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DETERMINATION OF ANTIBIOTIC RESIDUES IN EGG SAMPLES BY ELISA

MASTER THESIS

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

30.12.2016

YEŞİM KUTLU

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LIST OF SYMBOLS AND ABBREVIATIONS

CDC: Centers for Disease Control and Prevention

FDA: Food and Drug Administration

HPLC: High Performance Liquid Chromatography

l: litre

Min: Minute

OIE: World Animal Health Organization

PBS: Phosphate-buffered saline

ppb: per part billion

WHO: World Health Organisation

µ: Mikro

ABSTRACT

Kutlu, Y. Determination of antibiotic residues in egg samples by elisa method. Yeditepe University, Institute of Health Science, Department of Nutrition and Dietetics, MSc thesis, İstanbul.

Antibiotics are drugs used in the treatment of infectious diseases. The most basic reason for not using in some foods is to prevent passing on to microorganisms found in the normal flora of animals .Microorganisms such as *Salmonella* and *Campylobacter* can be found in the gastrointestinal system, feathers and skin of poultry.Since the microorganisms are likely to be found in chickens,especially egg shells,some tetracyclines and similar antibiotics are given via drinking water. Because of unconscious uses of antibiotics,residues of antibiotics can be occur over the limits from time to time.

In our study antibiotic level in eggs with highly day consuption were investigated. Organic and normal egg samples were collected and levels of chloramphenicol and tetracyclines were determined by ELISA method in the laboratory.In all four samples , residues of tetracycline were not found .These four samples are not packaged, but sold outdoors.In other not packed egg samples, it is determined as 0.4-70.8 ppb,and it is in the limits of maximum residue.But,for packed egg samples it was determined between 0,01-3,4 ppb. In 36 samples, none of the chloramphenicol residues were found,while the residues are detected in the four open samples. However, because of the residues of tetracycline are also over the limit, it is thougt to be cross react and can not be ascertained without being confirmed by HPLC management.It is pleasing that the level of the residues on the egg samples is low, it is a fact that that is necessary to increase the number of studies by enlarging the sample.

ÖZET

Kutlu, Y. (2016). Yumurta Örneklerindeki Antibiyotik Kalıntı Düzeylerinin Elisa Yöntemi ile Belirlenmesi. Yeditepe Üniversitesi, Beslenme ve Diyetetik Anabilim Dalı, Yüksek Lisans Tezi, İstanbul.

Antibiyotikler enfeksiyon hastalıklarının tedavisinde kullanılan ilaçlardır. Bazı gıda değeri olan besinlerde kullanılmalarının en temel nedeni ise hayvanların normal floralarında bulunan mikroorganizmaların hayvansal besin ürünlerine geçmelerini önlemektir. Kümes hayvanlarının hem gastrointestinal sistemlerinde hem de tüy ve derilerinde *Salmonella* ve *Campylobacter* gibi mikroorganizmalar bulunabilmektedirler. Tavukların özellikle yumurta kabuklarında bu mikroorganizmaların bulunabilme olasılı yüksek olduğu için içme suları aracılığı ile bazı tetrasiklin ve benzeri antibiyotikler verilmektedir. Ancak bilinçsiz kullanımları sonucu tavukların hem etinde hem de yumurtalarında zaman zaman limitlerin üstünde antibiyotik kalıntıları bulunulabilmektedir.

Çalışmamızda da günlük tüketimi yüksek olan yumurtalarda bulunan antibiyotik düzeyleri araştırılmıştır. Organik ve normal yumurta örnekleri toplanmış ve laboratuvarda ELİSA yöntemi ile kloramfenikol ve tetrasiklin düzeylerine bakılmıştır. Toplamda dört örnekte limitlerin üstünde tetrasiklin kalıntısına (120-170 ppb). rastlanmamıştır. Bu dört örnek paketli olmayıp, açıkta satılan yumurta örneklerindendir. Diğer açık yumurta örneklerinde 0,4-70,8 ppb olarak saptanmış olup, maksimum kalıntı limitleri içindedir. Paketli yumurta örneklerinde ise 0,01-3,4 ppb arasında saptanmıştır. 36 örnekte kloramfenikol kalıntısına rastlanmazken, açıkta satılan 4 örnekte kalıntı (0,9-3,7) tespit edilmiştir. Ancak bu örneklerde aynı zamanda tetrasiklin kalıntılarının da limitlerin üstünde olması nedeni ile çapraz reaksiyon olduğu düşünülmüş ve HPLC yönetimi ile doğrulanmadan kalıntının varlığından emin olunamayacağı kanısına

varılmıştır. Yumurta örneklerinde kalıntı düzeylerinin az olması sevindirici olmakla birlikte, örneklem genişleterek çalışmaların arttırılması gerekli olduğu bir gerçektir.

I. INTRODUCTION and PURPOSE

Antibiotics are used to prevent infectious diseases and to encourage the growth and yield of food-worthy animals. In addition to antibiotics, numerous medicines, hormones, vitamins, minerals, etc. are used to control parasitic diseases and support nutrition.Regardless of the purpose for which they are grown, almost all animals are exposed to one or several drugs once or several times during their lifetime (1, 2).

Antibiotics began to be used towards the 1950s, which has played a role in the reduction of deaths and illnesses caused by infectious diseases. However, according to recent studies excessive use of antimicrobial drugs leads to provision of resistance against ginseng and use of antibiotics for a long period of time is a threat to public health (3).

Drug use in animals has become an indispensable practice today. The Consumer Union has reported that excessive use of antibiotics in edible and developing animals affects public health negatively.People are at risk, because of the potential presence of both antibiotic-resistant bacteria in meat products and the fact that these bacterias migrate to the environment and baterıas which make people sick (3).

In modern livestock applications, the use of veterinary medicines is utmost importance for the efficient and safe production of meat, fish, milk, eggs and honey. The use of these drugs is subject to strict licensing and approval procedures with a system similar to that of medicinal products. In many countries inculiding Turkey, the use of growth hormones (which are used to speed up to put on weight for animals) and drugs that release toxic residues in animal products is prohibited. The presence of detectable levels of such prohibited products is considered as a violation of the law. Stilbens, stilbene derivatives, anti-thyroid agents, steroids, ascorbic acid lactones (including zeranol) and ß-agonists are amongst the prohibited drugs. In addition, the use of natural hormons as growth hormones is prohibited in the EU and Turkey. In the US, use of natural hormons is allowed (4).

Turkey is allowed to use many medicines despite these restrictions and prohibitions. Among the medicines that are allowed to use, antibacterial agents such as sulphonamides,antihelmintics,anticoccidials (including nitroimidazoles), sedatives and non-steroidal anti-inflammatory drugs. Some veterinary medicines are also used as preventive agents for the treatment of diseases, and such medicines are generally used by mixing with food (4).

In order to protect human health, the mandatory period between the administration of the drugs allowed for use and the cut-off of the animal (duration of excretion) was introduced. Many health organizations including the American Medical Association, the American Public Health Association, the Infectious Disease Society of America and the World Health Organization (WHO) has been calling for a significant reduction in the use of antibiotics used in food production in animals. Use of veterinary medicines attracts public attention and is a matter of debate. However, the use of these drugs is inevitable in modern livestock techniques. The greatest concern in this regard is the antibiotic resistance of microorganisms as a result of widespread use of veterinary drugs. The source of this concern is that the resulting antibiotic resistance also affects people. In this case, the effect of drugs used in humans is reduced. The anxieties have led to the reconsideration of the non-human use of certain antibiotics, and the use of enroflakasin in chickens was banned in the United States in 2005 . The World Health Organization (WHO) has requested the ban on the use of a number of antibiotics critical to the treatment of humans in animals (4).

II. LITERATURE REVIEW

Due to antibiotic resistance concerns, the use of antibiotics as growth support in the EU is prohibited. Compared to the risk of antibiotic resistance, the risk of toxicity caused by the remedies caused by drugs permitted for use in foodstuffs is very low. However, the EU has set the Maximum Residue Limits (MRLs) of veterinary drugs in animal foodstuffs to prevent people from exposure to harmful levels of residues. The regulation of the EU Commission on Active Pharmacological substances of 37/2010 and 22.12.2009 lists the maximum residue limits in foodstuffs of animal origin (4).

Over the past decade, there has been a lot of panic about veterinary medicines (banned substances or residue levels above the MRL). Among these panics, chloramphenicol in shrimps imported from Asian countries and nitrofurans in Brazilian and Thai chicken are the most known. Tetracyclines are among the most widely used veterinary drug groups in many countries due to their easy availability and wide spectrum. For these reasons, a large number of animal food remains are found. In Chinese honeys, the problem of chloramphenicol was frequently observed, and as a result there were problems in the EU the prohibition of Chinese honey. In 1997-1998, there was a bacterial outbreak in China, effecting the whole honey industry. Chloramphenicol (along with streptomycin) is widely used by honey producers because China is not a country with effective regulations on veterinary drug use. As a result of the chloramphenicol problem, China's exports to the EU countries in 1997 fell to 50 billion tons.The main source of the remains found in honey products are antibiotics (residues in honey and bee stock) and permanent lipophilic acaracids (residues in honey wax and bee resin).Recent studies on honey have found widespread antibacterial drug residues. Among the active substances detected the sulphanamides are in the first group, and tetracyclines, streptomycin, tylosin and chloramphenicol are the second group.In this regard, data on the problem in Turkey is very limited (4).

