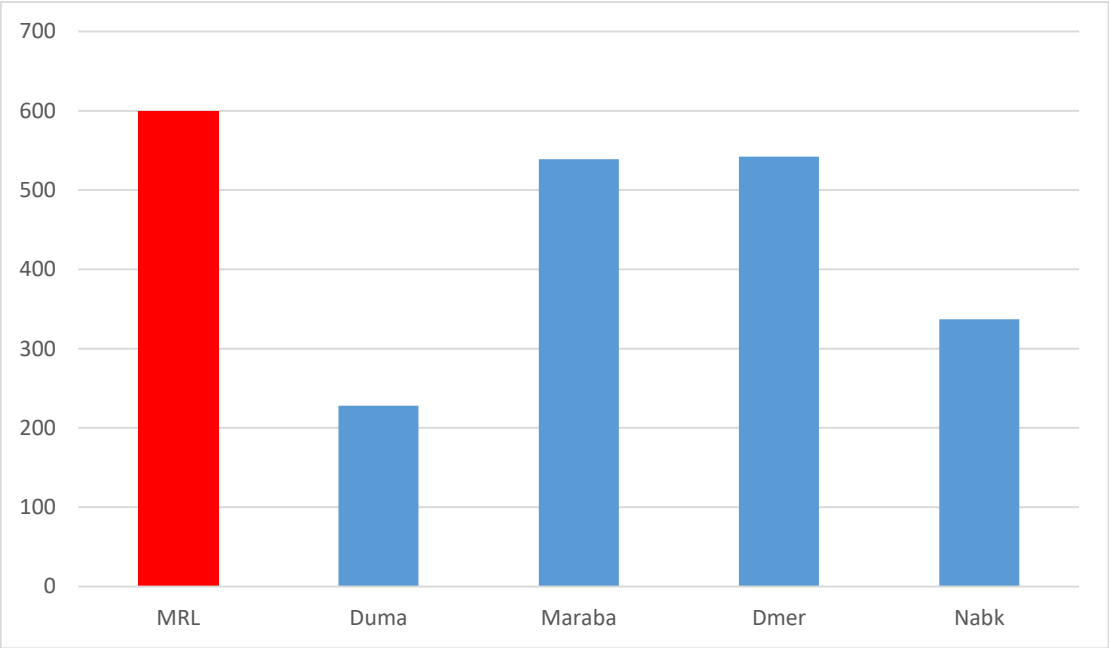

Figure 3. Mean of doxycycline residues in broiler kidney samples.

From a health point of view, we note that the average concentration of positive kidney samples and the general average of doxycycline residuals was lower than the local permissible limit (600 µg/kg wet weight), except for the mean of doxycycline residuals for positive samples in Maraba area, which exceeded the permissible limit (647.11). µg/kg in wet soil), while the

general mean of doxycycline residue concentration was lower than the permissible limit (Table No. 2 and Figure 3).

Statistically, the differences in the mean concentrations of doxycycline residues in kidney samples among the studied regions were not significant ($P>0.05$), except for the differences between Duma and Al-Dmer regions, which were significant ($P<0.05$).

