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STUDY OF ANTIBIOTIC REMOVALS IN VARIOUS HONEY SAMPLES WITH DIFFERENT METHODS

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Bu tez Yeditepe Üniversitesi Lisansüstü Eğitim-Öğretim ve Sınav Yönetmeliğinin ilgili maddeleri uyarınca yukarıdaki jüri tarafından uygun görülmüş ve Enstitü Yönetim Kurulu'nun 10.08.2017 tarih ve 2017/1508 sayılı kararı ile onaylanmıştır.

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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.

23.05.2017

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LIST OF SYMBOLS AND ABBREVIATIONS

CDC: Centers for Disease Control and Prevention

FDA: Food and Drug Administration

HMF (Hidroksimetilfurfuralı)

HPLC: High Performance Liquid Chromatography

l: litre

µ: Mikro

Min: Minute

OIE: World Animal Health Organization

PBS: Phosphate-buffered saline

ppb: per part billion

WHO: World Health Organisation

ÖZET

Serim, E. (2017). Çeşitli Bal Numunelerinde Antibiyotik Kalıntılarının Farklı Yöntemlerle Araştırılması. Yeditepe Üniversitesi, Beslenme ve Diyetetik Anabilim Dalı, Yüksek Lisans Tezi, İstanbul.

Günümüzde hayvancılık uygulamalarında, veteriner ilaçlarının kullanımı, verimli ve güvenli et, balık, süt, yumurta ve bal üretimi için son derece önemlidir. Bu ilaçların kullanımı, tıbbi ilaçlardakine benzer bir sistemle, sıkı bir lisanslama ve onay prosedürlerine tabidir Hayvanlarda hastalıkların önlenmesi ve gelişimlerinin hızlanması için ilaç ve diğer kimyasal maddeler kullanılmaktadır. Ancak gıda değeri olan et, süt, yumurta, bal gibi besin maddelerinde izin verilen limitlerin üzerinde bulunması durumunda kalıntı olarak kabul edilmektedir.

Çalışmamızda 20 adet paketli, 20 adet te açıkta satılan bal numuneleri toplanmıştır. Açık örnekler Türkiye'nin arıcılık yapılan çeşitli illerinden (Erzurum, Siirt, Malatya, Kars, Gümüşhane, Çanakkale, Artvin, Erzincan, Giresun, Trabzon, Rize) toplanmıştır. Paketli örnekler ise piyasadan elde edilmiştir. Alınan numuneler soğuk zincirde steril şartlarda laboratuara ulaştırılmıştır ve çalışma gününe kadar 2-8°C'de saklanmıştır. Öncelikle MeRA Test ile örneklerdeki antibiyotik kalıntıları kalitatif olarak saptandı. Daha sonra tüm örneklerin elisa yöntemi ile Tetrasiklin düzeyleri kantitatif olarak belirlenmiştir. Bu test ile sonuçlar kalitatif olarak saptanılması hedeflenmiştir. Bu kit et ürünlerine optimize edilerek tasarlanmıştır. Üretici firma tarafından bal örneklerindeki antibiyotik kalıntı tayini için kullanımını önerilmiştir. Çalışılan 40 adet numunenin analizi yapılmış ve açıkta satılan örneklerin 19 tanesinde; paketli olarak satılan örneklerin ise 16 tanesinde pozitiflik saptanmıştır. Elisa ile kıyaslandığında Tetrasiklin kalıntısı için MeRA testin ucuz, hızlı ve pratik bir test olduğu saptanmıştır.

Sonuç olarak ülkemizde bal üretiminde halen kontrolsüz şekilde antibiyotik kullanımının devam ettiği görülmektedir. Kaliteli bal üretimi için daha dikkatli davranılmalıdır.

Anahtar Kelimeler: Bal, Tetrasiklin, Antibiyotik Kalıntı, Elisa.

ABSTRACT

Serim, E. (2017). Study of Antibiotic Removals in Various Honey Samples With Different Methods. Yeditepe University, Institute of Health Science, Department of Nutrition and Dietetics, MSc thesis, İstanbul.

In today's livestock applications, the use of veterinary medicines is very important 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. Medicines and other chemical substances are used for the prevention of diseases and acceleration of development in animals. However, it is considered to be a residue if it exceeds the limits of food, such as meat, milk, eggs, honey.

In our work, we collected 20 pieces of packaged, 20 pieces of honey sold in unpacked. Unpacked samples were collected from various cities beekeeping of Turkey (Erzurum, Siirt, Malatya, Kars, Gümüşhane, Çanakkale, Artvin, Erzincan, Giresun, Trabzon, Rize) . Packaged samples were obtained from the market. Bought samples were delivered to the laboratory in sterile conditions in the cold chain and stored at 2-8 ° C until the working day. Firstly, the antibiotic residues in the samples were qualitatively detected with the MeRA Test. Tetracyclin levels of all samples were then quantitatively determined by elisa method. This test is intended to qualitatively determine the results. This kit is designed to be optimized for meat products. 40 samples were analyzed and the positivity was found 19 samples sold in open and in 16 samples of packaged samples. Compared with Elisa, the MeRA test was found to be a cheap, fast and practical test for Tetracycline residues.