Scientists concluded that there was a link between the antibiotics used in animals more than 20 years ago and the reduction in the productivity of drugs used for human beings. The institute of Medicine in 1988, even if they are discontinuous until now.Salmonella species were passed on to humans through food products, from farm animals treated with antibiotics at less considerations and were exposed to Salmonella bacterial infections (3).

In 2003 The World Health Organization, Food and Agricultural Organization (FDA) ve World Animal Health Organization (OIE) states that there is a negative condition on human health because of the resistant organisms that result from antimicrobials that people do not use. Increased incidence of untreated infections, frequency of unsuccessful treatments, severity of infections, and even deaths in some cases can be counted among these cases (3).

Between 2007 and 2010, 6 warnings about honeys of Turkey origin were published in the EU and sulfadimidine and sulphametazine were encountered in these declarations. It is not only the EU countries that affect Turkey's export of veterinary drug residues in animal products. Veterinary drug remnants have great prospects in the export of large amounts of poultry meat to Russia, which has been in the news in recent days. Russia regulates antibiotic residues under the name;maximum permissible concentrations. These limits are 0.01, 0.5 and 0.5 ng / g, respectively for tetracycline, penicillin and chloramphenicol. There is a obligation to obey the extremely low limits for export to Russıa. Failure to comply with the limits may result in major economic losses (4).

In 2010, The Food and Drug Administration (FDA) and the Infection Disease Prevention and Control Center (CDC) proves that there is a link between the reduction of antibiotic productivity for humans and the routine use of antibiotics for meat production. It has been noted that there is strong scientific evidence of antibiotic resistance in humans with the use of antibiotics in animals by the CDC (3).

In late 2012 The FDA indicates that the increase in the number of microbial resistant bacteria that result in excessive and misuse of antimicrobial drugs is greater than that of microbial susceptible bacteria. This increase leads to an increase in the number of people infected by resistant bacteria. At the same time, the FDA prohibited the use of cephalosporin antimicrobial drugs in animal food production. As a result of exposure to antimicrobials for animals used in food production, feeding people with food containing bacteria resistant to microbes threatens public health. Despite all this, livestock industry despite the fact that antibiotic-resistant bacteria used in the farm that it is not an important factor in terms of human health to be used in the common conditions required. As a result, the use of penicillin and tetracyclines as forage additives is forbidden at the end of the 1970s. The use of antibiotics in forage in Turkey only is allowed the inclusion of substances with specific properties such as avoparcin, simplex, flavophospholipol, monensin, spiramycin and virginamycin (3).

Among the most important hazards that penicillin-derived antibiotic residues in animal-derived foods, including meat, can cause, skin reactions in consumers from anaphylactic shock to deaths. Tolerance levels determined in relation to drug residues in foods indicate amounts in raw tissues and organs. It is stated that antibiotic residues in animal foods become harmless by breaking down during cooking, roasting, frying or cold storage or by turning them into ineffective metabolites. However, the unconscious use of antibiotics for whatever reason causes a danger both economically and in terms of human and animal health (5).

II.1. Legal Regulations About Antibiotic Residues

Prevention of economic losses that may lead to drug residues and protection of consumer health; Unity and harmony are ensured between The World Health Organization, the Food and Agriculture Organization (FAO), the Food and Drug Administration in America between the relevant units of the European Union and the activities and practices carried out by important public institutions such as the Ministry of Health, Ministry of Food, Agriculture and Livestock in our country.

There are a number of regulations and changes and updates on veterinary medicines in the European Union. Basic regulations regulate the use of hormonal, thyrostatic, and β-agonists in animals, creating legal grounds for prohibiting substances used as growth support. A directive on the approved medicines has been recently updated and provided a comprehensive list of permitted medicines and the maximum residue limits (MRL) associated with them.

All of these directives are adapted to Turkish Laws. In the 2002/30 numbered communiqué of the Turkish Food Codex, Maximum Residue Levels in Animal Food Material have been determined. The violation of the residue levels and the legal infrastructure are regulated by Law No. 3285. If a prohibited substance is found in the inspections of the farm, the related matter is confiscated and legal proceedings are carried out on the concerned persons. During periods when legal breakthroughs are not anticipated in live animals sent to the slaughterhouse, the animal is prevented from being slaughtered and the animal is under official supervision during this time. In accordance with Law 5179, the prohibited substance or residue over MRL level are found on the cuts or animal products and fish ,the product is withdrawn from the market and confiscated and money and jail sentences are applied. In addition to these, there are two different legal arrangements in the EU. The first directive sets out the Union and National Reference Laboratories to work on veterinary drugs and the second directive sets the number of mandatory screening tests per animal species, each member country, by regulating drug residue monitoring programs, which are mandatory for member states. Screening tests play an important role in national monitoring programs. In the slaughterhouses of the member countries, screening analyzes are carried out on a large number of urine, bile and stool samples, within the framework of national residue monitoring programs. Veterinary Research Laboratories within the Ministry of Agriculture in Turkey conduct National Reference Laboratories in their area of expertise. As well as Turkey since 2000; the National Residue Monitoring Programs started to be implemented about honey, fish, poultry and eggs, and the results were regularly reported to the EU (4).

Antibiotics are widely used in the treatment of infectious diseases and to promote the growth and productivity of livestock farm animals. B-lactam, tetracyclines, chloramphenicol, macrolides, spectinomycin, lincosamide, sulfomanid, nitrofuran, nitroimidazole, trimethoprim, polymyxin, quinolone and macrocyclic group drugs are the most commonly used medicines for the indicated purposes. However, these medicines are used as unfavorable forms and illegal uses ,and it leads to residues in meat, milk, eggs, honey and other edible tissues of animals (6).

Antibiotic residue may cause allergic reactions in humans as well as antibiotic residues also cause serious conditions such as increased antibiotic resistance in pathogenic bacteria that can lead to dangerous health problems. In addition to these ,residues may lead to lower guality levels of fermented food. Due to all these dangerous and serious problems, the identification of drug residues in foodstuffs is an important issue for consumers (6).

Nowadays, many advanced and quantitative analytical methods are used to detect antibiotic residues in different foodstuffs. ELISA, Charm II, GC, HPLC and LC-MS / MS methods are used. To ensure effective food safety there must be avoidance of the use of antibiotics unconscious in the field and possible antibiotic residues in the foods should be frequently monitored by the responsible legal authority (6).

Due to the lack of effective control over the production, distribution and consumption or use of veterinary medicines in our country, our country is experiencing serious economic losses every year. However, the objectionable environment and food contamination caused by veterinary medicines threaten the health of the people. The maximum residue limits considered suitable for inclusion in many medicines are in line with the EU legislation, has been prepared within the scope of the Turkish Food Codex by the communiqué published in the Official Gazette No. 247739 dated 28.04.2002. Published in Official Gazate dated 19.01.2005 and numbered 25705 National residue monitoring program is implemented on the basis of the Regulation on Measures to be taken to Monitor Certain Substances in Livestock and Animal Products and Their Properties. The number of samples to be searched according to animal species and food grade were prepared in accordance with the EU directive 96/23 / EC .Ankara and İzmir Provincial Control Laboratories and Etlik, Bornova and Pendik Veterinary Control and Research Institutes were authorized in observing residue by the Ministry (7).

A list or group of substances to be sought in residue monitoring programs and residue-seeking foodstuffs are expressed in EU directive 96/23 / EC.Applıcatıons in Turkey Regulation on Measures to be taken for the Monitoring of Certain Substances and their Properties in Live Animals and Animal Products was dated 17.12.2011 and No 28185). According to this regulation, the prohibited substances are listed below:

Group A. Materials that are anabolic and not permitted to be used

1. Stilbenes, stilbene derivatives, salts and esters

2. Antithyroid substances

3. Steroids

4. Resorcic acid lactones, including zeranol

5. Beta-agonists

6. The substances listed in Annex IV to EEC / 2377/90 (Aristolochia species and preparations therefor, dapsone, dimetridazole, furazolidone, chloramphenicol, chloroform, chlorpromazine, colchicine, metronidazole, Nitrofurans, ronidazole).

GROUP B. Veterinary Drugs And Contaminants

1. Antibacterials, including sulphonamides and quinolones

2. Other veterinary drugs

- Anthelmintics
- Antichoxidial substances, including nitroimidazoles
- Carbamates and pyrethroids
- Sedatives
- Non-steroidal painkillers
- Other pharmacologically active substances

3. Other substances and environmental contaminants

- Organic chlorinated materials, including PCBs
- Organic phosphorous materials
- Chemical elements
- Mycotoxins
- Painters
- Others

II. 2. Antibiotics

Antibiotics are substances that can inhibit or kill the growth of microorganisms. They are widely used in the treatment and prevention of infectious diseases. They are used as therapeutics to protect the health and well-being of animals and people. Some antibiotics are caused by microorganisms, but most of them are now artificially produced (8).