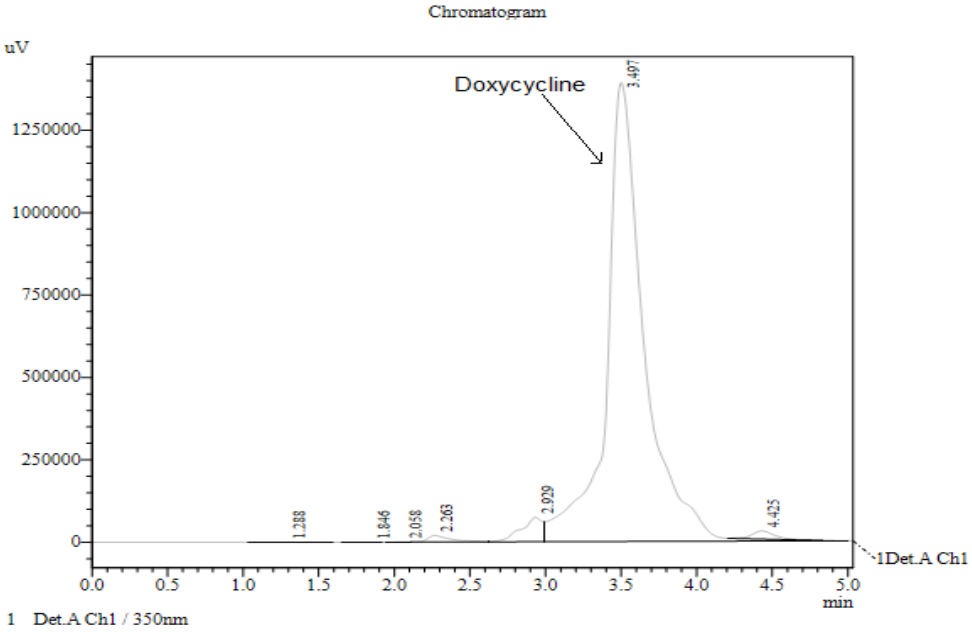

Figure 4. Chromatographic scheme of doxycycline residues alone in one of the kidney samples (Maraba).

The concentration of chlortetracycline residues in broiler kidney samples:

Table No. (3) indicates the concentration of chlortetracycline residues in broiler kidney samples in the four studded areas.

We note, as is clear in Table (3), that there are only 12 positive kidney samples in all studded areas out of 24 samples (50%) Where there was only one sample in the Duma region (0.69 µg/kg wet weight), two samples only in the Nabk area (7.46-12.65 µg/kg wet weight) and three positive samples in the Ma`raba area, where among these samples there was a sample that contained The lowest concentration was recorded in the kidneys (0.13 µg/kg) and the sample had the second highest concentration among the positive samples (13.09 µg/kg wet color) in all studied areas. As for the kidney samples collected from Al-Dmer area, they were all positive for the presence of chlortetracycline residues, with a concentration ranging between 0.68 and 18.09 µg/kg wet weight.

We also note that all the positive kidney samples collected from the four areas in Damascus countryside did not exceed the locally permitted concentration of chlortetracycline residues

(600 µg/kg wet weight).

Also, in terms of comparing the general average of chlortetracycline residues, we note that the lowest general average was in Duma (0.11 µg/kg wet weight) followed by the general average in Maraba (2.42 µg/kg wet weight) and in the third place was the concentration of chlortetracycline residues in kidney samples. Broilers in Al- Nabk region (3.55 µg/kg wet weight) and the highest average concentration was in Al-Dmer region (6.88 µg/kg wet weight) and all of these averages were below the locally permitted limit (600 µg/kg wet weight).

Table No. (3). Concentration of chlortetracycline residues (µg/kg wet weight) in broiler kidney samples:

	Duma A	Maraba B	Dmer C	Nabk D
1	0	13,09	0,68	0
2	0	0	18,09	0
3	0,69	1,27	12,82	12,65
4	0	0,13	0,72	0
5	0	0	1,51	0
6	0	0	7,45	7,46
Average positive samples	0,00±0,39	7,18 ±4,83	7,30 ±6,88	3,67 ±10,6
overall average	0,28± 0,11	5,25 ±2,42	7,30 ± 6,88	5,44 ± 3,35

Note: The presence of two letters is evidence of significant differences between the groups that represent them.

It was also noted that all samples that showed positive for chlortetracycline residues in broiler kidney samples were associated with the presence of residuals of doxycycline (Fig. 4) or of oxytetracycline, and we did not find any sample that contained residuals of chlortetracycline.

Statistical analysis of the data we obtained indicates that the differences in the mean concentrations of chlortetracycline residues in the kidney samples between the studded regions were not significant ($P>0.05$), except for the differences between Duma and Al- Dmer regions, which were significant ($P<0.05$).