As a result, it is seen that the use of antibiotics is still uncontrolled in honey production in our country. Be more careful for quality honey production.

Key words: Honey, Tetracycline, Antibiotic Residue, Elisa.

I.INTRODUCTION AND PURPOSE

Honey is an important source of nutrient for the living. Today, honey production is growing in line with increasing consumption potential. Beekeeping is a breeding type that can be carried out in harmony with various branches of agriculture and is not adhered to the soil. Turkey has very suitable conditions for beekeeping in terms of its climatic characteristics. Among the bee products, the most known is honey with 99. 4%. This is followed by pollen (61. 6%) , arısütü (52. 8%) and wax (46. 4%) respectively. On the other hand, bee stover (16. 3%) and propolis (8. 9%) are less common (1).

The plant nectar is harvested by honey bees (Apis mellifera) from the secretions of live parts of plants or the secretions of plant - absorbing insects that live on live parts of plan, then combines its own ingredients to change it, reduces the water content and matures it by storing it in the honeycomb.

Nothing is added to honey including food additive substances. Honey, except for bakery, alien flavour and odor not belonging to honey, fermentation has started, acidity has been artificially altered or it can not be heated to break down or significantly inactivate the natural enzymes it contains. Subject to the provisions regarding filtered honey, pollens or other honey-specific components can not be removed except for losses that are unavoidable during the separation of foreign organic or inorganic substances (2).

After the streptomycin, an antibiotic against bee diseases, was detected in the honey, the risk of contamination comes from the agenda of honeyand then it has since become a commercial product requiring complicated analyzes. Therefore, nowadays, the use of drugs in beekeeping is a critical issue whether consumers, or even food control authorities, are increasingly focusing on it.

Beekeeping in our country is a widely-established and growing sector as it is all over the world. Commercial products such as honey, pollen, bee milk and propolis produced as an important result of beekeeping activities can be found both in domestic market and foreign market, provide significant contributions to the country's economy. The honey production in our country is approximately 50.000 tons (± 10,000 tons). This figure varies according to climatic conditions. The quality, aroma and flavour of the honeys produced in our country

are not found in other countries in the world. The annual total honey production, is very limited even in the higher countries such as China and Argentina. The place of Turkish honeys in the world market is showing some developments but negativeness, too. At the beginning of these adversities, the problem of veterinary medicine residues, which constitutes a major obstacle to exports. It is known that, due to the prevention of various diseases seen in honey bees, various drugs used lead to residual problem in honey samples. This situation emerges as a very important problem in our country especially in honey export to Europe and America (3).

In the results obtained in studies conducted in our country, it has been found that some beekeepers have been using drugs, especially those used in the poultry sector, for both tetracycline and sulfadimidine. This extremely misapplication has led to an increase in antibiotic residues in the tetracycline group in our country honeys(3). For this reason this work is planned. In our study, it was aimed to investigate the residues of tetracycline in 40 honey samples.

II. LITERATURE REVIEW

According to national and international laws and regulations; The honey must not be added to the outside or one of the components forming the honey should not be removed. Falsification by the addition of commercial glucose and starch reduces both the nutritional value of honey and can give harm the health of consumers. Honey samples produced in Turkey were examined by Kahraman and et all. No commercial glucose and starch were reported in any of the analyzed honeys. Aydin and ark. in their study, they identified commercial glucose at 10 of the 20 honey samples analyzed. No objectionable properties, flavour or aroma should occur that may affect honey quality during processing, heating and storage of honey. Fermentation or gas formation should not have begun. During processing, it is necessary to apply heat treatment in order to protect the honey from frying and fermentation, to facilitate extraction and filtration and to increase the viscosity of the honey. The duration and temperature of the heat treatment must be checked. Heat treatment causes the loss of enzymes in the honey and increases the amount of HMF (hydroxymethylfurfuryl), thus causing the honey to lose its freshness. The other reason for the loss of quality is that the other is kept for a long time under inappropriate conditions. Honey must be kept in corrosion-resistant cups due to its acid character, as it is hygroscopic and can not attract odors from the environment. The harmful effects of various microorganisms which are mixed in soil and plants are prevented by the antibacterial features of honey. However, *Clostridium botulinum* spores are dangerous for infants up to one year of age. Bacterial spores can survive in honey but do not form toxins. Because the intestinal flora of infants is sensitive to the colonization of C. botulinum, the bacteria taken with the ball may form a toxin in the bowel. In this case infant botulism occur by symptoms such as constipation, uncontrollable head, weakness and reflex loss. The improvement takes long, but the symptoms are getting out of the way after the treatment. Although the mentioned cases are extremely rare, it is suggested that young children of one year should not be given honey (1).