Within the framework of the national residue request scheme, antibacterial medicines such as quinoline group and sufanomide group drugs are analyzed in poultry meat products, milk and fish. Chemotherapy is a term used by German researcher Paul Ehrlich in the late 19th century, Chemotherapy is a therapeutics to prevent to growth or kill the bacteria, internal and external parasites, viruses, protozoa, etc. that are present in the body without harming or or little damage to host because of the numerous factors that can lead to disease in the body, such as helminths, protozoa, fungi, bacteria, viruses, insects, etc. For he first time in 1871 It was Pasteur for the antibiotic phenomenon which expresses that the various microorganisms synthesize and release to the culture medium obstructs or kills the development of other disease-causing agents. Term of Antibiotic is defined as, even at extremely low densities, brought into the field by microorganisms such as fungi, or synthetically prepared, are substances which prevent or kill the growth of bacteria. As with other medicines, antibiotics may cause more or less undesirable effects in the patient. Drug allergy, interferes with other drugs, leading to increased severity and frequency of adverse effects, causes disruption of normal mycloflora balance in the patient, leading to the emergence of resistance in antibiotic bacteria, leads to suppression of the immune system in the patient, causes the immune system to be suppressed or degraded in the patient, leads to tissue damage at the site of injection and leads to drug residues in edible tissues and organs (9).

II.2.1. Classification of Antibiotics

Antibiotics can be classified by both the movement mechanism and the chemical structures. They are molecules with different functions in the same molecule. Thus, antibiotics at different pH conditions can be neutral, cationic or anionic. Antibiotics are divided into different subgroups. Fluoroquinolones, tetracylines, aminoglycosides, β-lactams, macrolides and amphenicol are some of these (8).

Beta-Lactam Antibiotics

Beta-lactam antibiotics are a large group of antibiotics with antibacterial activity, chemical structure and pharmacokinetic properties. Common features of this group of members are the presence of a beta-lactam ring in all its structure, the mechanisms of effect and the ways of resistance against them. Antibiotics included in this group; Penicillins, cephalosporins, monobactams, carbapenems and betalactam betalactam inhibitors. All beta-lactams act by blocking peptidoglycan synthesis by blocking transpeptidase activity of penicillin-binding proteins (PBP) responsible for cell wall synthesis in bacteria. As a result, bacteria that can not synthesize the cell wall lysis and die. Beta-lactam antibiotics are bactericidal (10).

Antibiotics play a critical role in the treatment of bacterial infections. In the research on antibiotics and their intensive use, the discovery of penicillin followed the extraordinary advancements (8).

Penicillins

Penicillin is the first most important antibiotics found. Result of studies on the penicillin main molecule whıch has a spectrum of effect narrow, ampicillin, amoxicillin, azlocillin, carbenicillin were found and begin to be used for treatment (11).

The first information on penicillin was given by A. Fleming in 1928. The investigator found that bacteria in the petri dish did not produce bacteria around the penisillium mushroom that was transmitted to the staphylococcal colon and he recorded it as an antibiotic. The mushroom forms a substance that inhibits the reproduction of staphylococci and excrete, and A.Fleming gave it the name of penicillin. Di Vigneaud synthesized penicillin in 1956 and one year later developed methods related to the synthesis of 6-amino penylascanic acid (6 -APA), the main core of the group penicillin, consisting of Batchclor, Doyle, Meyler and Robinson (8). Penicillin is largely derived from Penicillium notatum and Penicillium chrysogenum fungi. More than 40 penicillin derivatives are prepared until today. Some of them were obtained from natural environment, Some of them were biosynthetically formed by incorporating pre-materials into the culture medium, some have been reported to be semisynthetic by attaching different groups to 6-PA (11).

Penicillic acid is formed by cleavage of the β-lactam ring in 6-APA by β-lactases, which requires protection of the integrity of 6-APA for activity against bacterias. It has no effect on bacteria but it combines with proteins in the body to give it an antigenic characteristic which pose a serious problem, It is reported that penicillin acid causes to problems from penicillin allergy. The efficency of natural penicillins is evaluated by Oxford Unit or International Unit (IU) (11).

Once the penicillins are absorbed, they are usually distributed in the body's extracellular fluid. Because of the fact that they are ionized and dissolve well in water, they overtake biological membranes. 65% of the circulating benzylpenicillin is bound to the album, 10% enters the red blood cells. It does not easily break down in the body, and about 90% is removed from the kidneys unchangedly; The concentrations in the milk are 13 to 30% of the dose of plasmadine. Excretion of Ampicillin and cloxacillin is higher with milk (24-30%).This affects the production of dairy products negatively and is important for causing allergies in people sensitive to penicillin (11).

Tetracyclines

The first member of these drugs, chlortetracycline, was obtained from Duggar in 1948 by Streptomyces aureofaciens, and 1 year later by oxytetracycline Finlav from Streptomyces rimosus cultures. In 1952 tetracyclin was prepared semi-synthetically with 1 mole of chlorine away from the chlortetracer. Tetracyclines are amphoteric substances, that is, salts with acids and bases. Tetracyclines very poorly soluble in water at pH 7. It has been reported that oxytetracycline from circulating drugs binds 20-40% of plasma proteins, 45-65% of tetracyclin, and 50-70% of chlortetracycline. This group of antibiotics consists of four hydrocarbon derivatives. The subgroup of polyketides with Octahydrotetracene-2-carboxamide skeleton, Polycyclic naphthacene carboxamide derivatives are known e.g. Oxytetracycline, cholortetracycline, doxycycline and tetracycline (8).

Tetracyclines are reported to be partly biotransformed in the body, and the most abundant metabolites in urine, tissues, and stools are tetracyclines. It has been reported that all tetracyclines are 5-10 times higher in density than plasma in bile (11).

Tetracyclines are largely excreted in the urine and secondarily in the bile. In this way, some of the tetracyclines coming into the intestines were reabsorbed and entero-hepatic circulation was involved, and effective concentrations pass to milk. Compared to the sort of compound , the amount of passing to milk changes but it is present in the milk at 5%. Whereas the level of chlortetracycline is equal to or higher than that of plasma (11).

All tetracyclines pass to the placenta and participate in fetal circulation, so that they also pass to the prostate, joint and eye fluids and milk and eggs to a considerable extent, It has been reported that it may cause developmental disorders and deformations besides coloring in fetus, bones and primar tooth (11)

Tetracyclines cause stomach intestinal irritation at different stages, this can lead to significant nausea, vomiting, pain, anorexia and diarrhea in simple pancreas. It has been reported that these effects of tetracyclines can be prevented by ingestion with nutrients other than dairy products or with antacids containing no calcium, magnesium and aluminum (11). Tetracyclines which has a broad spectrum antibiotic, because of its high antimicrobial efficacy, are also used in the treatment of various diseases and in veterinary cautery. It is used therapeutically in different types of infectious diseases in a large part of bacterial patients such as respiratory system diseases.

Aminoglycosides

Aminoglycoside antibiotics play an important role in the treatment of infections in the clinic, especially in the hospital. Aminosugars are present in the structure of all aminoglycosides except for the spectinomycin. Although the main structure of the spectinomycin is aminocyclitol, this group ıt belongs to this group. These antibiotics are divided into two main groups according to the presence of streptidine or 2-deoxystreptamine. The only compound with clinical significance in the streptidin-containing group is streptomycin. The group containing 2-deoxystreptamine can be divided into two in itself. These include neomycin, paromamycin, lividomycin, and butyrosamine, which bind 2-deoxystreptamine at the 4th and 5th positions, or bleomycin, amikacin, tobramycin, gentamycin, sisomycin, which are bound in the 4th and 6th position (12).

Aminoglycosides are also effective against resistant-peniciline microbes. But according to other antibiotics, toxic effects limit their more frequent use. In this respect, particular attention should be paid to the duration of the treatment. Inadequate penicillin and cephalosporins in the treatment of infections caused by various microbes have led specialists to investigate new antibiotics. Unlike penicillin, which is the result of coincidence, the finding of streptomycin is the result of studies conducted in a way that is purposeful (13).

As with other antibiotics, it has been determined that the use of streptomycin also leads to come out of resistant microbes, and new antibiotics called aminoglycoside have been developed by streptomycin semisynthetic. They have some properties in terms of antibacterial effect and possible side effects. Aminoglycosides are used as treatment for eye ulcers, severe urinary tract infections, as well as endocarditis and bacterial therapy (8).

Fluoroquinolones

Typically, they have a fluorine atom attached to the central ring in 6 positions. Eurofloxacin, ciprofloxacin and norfloxacin are among the fluoroquinolones. Fluoroquinolines are a class of active antibacterials against important synthetic, gram-positive and gram-negative antibacterials. In addition, these bacteria have activity against mycobacteria, mycoplasmas and rickettsia. This group of antibiotics can easily enter the cell, which is why they are frequently used in the treatment of intracellular pathogens. Fluoroquinolone antibiotics, such as ciprofloxacin, are used in the treatment of infectious diseases in humans (8).

Table 1: Maximum Residual Limits of Veterinary Wastes (MRL) (µg / kg) (14).