6.Discussion:

The results we obtained indicate that there is a discrepancy in the values of the studied tetracycline residues in the broiler kidney samples collected from the four study areas in the

Damascus countryside. Only 12 positive samples in all studied areas with relatively low concentrations compared to oxytetracycline and doxycycline residues. These light concentrations of chlortetracycline detected in positive samples are evidence of the lack or lack of use of this compound in broilers in the regions and time of the study because they do not correspond to the concentrations detected for oxytetracycline and doxycycline, and the presence of these positive samples can explain For the residuals of this compound, there may be light concentrations of this compound pre-existing with the raw material of doxycycline or oxytetracycline from the source resulting from some contamination during manufacturing or as a result of contamination of the doxycycline or oxytetracycline preparation during their manufacture, whether in the form of powder or in the form of A liquid with the chlortetracycline compound, or it was obtained with the intention of profit (cheating) due to the difference in the price of chlortetracycline from doxycycline or oxytetracycline, or it may have actually been used.

Our results agree with the results of the researcher Rami (2018) in terms of the percentage of positive kidney samples for doxycycline and oxytetracycline , where he indicated that there are 19 positive samples out of 24 samples collected from Homs countryside, and they differ with of this researcher in terms of high values of The concentration of chlortetracycline residues, as there were samples that contained high concentrations, and some of them exceeded the permissible limit locally.

It was noted through the results that we obtained that there is an intensity in the use of oxytetracycline, through the number of positive samples for the remnants of this compound (19 samples out of 24), and that there is no adherence to the health conditions for applying the treatment such as the use of large doses and for long periods, and also there is a lack of health commitment to the time Marketing after the last treatment (at the time of drug withdrawal) 4 out of 19 positive samples were observed (21.05%) It exceeded the local permissible limit (600 µg/kg), where the highest concentration of this compound residue in kidney samples reached 693.53 µg/kg wet weight (Al-Nabk).

It was also observed from the results that there is a discrepancy in the number of positive samples for oxytetracycline residues between each region and a variation in the concentration of residuals between regions in the same region between the samples, meaning that there is heterogeneity in the source of broilers, which led to a difference in drug residue concentrations where the residues were Oxytetracycline in some areas is low (Duma) and in other areas is high (Dmer) also in the same region, where a discrepancy was observed in the concentrations of residuals, and this was indicated by the high values of the standard deviation.

As for the residual doxycycline in the kidney samples collected from the four study areas, it was also noted that there is a large intensity in the use of this compound and randomness in treatment and marketing, as it was noted that 87.5% Of the samples (21 samples) were positive for residual doxycycline, as all samples collected from Al-Nabk and Al-Dmer areas were positive. It was also noted that 40% (9 samples) of the positive samples in which the concentration of residual doxycycline exceeded the locally permissible limit (600 µg/kg), and these samples were distributed in three regions, two samples in Al-Nabk region, three samples in Al-Dmer region, four samples in Maaraba region and the highest concentration It was recorded for these residues was 1118.62 µg/kg wet weight in Maaraba area.

On the other hand, we also notice a discrepancy in the concentrations in one region and between regions, and this is evidence of the multiplicity of sources of broilers, that is, not from one farm. In all cases, we can say that there is a clear lack of commitment to the rules of treatment and marketing of broilers, and this may be the result of ignorance of the rules Health or fear of economic losses, in this regard the researcher (Nonga et all, 2009) indicated that 95% Some poultry farmers in Tanzania market their chickens before the recall period ends due to fears of economic losses.

In this regard, our results are consistent with what the researcher (Rami, 2018) indicated in a study similar to our study on tetracycline residues in broiler kidney samples, where the distribution of tetracycline residues was as follows: For chlortetracycline 16 positive samples (29.09%) And for oxytetracycline 17 samples (30.91%) While there were 22 positive samples for doxycycline (40%) The highest percentage is for doxycycline residues, followed by oxytetracycline, and then chlortetracycline. This researcher indicated that there is a discrepancy in the concentration of tetracycline residues between the sampling areas and between samples in the same region. It was noted that there was no commitment to the withdrawal period, but our results differ with the results of this researcher in terms of The values of tetracycline residue concentrations and the number of samples whose residue concentration exceeded the permissible limit.

Similar results were reached by the researcher Sharif and others (2021) in terms of the intensity of the use of doxycycline in the poultry sector and the non-compliance with the period of drug withdrawal, where he indicated that out of 94 liver samples collected from the markets of Lattakia city, there were 78.5% A positive sample for residual doxycycline was 83.3% The residue concentration exceeded the permissible limit.