ANTİBİYOTİK	MRL		ANTİBİYOTİK	MRL
Benzyl penicillin	4		Sulphonamides	100
Ampicllin	4		Trimethoprime	50
Amoxycillin	4		Spiramycin	200
Oxacillin	30		Tylosine	50
Cloxacillin	30		Erythromycine	40
Dicloxacillin	30		Qinalones	75
Tetracycline	100		Polymyxine	50
Oxytetracycline	100		Ceftiofur	100
Chlortetracycline	100		Cefquinome	20
Streptomycin	200		Nitrofurans	0
Dihydrostreptomycine	200		Nitromidazoles	0
Gentamycine	200		Other chemotherapeutics	0
Neomycin	100			

Chloramphenicol

Chloramphenicol, which was first used in 1949, is a cheap antibiotic. It is the first option in the treatment of enteric fever and many other infections, especially in developing countries. It is still one of the alternative therapies in the treatment of highly resistant bacterial infections. It is also an alternative treatment for anthrax and plague treatment.

Chloramphenicol enters the cell energy-dependent. It is protein synthesis inhibitor, bacteriostatic (bacterial growth limiting) antibiotic. Bactericidal effect on meningial pathogens such as *H.influenzae, Streptococcus pneumoniae* and *N.meningitidis*. The spectrum of action is broad, affecting many microorganisms including bacteria, spirochetes, ricketsia, chlamydia and mycoplasmas. It is generally susceptible to chloramphenicol, including *Salmonella* spp, *Salmonella Typhi*. But since 1989, resistance has rapidly increased in Korea, Vietnam. Genes of resistance are plazmidal. The resistance of Ampicillin, chloramphenicol and trimethoprim/

sulfamethoxazole are together. The most important meningitis agents in childhood (*H.influenzae, S.pneumoniae* and *N.meningitidis*) are generally susceptible to chloramphenicol. Clinically, *H.influenzae* resistance is around 0.6%. In Canada, more than 99.2% of isolates are susceptible to chloramphenic regardless of the presence of beta-lactamase. Respiratory isolates of *S. aureus* and *S. pneumoniae* are sensitive to chloramphenicol in 81.6% and 91%. Chloramphenicol is one of the most important antibiotics (including *Bacteriaoides fragili*s) that are effective against anaerobic bacteria (15).

II.3. Antibiotic Use In Food Valuable Animals

Antibiotics are widely used in the treatment of infectious diseases and to promote the growth and productivity of livestock farm animals. B-lactam, tetracyclines, chloramphenicol, macrolides, spectinomycin, lincosamide, sulfonamide, nitrofuran, nitroimidazole, trimethoprim, polymyxine, quinolone and macrocyclic group drugs are the most commonly used medicines for the indicated purposes. However, because of their unfavorable forms and unlawful uses, the residuescomes out in meat, milk, eggs, honey and other edible tissues of animals. Antibiotic residue may cause allergic reactions in humans, It also causes serious conditions such as increased antibiotic resistance in pathogenic bacteria that may lead to dangerous health problems. In addition, residues may lead to lower quality of fermented food. Due to the all these dangerous and serious problems, the identification of drug residues in foodstuffs is an important issue for consumers. Today, many advanced and quantitative analytical methods are used to detect antibiotic residues in different foodstuffs.

The safety of the food we consume is the main theme of consumer health. The maintenance of the highest level of safety in this area is not only vital to public health, It also plays a role in protecting consumers' belief in branding and statutory auditing in the field of food. There is a risk of residual, created in the foods we consume and not only development of resistant bacteria strain but also the risk of transfer to pathogen bacterias ıt Is a worrying situation for consumers. It is also an inevitable fact that veterinary medicines are the result of uncontrolled and unconscious use, contamination of urine, blood, wastewater and other water resources and the soil, and therefore the environment we all live in. Veterinary medicines, especially antibiotics, are the most necessary and effective elements of our country and world intensive animal food production. As a matter of fact, approximately 80% of the animals currently used in food production, some part of their lives or many times are treated with medication.

In the Member States of the European Union in 2004; 4.6 tons of hormones, 194 tons of antiparasitics, 221 tons of metabolism regulators and 5,393 tons of antibiotics and a total of 6.051 tons of veterinary drug active substance are used. According to 2006 data in Turkey, in terms of main drug groups in veterinary medicine ,77% of the total consumption is used for bacterial (33%) and drugs used for fight against parasitic diseases (28%) and (16%) which support the increase of animal yield. When we look at this number, we can see the dimensions of drug use in veterinary medicine field.

Antibiotics, together with other veterinary drugs, were first used as feed additives in the 1950s to prevent and control diseases. It is added to animal feed and drinking water to remove the stress effects of environmental changes, vaccination and other management practices, and to increase growth. Over 40,000 antibiotics have been discovered during this period, and about 80 of them have been used in veterinary-agriculture and fisheries. ıIt is not completely checked that our country obey the legal requirements or not , to have high yield and growth from hormones, drugs and antibiotics. In this context, the techniques used in intensive production often put animal rights and health, and human health, into the second plan. Most frequently used antibiotics in veterinary medicine B-lactam (penicillins and cephalosporins), tetracycline group, chloramphenicol, macrolides, spectinomycin, lincosamide, sulfonamide, nitrofuran, nitroimidazole, trimethoprim, polymyxin, quinolone, and macrocyclic (ansamycin, glycopeptides and aminoglycosides) groups.

Although these effects can not be fully explained by the antibiotics and similar substances used as growth factors in general, some hypotheses about this situation should be mentioned. These drugs used in production,

1) Inhibits the production of toxic metabolites that prevent the absorption of nutrients
2) Prevent the development of pathogenic microorganisms in the gastrointestinal tract

3) It is thought that they are effective in increasing growth and yield in food-worthy animals by reducing or preventing subclinical infections.

Antibiotics accumulate in animals, especially renewable internal organs such as kidney and liver, other organs and muscles, and can also pass on animal products such as milk, eggs and honey. Remaining in foodstuffs occurs when animals are given a high dose of drug, at the same time using feed and water with medication and withdrawal times are not observed. Dismissal of applied animals (method, duration, license status, etc.) as a butchery without waiting for a certain period after the end of drug administration has a negative effect on public health and Public health is, too , negatively affected by consumption of nutrients such as milk, eggs and honey from animals exposed to uncontrolled administration (1).

II.3.1. Antibiotic Use in Poultry

Intensive feeding programs in broiler chick breeding aim at fast put on weight in short time in animals. For this purpose, as well as increasing the nutrient content of the rations, it is possible to add growth factors to the rations. Antibiotics play an important role in the development of feed additives in poultry farming. First, that antibiotics had anabolic effects in the 1940s in the USA is shown when ıt ıs added to certain quantities in chick rations it was observed that the increase in live weight gain. The effects of antibiotics and similar substances used as growth factors are not fully explained.

As a result of excessive and inappropriate use of antibiotics and the development of bacteria resistant to these substances, It has been reported by the European Union that the use of antibiotic-derived growth factors in poultry farming has been prohibited extensively in 1998-1999.

In recent years, researchers have searched for natural and development of accelerators that could be alternative antibiotics. For this purpose, the alternative use of probiotics, organic acids and enzymes has been updated.

Many studies show that the routine use of antibiotics on farms has led to the development of antibiotic-resistant bacteria in plants. At the end of the FDA applications of flouroquinolone group antibiotics, including ciprofloxacin, used in livestock production since 1995, some of the most dramatic evidence has emerged. In

1999, approximately 20% of the chick breast samples contained Campylobacter, ciprofloxacin-resistant, disease-causing bacterium. After long-term use of the field In 2005, the FDA banned drug use. In 2010, Ciprofloxacin resistance decreased to 13.5% in chicken breasts containing approximately 30% C. coli resistant to ciprofloxacin. For this reason, when we feed the animals with antibiotics, the bacteria in and around the animals are exposed and most of them die. But the drug can not always kill some. They live and multiply.

There are some antibiotics resistant bacteria in the farm, but they do not affect pesople. These bacteria have two main pathways. There, antibiotic-resistant bacteria can abandon the farm and infect people.From these roads,the first road is the direct route from the livestock and farm animals, and the other is the indirect route from the livestock.

Most of the bacteria resistant to antibiotics found in the farm are transported to the kitchens through uncooked meat and livestock here. In 2006-2010 The presence of antibiotic-resistant pathogens in consumer reports of chickens in the production of farm animals has emerged. In both years, more than two-thirds of the chicken samples were contaminated with Salmonella and Campylobacter, more than 60% of these bacteria are resistant to one or more antibiotics.

Industries argue that this should not cause concern, because people know that livestock must be completely cooked. Despite these facts, packages may drip into the refrigerator or the cutting boards may become dirty. There is no definite information about to how and how often this condition occurs, especially in cases of difficult illnesses to be treated.

CDC intervene when antibiotic-resistant bacteria occasionally erupt. In 2011, milled turkey caused a death due to Salmonella-resistant bacteria from four different antibiotics (gentamicin, tetracycline, streptomycin and ampicillin) and 136 diseases. Another case in 2011 There were 7 cases of hospitalization and deaths from multiple antibiotic-resistant Salmonella strains found in sulfisoxazole in beef meat milled in the Hannaford food store chain in the North East American states. Antibiotic-resistant bacteria can also spread with environmental transmission from the farm and threaten public health. This can occur in a variety of ways, especially workers or farming rivers. Bacteria resistant to antibiotics originating from the farm are able to exchange genetic material after they leave here and these bacterıa have theır resıstance to make other bacterıoles, whıch have no antıbıotıc contaıners ın dıfferent genes and types. This can happen in lakes, in wild animals, and even in the human digestive system.

Employees engage with resistant bacteria, especially from animals and other places. In a study of poultry farmers on the peninsula of Delmarva, 32 times more gentamycin-resistant Escherichia coli, 5 times more multidrug-resistant E. coli were found to be involved when compared to other community members. While ın another study, methicillin resistant Staphylococus aureus was found in 64% of workers and 70% of pigs in the central region of the United States.It has been observed that these bacteria are not found in another state-of-the-art laboratory that warns that pigs and humans can carry methicillin resistant Staphylococcus strains.In addıtıon to,resistance-bacterias can be carried by fertilizer applıcations for productivity,animals carrıed on trucks,wıngs departures from pig factories,flies sticking to fertilizer.

In many environments, bacteria can easily replace antibiotic-resistant gene codes with neighboring bacteria.*Genes resistance to Antibiotic , especially plasmids, transposons and integrons are often placed on mobile genetic elements that can be carried easily between different or the same type of bacteria that facilitate the spread of resistance by multiple drugs (3, 12).

II.3.2. Causes of Antibiotic Residues in Animal Foods

Antimicrobial substances applied to animal food remain for some reasons in the bodies of animals and pass on animal products. These reasons are listed below;

II.3.2.1. Not paying attention to the time of wiping the body from the residue (drug).

Factors such as the waiting period prior to cutting, the duration of not using eggs and pills, and the duration of non-catching fish affect the duration of the purifiying body's antibiotic remediation (2).

a) Waiting Time Before Cutting

In the foodworthy and organs, animals given drug, should not be cut off until drugs or chemical residues of importance due to undesirable or toxic effects are brought to safe levels for consumers. It also means the time that it takes to stop the treatment in animals and to stop it as a butcher. At the end of this period, it is accepted that chemical residues and antibiotics found in eaten parts of animals are at a level that can not affect human health negatively.

The waiting period pre- slaughter is valid for the animal is approved to be used in the recommended dose, target animal type, administration route and dose range. The waiting period pre-slaughter is determined as the result of experimental studies in healthy animals (Table 2). This time varies from several days to a few weeks depending on various factors such as the type of drug, manufacturer, formulation and type of formulation (Such as water-soluble and dispersible powders, long-acting short-acting injectable solutions), the movement of the drug in the body, the route of administration, the type of animal, accordingly, there may be more than one pre-cutting wait time for an active ingredient.

The same applies to milk and eggs. The pre-slaughter duration for the medicinal product may not match the predetermined duration in healthy animals, due to the movement in the body may change since the drug is used in the patient's animal. When such a situation occurs it must be evaluated by the doctor. If there is no record of the drug, the pre-slaughter waiting period for poultry and mammals is temporarily set at 28 days.

Table 2: Waiting Times of Some Antibiotics Before Slaughter (2).

TYPE OF DRUG	TYPE OF ANIMAL	METHOD	WAITING TIME (DAY) MEAT
Diminazen	Gevişenler	Enjeksiyon	28
Sulfaquinoxaline	Kanatlı	Oral	10
Sulfadimidin	Gevişenler	Enjeksiyon	10
	Gevişenler	Oral	12
	Kanatlı	Oral	14
Amprolyum	Kanatlı	Oral	3
Imidocarb	Gevişenler	Enjeksiyon	28
Lasalosid	Kanatlı	Oral	5
Buparvaquon	Gevişenler	Enjeksiyon	42
Tetrasiklinler	Gevişenler	Enjeksiyon	15
	Gevişenler	Uterus içi	7
	Kanatlı	Oral	7

b) Period of Not Using Eggs and Milk

As long as the use of medicines continues in dairy animals and laying hens, even if the application is interrupted, such foods will not be consumed until the drug residue or metabolite residues are reflected in milk and eggs for a while until the drug residues are found to be unaffected by the consumer's health negatively. For example, organic some medicines pass easily and are discarded for a long time, If there is no relevant record, it is imperative not to use the eggs and milk obtained temporarily for the duration of the milking and for the seven days following the milking for human consumption (9).

c) Period of Not Fishing

The physiology of fish varies according to the temperature of the water. Since the metabolism of nutrients and the pharmacokinetics of drugs are closely related to the water temperature, it is difficult to determine the duration of hunting after the use of medicines. For this reason, many times, waiting period for the drug is determined by moving from a general principle. According to this, a 500-degree day unit is taken into consideration, and this number is divided by the temperature of the water and the waiting time for the substance or the duration of hunting of the fish is determined (6).

II.3.2.2. Pharmaceutical Shape

Drug formulation is extremely important in terms of the danger of causing residues. Tablets, growth factors and intramammary preparations, especially those for injectable medicines, are more important than others. Long-acting-slow release formulations (long-acting forms of drugs such as penicillin, amoxicillin, oxytetracycline) are particularly important in injectable preparations (9).

II.3.2.3. Route of application

The route of administration of the drugs follows the sequence indicated in the pharmaceutical forms. Injection, oral tablets and adding to forage are more important than other routes in terms of inducing intramembrane application residues. Especially when long-acting medicines are used, there is a risk of long-term residues at the site of application, even when the drug is below the level allowed for plasma, tissue, and level in the organs (9).

II.3.2.4. Type of drug

Among the drugs that lead to the residue are mainly antibiotics such as streptomycin, penicillin, oxytetracycline, gentamycin, neomycin, sulphonamides, ivermectins. Penicillin, oxytetracycline is the main reason for causing traces in medicines such as long-acting salts or medicines are used to make. Drugs such as streptomycin, neomycin, and gentamycin are due to the fact that they are associated with special interest in the kidney (9).

II.3.2.5. Non-Tag Drug Use

The use of this condition is due to the use of the unapproved drug(Increasing the dose, changing the route of administration, shortening the dose interval) or formulation in the food animal or the use of the approved drug or formulation in an unsuitable form for the animal. As a general rule, It must not be used in food-worthy or food-producing animals in a unsuıtable manner for any medication package, but in some cases the doctor may have to use non-label drugs. The main reasons for this are the lack of a licensed drug that can be used to prevent the current disease, and the fact that the licensed drug is ineffective at the recommended dose. For this reason, higher doses (non-labeling) may be required to achieve the desired effect (9).

II.3.2.6. Use of Medicines

Since there are no licensed medicines in veterinary medicine, some of the licensed medicines for human medicine are compulsory. Since there is no regulation related to residues in these medicines, when they are used in food-worthy animals, they are in danger of contamination in food. These medicines should not be used unless required; If they are used, the animal must be observed due to the danger of the residue by the physician (9).

II.3.3. The Effect of Antıbıotıc Remains On Formation And Human Health

As with other medicines, antibiotics cause more or less undesirable effects in the patient; The main ones are listed as follows.

Drug allergy, Interaction with other medicines causes the severity and frequency of adverse effects of the end result in impaired normal microflora balance in the patient,leading to tissue damage at the site of injection, leading to drug residues in edible tissues and organs.

Drug allergy: In this situation, also known as type I allergy, almost by all of the antibiotics, especially beta-lactams, can be caused; The event mediated by IgE-type antibodies (found in the mast cell membrane) occurs from a mild skin rash to anaphylactic shock to death. Penicillins and sulphonamides are at the head of drugs that cause the most serious allergic reactions. During the use of antibiotics, some organs and tissues may have severe irreversible organic defects (such as deafness).

Nervous disorders: During the use of aminoglycosides, about 1% of the patients are disordered in hearing and balance organs. Aminoglycosides, linkozamides, polymyxin, colistin, and capreomycin cause impairment of the stimulation of neuronal junctions, penicilins, nalidiksikasit,cycloserine,etionamid,quinolones cause tostruggle; isoniazid, cycloserine, ethambutol cause to inflammation environmental nerve; Colistin, chloramphenicol, streptomycin, sulphonamides, sulphonamide-trimethoprim mixtures and nalidixic acid paresthesia; Isoniazid and ethambutol may cause visual impairment. The frequency of the unwanted effects ranked is 1% or less.

Gastrointestinal tract disorders: Tetracyclines, sulphonamides, chloramphenicol, cephalosporins, ampicillin, isoniazid and quinolones can cause nausea, vomiting and exile by scaring the digestive tract mucosa. In addition, broad-spectrum drugs may cause superinfection. Likewise, antibiotics may interfere with the intestinal flora, resulting in decreased synthesis of the K and B vitamins.

Renal failure: Aminoglycosides, mainly polymyxins, simplexin and amphotericin B, to some extent, sulfonamides may cause damage to the kidneys.

Liver damage: Drugs such as erythromycin estolate, novobiocin, isoniazid, chlortetracycline, triacetyloleandomycine, chloramphenicol may cause liver damage at various stages.

Bone marrow suppression: As a result of long-term use of chloramphenicol, bone marrow suppression can occur at a rate that will eventually decrease in all blood-forming cells.

Endotoxic shock: Especially in severe Gram-negative bacterial diseases, if treatment with high doses of antibiotics is initiated, This event is a reaction caused by endotoxins released from degrading bacteria, followed by narrowing of the arterioles and enlargement of the venules, also known as septic shock. Such events may occur during treatment with chloramphenicol, typhoid, penicillin with syphilis, streptomycin with plague and isoniazid.