There are also discrepancies in the results of researchers in a number of other countries, where between Elbayoumi and others (2018) after collecting 60 muscle, liver and kidney samples (20 samples of each type) broilers collected from Menoufia Governorate in delete Egypt in order to investigate the residuals of doxycycline. And oxytetracycline, the percentages of positive samples for residual doxycycline in the kidneys were 70 %.
The concentration of doxycycline residues in kidney samples ranged between 25.1 and 3661.6, with an average of 1305.59 µg/kg wet weight. As for oxytetracycline residues, the percentage of positive samples in the kidneys was 45 %.
The concentration of oxytetracycline residues in the kidney samples ranged between 26.8 and 412.7, with an average of 162.23 µg/kg wet range. This researcher also pointed out 30% Of the kidney samples, the concentration of residual doxycycline exceeded the permissible limit according to the Egyptian specifications. In Bangladesh, the researcher (Sattar et al ،2014) stated that out of 50 kidney samples collected, 12 were found (24%) Positive for tetracyclines.
Our results differ with the results of researcher Abdel-Mohsein and her colleagues (2015) in terms of the intensity of chlorine tetracycline use, as she analyzed chlorine tetracycline residues in 94 bird kidney samples collected from poultry farms in the Arab Republic of Egypt from several farms, and all tested samples were positive for chlorine. Tetracyclines with an average residue concentration of 690 µg/kg wet weight. This is due to the nature of each country and the extent to which each antibiotic is used.
It can be said that the presence of residual tetracyclines in the collected kidney samples is evidence of the intensive use of this group of antibiotics as a result of the need for them in treatment due to their great effectiveness and wide spectrum in eliminating bacterial pathogens that are widely spread in broilers, but as a result of incorrect use of In terms of applying large doses and for long periods, or using raw materials instead of using manufactured preparations (due to price differences) and non-compliance with the time of drug withdrawal either because of ignorance or because of fear of economic losses when pandemics occur, or with the aim of achieving more profits when marketing is appropriate, All this causes the arrival of poultry products of meat and internal organs that can be consumed in an unhealthy and unsafe manner. This phenomenon is often seen in developing countries, as we have previously noted from the research results in references.
On the other hand, when there is safe marketing, adherence to treatment, and the time of drug withdrawal, we note that there are products that are safe for human consumption, and this can be observed in developed countries and in this field, the researcher Bartkiene and

others (2020) indicated that out of 20 broiler muscle samples collected from sales outlets In Germany and Lithuania, only three samples were positive for antibiotic residues, two of them contained residual enrofloxacin and the third contained residuals of enrofloxacin and doxycycline, and all concentrations were lower than the European limit.

From the foregoing, we note that the percentages of tetracyclines use vary from one compound to another, from one region to another, and from one country to another, and the concentrations of residuals of these compounds in the kidneys of broilers vary greatly according to regions and according to the season and in all cases and as long as there are positive samples and samples containing Unsafe concentrations of tetracycline residues and they may contain antibiotics from another group. There is a weakness in strict health control, whether in the trade of antibiotics or in the mechanism of treatment with them, or in the failure to adhere to the appropriate withdrawal periods for each drug compound separately.

7.Conclusions:

1. The presence of tetracycline residues in 22 out of 24 broiler kidney samples collected from the studied areas in Damascus Countryside Governorate.
2. Among the positive samples for tetracycline residues, there were 13 kidney samples that contained tetracycline residues above the permissible limit, four of them for oxytetracycline residues and nine for doxycycline residues, while the chlortetracycline residues were lower than the permissible limit in all samples.
3. There is a discrepancy in the use of tetracyclines between each region and another and between each compound and another, but in general there is an intensity of use and a failure to follow the health rules in marketing, especially at the time of withdrawal.

8.Recommendations:

Emphasizing the need to conduct periodic surveys on veterinary drug residues at the national level in cooperation between research and community institutions, and the need to develop appropriate solutions to rationalize the use of antibiotics to obtain safe and sound food for humans.

Commitment to the period of withdrawal of antibiotics when used curatively or prophylactically in the poultry sector.

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