Resistance to antibiotics: The resistance status among bacterial populations exposed to antibiotics is among the most popular topics in veterinary medicine and human medicine. Bacterial resistance generally means that the bacteria are not affected by the drug. Clinically, drug resistance is the type of bacteria known to be susceptible to plasmid-mediated doses of treatment at the dose of the drug, meaning that the strains can live and multiply. The influence of such bacteria requires that the drug is sometimes found in the plasma at concentrations that are normally sensitive to several, even dozens of times higher densities (9).

II.3.4. Analysis Methods Used in Monitorıng Drug Residues

There are numerous and different types of drug residues in animal wastes, and many analyzes are required for effective monitoring. For this reason, the use of screening assays is essential (16).

In the identification and characterization of microorganisms, a long-standing antibody-antigen reaction is applied. Immunological methods are preferred when determining food contaminants such as mycotoxins, pesticides or veterinary drugs with low molecular weight. The antigen-antibody reaction is a powerful system for rapid identification of all pathogens. Some systems are automatized at high speed while others are simple to use. These tests can be classified as follows (17).

II.3.4.1. Latex Agglutination Tests

Antibody coated and dyed latex or colloidal gold particles are used for rapid serological identification or identification and typing of pure bacterial culture isolates. Visible agglutination is formed after antigen and antibody conjugation. The reverse latex agglutination tests are for soluble antigens and are often used to search for toxins (17).

II.3.4.2. Automatic and Manual ELISA (Linked Immunosorbent Assay) Methods

The most common application is the use of Enzyme-Linked Immunosorbent Assay (ELISA) systems. This technology is extremely sensitive due to the use of antibodies developed according to the target molecule. Because of its high orderability, the results of the analysis are reliable. It is possible to analyze a large number of samples for different drug residues as soon as they contain easy sample preparation procedures (16). In this method, the antibody is labeled with an enzyme and the immunological reaction is measured as an enzymatic activity result. The most commonly used sandwich ELISA method is the ELISA test, although there are different forms such as direct, indirect and sandwich ELISA. Several ELISA tests have been developed to identify pathogenic microorganisms and toxins Many ELISA kits currently in use have a high standard and automatically increase speed and efficiency for their work and reduce human error (17).

II.3.4.3. Lateral Migration Immunoassay Method

Another development in the field of immunology is the use of Laterel Flow Technology which is based on the antigen-antibody relationship. It's a real quick test. Bacillus anthracis has been developed for the rapid detection and identification of various samples of pathogens such as E. coli 0157, Salmonella, Listeria and Avian influenza (17).

II.3.4.4. Immuno-Magnetic Separation (IMS) Technology

The IMS system saves at least one day from the enrichment and pre-enrichment steps by identifying pathogens from the grains. Recently several diagnostic systems (ELISA) have been combined with the immuno-magnetic coating system. In this case, the incubation period was shortened and the sensitivity increased (17).

II.3.4.5. Fast Scan Kits

Some fast screen kits are available for the detection of antibiotic residues. MeRA test is one of them. Some antimicrobial agent groups, such as beta-lactams and tetracyclines, are sensitive to heat; Molecules belonging to these chemical classes are inactivated shortly at the growth temperature of thermophilic bacteria.The MeRA test involves a rapid pre-incubation step that allows growth and multiplication of Geobacillus stearothermophilus. Following this step, the interaction between the vegetative form of *G. stearothermophilus* and heat-sensitive antibiotics, if present in the sample, is carried out at room temperature. Finally, the test tubes are subjected to a final incubation and color change is observed (18).

II.3.4.6. High Performance Liquid Chromatography (HPLC)

Chromatographic methods can be described as separation techniques involving mass transfer between the stationary phase and the mobile phase. One of these methods, liquid chromatography, Although this method was found at the beginning of the 1900's, it has been the subject of extensive research for the development of methods for residue analysis since 1960's. HPLC is a very sensitive method in which the liquid phase soluble chemical substance mixture can be easily and rapidly separated into its components. Today, HPLC is widely used in many areas. Its primary uses are chemical separation, purification, identification and concentration determination (19).

III. MATERIAL AND METHODS

III.1. Materyal

20 branded eggs sold in markets in Istanbul and 20 different egg samples sold outdoors in various district markets were collected. The eggs were delivered in the laboratory cold chain and stored at 2-8 ° C until the day of operation.

III.2. Method

Firstly, the antibiotic residues in the samples were qualitatively detected with the MeRA Test. Chloramphenicol and Tetracycline levels of all samples were quantitatively determined by elisa method. Chloramphenicol and tetracycline group are the most common antibiotics in food value foods.

III.2.1. Antibiotic Assay with MeRA Test Kit

MeRA is a microbiological test containing Geobacillus stearothermophilus spores for the detection of antimicrobial agent residues in the test. Some antimicrobial agent groups, such as beta-lactams and tetracyclines, are sensitive to heat, Molecules belonging to these chemical classes are inactivated shortly at the growth temperature of thermophilic bacteria. The MeRA test involves a rapid pre-incubation step which allows growth and multiplication of G. stearothermophilus. Following this step, the interaction between the vegetative form of G. stearothermophilus and heat-sensitive antibiotics, if present in the sample, is carried out at room temperature. Finally, the test tubes are subjected to a final incubation. This incubation step of the MeRA assay is a critical step in achieving extremely low detection limits (18).

Preparation of MeRA Test

1) Egg samples (Figure 1) to be tested were broken into different containers and beaten, 2 shargram samples and 6 ml distilled water were transferred to a 10 ml test tube (Figure 2) (egg: water ratio 1: 3).

Figure 1: Preparation of samples

2) The test sample in the tube was homogenized with the vortex for a few seconds.

3) The homogenized sample was centrifuged at 4000 rpm for 15 min

4) One sports disk was added to the food solution.

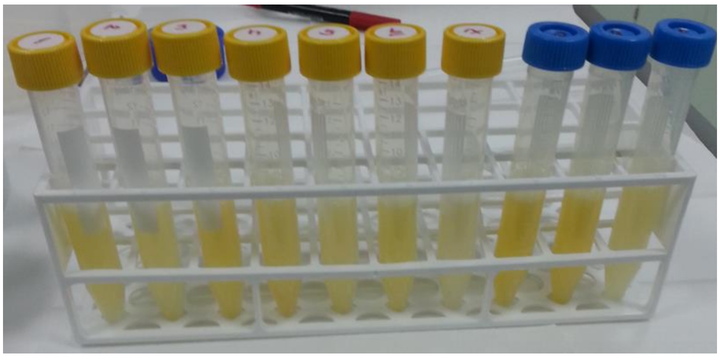


Figure 2: Appearance of prepared egg samples

5) Pre-incubation of the sports diskin was performed with a 20 min solution at 64 ° C

6) After the incubated solution reached room temperature after incubation, 1ml of homogenized supernatant (test sample) was transferred into the incubated solution,The solution was allowed to stand at room temperature for 20 minutes in order to effect the antimicrobial agent (if present) in the test sample.

7) The test specimen was incubated on a water bath or thermoblock at 64 ° C for 3 to 3.5 hours (Figure 3).

Figure 3: Incubation in water bath

8) If there is no color change after observing the color change in the tube (Blue-Green Color): The concentration of antimicrobial agent in question is considered above the detection limits. If there is color change (Yellow Color):In the example, the absence or concentration of the antimicrobial agent is considered below the detection limits (Figure 4) (18).

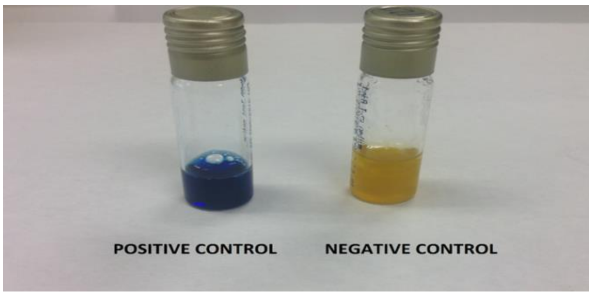


Figure 4: Positive and Negative Control

III.2.2. Elisa Test

Egg samples were also examined for antibiotic residues of tetracycline and chloramphenicol by elisa method.

III.2.2.1. Tetracycline Elisa Test Methodology

Preparation of the samples: Firstly, the egg samples were homogenized in a bowl.

A sample of 4 g of egg was transferred to 50 ml falcon tubes and 20 ml of 50 mM succinic acid was added. Then the balcony tubes were shaken in a 15 min shaker incubator at room temperature. It was then centrifuged for 15 min at 4000 g for 15 min. The supernatant after centrifugation was diluted 1/10 (100 µl supernatant, 900 µl PBS-Phosphate buffered saline). 50 µl of this mixture was used in the experiment.

Preparation of Standards: Standards were diluted to be concentrated. Each standard (50 µl) was diluted with 450 µl of sample buffer 1.

Preparation of Elisa Test: The solutions and plate in the kit were brought to room temperature before the operation and the following steps were followed step by step and the operation was completed (Figure 5).

Figure 5: Elisa test kit

1. The test sample was placed in the plate as much as the sample and standard
2. 2. 50 .mu.l of standard and samples were pipetted into wells.
3. 50 µl of anti-tetracycline antibody was pipetted into each incubator. It was then incubated for 1 hour at room temperature.

4. In the automatic elisa washer, 250 µl wash buffer was washed in each wash 3 times
5. Add 100 µl of conjugate to each buffer with the help of a multi-channel pipette, shake, and incubate at room temperature for 15 min (Figure 6).
6. In an automatic elisa washer, 250 µl wash buffer was washed in each wash 3 times
7. Add 100 µl of sucrose / chromogen to each medium, shake, and incubate at room temperature for 15 min.
8. 100 µl stop solution was added and Elisa was read in reader using a 450 nm filter.
9. A standard curve graph was drawn using the Rida Soft Win program (Figure 7).
10. Absorbance sample / zero values of tetracycline values in ppb were calculated using the standard absorbance x100 formula (20).

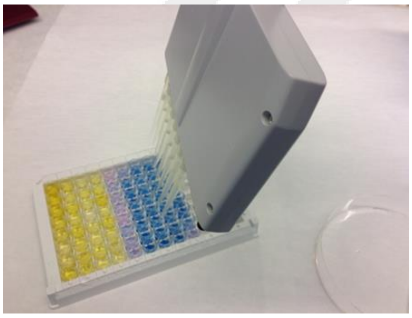
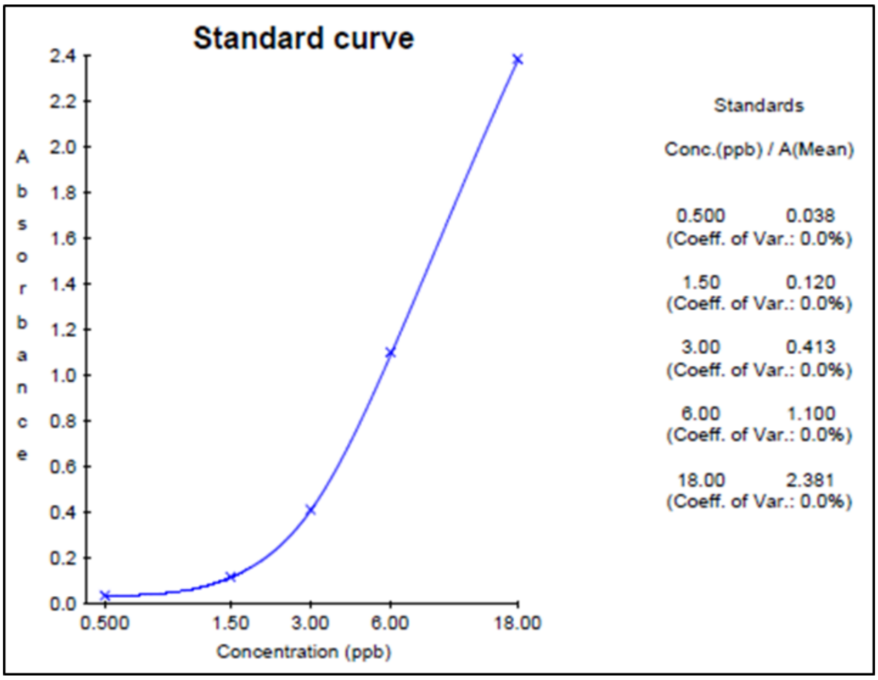


Figure 6: Performing Elisa Test



Graphic 1: Standard Curve Graphic of Tetracycline Elisa Test

III.2.2.1. Chloramphenicol Elisa Test Working Method

1 gr homogenized egg samples were transferred to the test tube. 6 ml of ethyl acetate was added and vortexed. Centrifuged for 1 min at 2000 g. 3 mL of ethyl acetate was discarded. The solvent was then removed with nitrogen gas at 50 ° C. 1 ml of isoactone / chloroform (2/3 ratio) and 0.5 ml of dilution buffer were added. After thoroughly mixing, it was centrifuged at 2000 g for 10 min. After centrifugation, 50 µl of the supernatant was used in the experiment.

Preparation of Elisa Test: The solutions and plates in the kit were brought to room temperature before the operation and the following steps were followed step by step and the work was completed.

The calibrators were used because they were ready for use.

1. 50 µl sample and calibrators were added to each of the probes

2. Add 50 µl of conjugate and shake the plate. A protective film was placed on the plate and incubated for 1 hour at room temperature.

3. The protective film was carefully removed. In an automatic elisa washer, 300 l wash buffer was washed 4 times in each wash.

4. Add 150 µl TMB substrate solution and incubate at room temperature for 30 min.

5. At the final stage, 50 µl stop solution was added and elisa was read in reader using a 450 nm filter.

6. Using the sample / zero standard absorbance x100 formula, the values of columbamic phenol ppb were calculated (21).

III.2.3. Bacteriological culture from egg samples:

Bacteriological culture was performed on all eggs. Species of the genus *Salmonella-Shigella* were screened. *Salmonella-Shigella* Agar (Salubris, Turkey) was used for isolation. *Salmonella* ssp strains were used positively in culture. Sowing was done by single colony sowing method, and the Petri dishes were incubated for 48 hours at 37 ° C. They were recorded as no reproduction ; in non-reproducible egg samples for 48 hours.

IV. RESULTS

IV.1. MeRA Test Results

This test was intended to qualitatively determine the results. This kit is designed to be optimized for meat products. It has been proposed by the manufacturer to use for antibiotic residue identification in egg samples. Forty samples were analyzed and no positive samples were found (Table 4).

Table 4: MeRA Test Results

Unpacked Sample No	Results	Packed Sample No	Results
1	Negatif	1	Negatif
2	Negatif	2	Negatif
3	Negatif	3	Negatif
4	Negatif	4	Negatif
5	Negatif	5	Negatif
6	Negatif	6	Negatif
7	Negatif	7	Negatif
8	Negatif	8	Negatif
9	Negatif	9	Negatif
10	Negatif	10	Negatif
11	Negatif	11	Negatif
12	Negatif	12	Negatif
13	Negatif	13	Negatif
14	Negatif	14	Negatif
15	Negatif	15	Negatif
16	Negatif	16	Negatif
17	Negatif	17	Negatif
18	Negatif	18	Negatif
19	Negatif	19	Negatif
20	Negatif	20	Negatif

IV.2. Elisa Test Results

As a result of elisa study with tetracycline (r-bioPharm), positivity was found in 4 of the egg samples sold out in the open, and no positive sample was found in packed egg samples (Table 5). As a result of the chloramphenicol ELISA test, no positive samples were found in any of the 40 samples (Table 6).

Table 5: Tetracycline Elisa Test Results

Tetracycline Elisa Test Results					
Unpacked Sample No	**Elisa Count (ppb)**	**Results**	**Packed Sample No**	**Elisa Count (ppb)**	**Results**
1	4,4	Negative	1	1	Negative
2	65,9	Negative	2	3,4	Negative
3	4,9	Negative	3	0,3	Negative
4	0,4	Negative	4	0,6	Negative
5	60,4	Negative	5	0,7	Negative
6	12,7	Negative	6	0,1	Negative
7	13,8	Negative	7	0,9	Negative
8	41,4	Negative	8	1,8	Negative
9	40,6	Negative	9	1,1	Negative
10	10,2	Negative	10	2,3	Negative
11	4,5	Negative	11	2,8	Negative
12	120	Positive	12	0,4	Negative
13	6,9	Negative	13	0,3	Negative
14	1,9	Negative	14	0,01	Negative
15	2,8	Negative	15	0,4	Negative
16	70,8	Negative	16	0,7	Negative
17	9,9	Negative	17	1,7	Negative
18	120	Positive	18	1,7	Negative
19	140	Positive	19	2,6	Negative
20	170	Positive	20	0,4	Negative

Table 6: Chloramphenicol Elisa Test Results

Chloramphenicol Elisa Test Results					
Unpacked Sample No	**Elisa Count (ppb)**	**Results**	**Packed Sample No**	**Elisa Count (ppb)**	**Results**
1	< 0,2	Negative	1	< 0,2	Negative
2	< 0,2	Negative	2	< 0,2	Negative
3	< 0,2	Negative	3	< 0,2	Negative
4	< 0,2	Negative	4	< 0,2	Negative
5	< 0,2	Negative	5	< 0,2	Negative
6	0,3	Negative	6	< 0,2	Negative
7	< 0,2	Negative	7	< 0,2	Negative
8	< 0,2	Negative	8	< 0,2	Negative
9	0,35	Negative	9	< 0,2	Negative
10	< 0,2	Negative	10	< 0,2	Negative
11	< 0,2	Negative	11	< 0,2	Negative
12	3,1		12	< 0,2	Negative
13	< 0,2	Negative	13	< 0,2	Negative
14	0,4	Negative	14	< 0,2	Negative
15	< 0,2	Negative	15	< 0,2	Negative
16	< 0,2	Negative	16	< 0,2	Negative
17	< 0,2	Negative	17	< 0,2	Negative
18	3,5		18	< 0,2	Negative
19	3,7		19	< 0,2	Negative
20	0,9		20	< 0,2	Negative

IV.3. Cultural Results: After 48 hours of incubation, none of the 40 specimens of *Salmonella* or *Shigella* species were observed.

V. DISCUSSION AND CONCLUSION

In modern livestock applications, the use of veterinary medicines is of utmost importance for the efficient and safe production of meat, fish, milk, eggs and honey. The use of these drugs is subject to strict licensing and approval procedures with a system similar to that of medicinal products (22-25).

Medicines and other chemical substances are used in animals to treat, prevent, control and accelerate disease progression Nutrient-bearing tissues and organs and unchanged metabolites that accumulate or are stored in products(Meat, milk, eggs, honey) obtained therefrom, degradation products, residues of free or bound substances (26-27).

The world is estimated to be between 100,000 and 200,000 tons of antibacterial drug market.Over the past 50 years, 1 million tons of antibacterial substance has been released into the biosphere, of which about 50% is veterinary and agricultural (12, 29).

Antibiotics began to be used in animal feeds in the early 1950s, and a new era in breeding began. . However, most of the antibiotics used as prophylactic or non-, have had to complete the life span due to the rapid increase in resistant strains occurring between pathogenic bacterial species in humans and animals (12,30,31)

Antibiotics are known to be more important in terms of residues than other pharmacologically active substances. The reasons for this are the overdose of the drugs prescribed for the animals and in particular the delivery of the drug to the animals without the compliance with the legal waiting period of the drug.

As a result, antibiotics can be found in antibiotics on the grounds that antibiotics do not completely disappear in the body or are not completely removed from the body. The tetracycline group (Tetracycline, oxytetracycline and chlortetracycline) is widely used in poultry farming due to the broad spectrum of antibiotics and low toxic effects.For this reason, the presence of tetracycline residues in chicken meat is important (32-35). However, antibiotic residues are found in the egg (36).

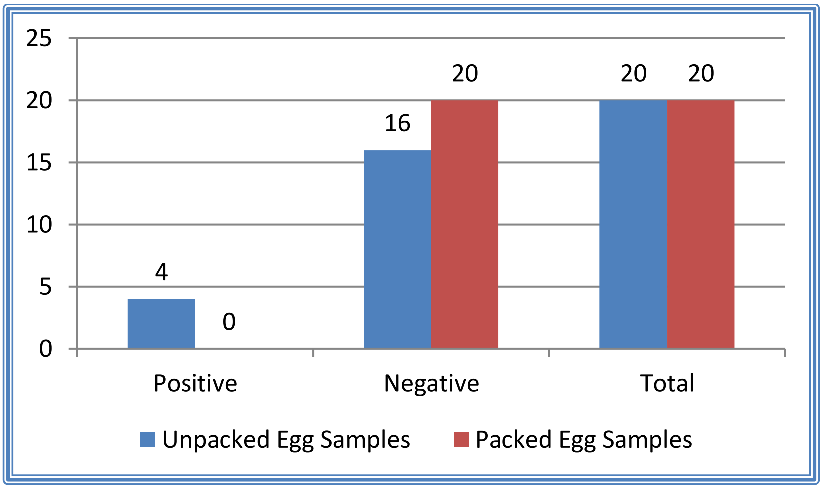
The only problem is not that antibiotics give harm human health. Compared to the risk of antibiotic resistance, the risk of toxicity caused by the remedies caused by drugs permitted for use in foodstuffs is very low. The resistance rates against antibiotics are increasing day by day.Nevertheless, the EU has set the Maximum Residue Limits (MRLs) of veterinary drugs in animal feedstuffs to prevent people from exposure to harmful levels of residues

The maximum amount of tetracycline available in the egg according to Turkish Food Codex is 100 (ppb) μg / kg. The eggs on this value should not be consumed for the reason that it is harmful for human health (4).

Studies on chicken meat in our country have occasionally found high levels of tetracycline antibiotic residues (37-39). In many countries as in our country, tetracycline levels are found above the limits in chicken meat (40-43). In our study, antibiotic residues were determined qualitatively with the MeRA test kit. This kit detects antibiotics such as Beta-lactams and tetracyclines in meat and meat products

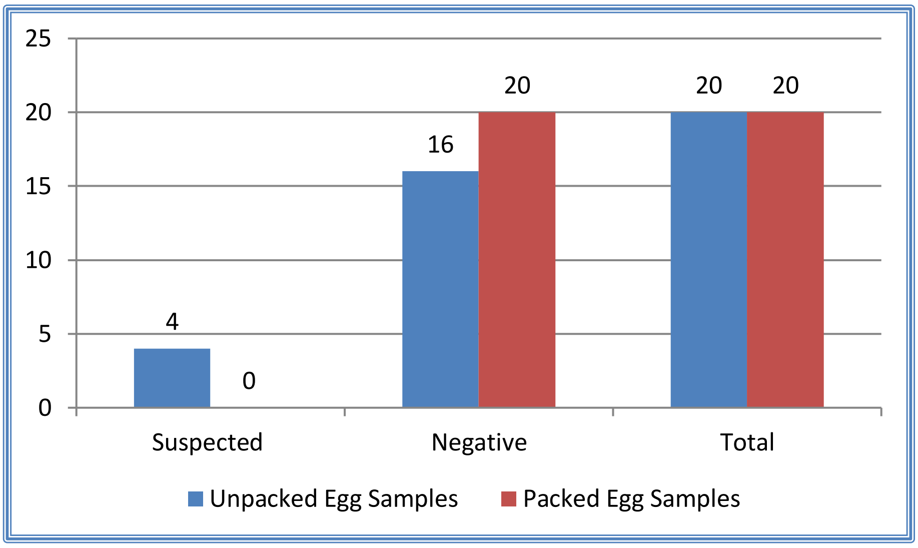
In our study with a total of 40 egg samples, no positive result was found with the MeRA test..This kit, which is highly successful in meat and meat products, is not suitable for egg samples, so more samples are required (4).

In our Tetracycline residue studies we have done with Elisa, forty specimens were found above the limits of four (120-170 ppb).). These four specimens are not packaged, but are eggs sold outdoors. As it is understood from this, the egg poses a risk for tetracycline. In other open egg samples it was determined as 0,4-70,8 ppb and it is in the limits of mucinous residue (Graph 2). For packed egg samples it was determined between 0,01-3,4 ppb.



Graph 2. Distribution of tetracycline elisa test results

There are not enough studies about the level of tetracyclin in the egg in our country. A study in India looked at levels of tetracyclines in egg samples taken on different days from the same chicken, Antibiotic residues were found above the limits in eggs, especially until the 5th day of administration of the antibiotic to the chicken. . On days 9 and 14, low levels of tetracycline were detected (44). No chloramphenicol remnants were found in any of the 40 specimens in our study. Chloramphenicol is one of the prohibited antibiotics. In our study, none of the packaged egg samples were found to have chloramphenicol in any of the four samples sold in the open. However, these values are very low and in these cases there are tetracycline residues above the limits in these examples so It is necessary to be approached with suspicion by the reason of cross reaction in ELISA method and to confirm by HPLC.



Graph 3. Distribution of chloramphenicol elisa test results

There are not many studies in our country since chloramphenicol is a banned antibiotic. In a study conducted in Konya in 1990, the presence of chloramphenicol residue was investigated in 50 egg samples by HPLC method and it was reported that no positive specimen was found (45).

In 1995, in a study conducted in Istanbul, chloramphenicol was detected in 6 of 324 samples (46).

In a study conducted in Ankara in 2011, 50 different groups of chickens were given florfenicol, a group of chloramphenicol at different doses and The extent to which the residues are present in the egg has been analyzed with different methods. Investigating the effects of preserving eggs in different ambient temperatures on residuals in eggs. There was a significant decrease in the levels of florfenicol and florfenicol amine residues in the eggs incubated both at room temperature (15-20 ° C) and refrigerated (+4 ° C) .but It was observed that there were still residues until 28th day. In the same way, the amount of florfenicol and florfenicol amine residues in the egg was significantly reduced by frying and boiling (1 and 5 min boiling) applied to the egg.But it was determined that these transactions did not completely remove the remains. . Thus, florfenicol and florfenicol amine appeared to be quite sensitive to heat.

As a result, it is recommended that florfenicol should not be used in laying hens or when it has to be used, it is recommended that the period of complete elimination of the drugs from the egg be complied with and that the egg containing florfenicol residue should not be presented to human consumption during this period (47) (at least 7 days when a single oral, intramuscular or subcutaneous route is used, 8 days when used 3 days or 5 days or at least 9 days when used orally). Bacteriological cultivation was done to detect the presence of *Salmonella* and *Shigella*. In such studies it has been observed that microorganisms do not penetrate into the egg through the pores of the crust. However, in the cracked ones, it is reported that the crust enters microorganism into the egg and causes decomposition (48-50). *Salmonella* and *Shigella* bacteria were not found in our bacteriological cultures.

According to the regulation that Turkish Food Code was published, the use of chloramphenicol in food was banned. For this reason, the absence of chloramphenicol residue in our study has been a welcome result.ın addıtıon to The presence of tetracycline residues in very few cases is also a pleasing condition, yet it reveals the unconscious use of antibiotics. This is harmful not only because of the accumulation of foreign substances in the body in terms of human health but also because microorganisms also cause resistance to antibiotics .With more training and supervision, such problems can be avoided and such studies must be continued frequently.

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