II.1. The Content of Honey

Honey bees gather honey by collecting sweet liquids called nectar which are found in flowers or created by insects on plants. Nectar is treated with various enzymes that convert nectar into honey in the honey mide, an altered part of the digestive system. Then the water is blown and convert into honey. According to the nectar obtained honey, color, flavor, odor and chemical composition shows variety. According to honey origin there are two group honeys; the first group is flower honeys (obtained from plant nectars) and the second group is secretions honey (obtained from the secretions of living parts of plants or the secretions of plant-absorbing insects living on live parts of plants) . Turkey is one of the most important secretion honey producers in the world. This honey is made by honey bees from the insect secretion named Marchalina hellenica, which feeds on pine trees in Turkey (1). The substances in content of honey are indicated in Tables 1 and 2 (12).

Table II.1. Content of Honey (g / 100g) (12).

	Blossom honey		Honeydew honey	
	average	min. - max.	average	min. - max.
Water	17.2	15-20	16.3	15-20
Monosaccharides				
fructose	38.2	30-45	31.8	28-40
glucose	31.3	24-40	26.1	19-32
Disaccharides				
sucrose	0.7	0.1-4.8	0.5	0.1-4.7
others	5.0	2-8	4.0	1-6
Trisaccharides				
melezitose	<0.1		4.0	0.3-22.0
erlose	0.8	0.5-6	1.0	0.1-6
others	0.5	0.5-1	3.0	0.1-6
Undetermined oligosaccharides	3.1		10.1	
Total sugars	**79.7**		**80.5**	
Minerals	0.2	0.1-0.5	0.9	0.6-2.0
Amino acids, proteins	0.3	0.2-0.4	0.6	0.4-0.7
Acids	0.5	0.2-0.8	1.1	0.8-1.5
pH-value	3.9	3.5-4.5	5.2	4.5-6.5

Tablo II.2. Substances in Trace Amount in honey (12).

Element	mg/100 g	Element	mg/100 g
Aluminium (Al)	0.01-2.4	Lead (Pb)*	0.001-0.03
Arsenic (As)	0.014-0.026	Lithium (Li)	0.225-1.56
Barium (Ba)	0.01-0.08	Molybdenum (Mo)	0-0.004
Boron (B)	0.05-0.3	Nickel (Ni)	0-0.051
Bromine (Br)	0.4-1.3	Rubidium (Rb)	0.040-3.5
Cadmium (Cd)*	0-0.001	Silicon (Si)	0.05-24
Chlorine (Cl)	0.4-56	Strontium (Sr)	0.04-0.35
Cobalt (Co)	0.1-0.35	Sulfur (S)	0.7-26
Floride (F)	0.4-1.34	Vanadium (V)	0-0.013
Iodide (I)	10-100	Zirconium	0.05-0.08

Dry matter is composed of carbohydrates about 95% of the honey, mainly fructose and glucose. Oligosaccharides are 5-10 % of the total carbohydrates, in total about 25 different di- and trisaccharides. The Glycemic Index (GI) of honey varies from 32 to 85, depending on the botanical source and botanical source is lower than sucrose (60 to 110). Fructose-rich honeys such as acacia honey have a low GI (Tablo 3). Also, there are small amounts of proteins, enzymes, amino acids, minerals, trace elements, vitamins, aroma compounds and polyphenols in the honey. Honey is shown to possess antimicrobial, antiviral, antiparasitory, antiinflammatory, antioxidant, antimutagenic and antitumor effects. Honey is an excellent source of energy for athletes because of its high carbohydrate content and functional properties. High doses of honey such as 50 to 80 g per intake can cause health promoting properties (12).

Tablo II.3. Glycemic index (GI) and glycemic load (GL) for a serving (25 g) of

honey (12).

	honey origin	Fructose g/100 g	GI	AC g/serving	GL (per serving)
Acacia (black locust)*	Romania	43	32	21	7
Yellow box	Australia	46	35±4	18	6
Stringy bark	Australia	52	44±4	21	9
Red gum	Australia	35	46±3	18	8
Iron bark	Australia	34	48±3	15	7
Yapunya	Australia	42	52±5	17	9
Pure Australia	Australia		58±6	21	12
Commercial blend	Australia	38	62±3	18	11
Salvation June	Australia	32	64±5	15	10
Commercial blend	Australia	28	72±6	13	9
Honey of unspecified origin	Canada		87±8	21	18
average		55	55±5	18	10
Sucrose (mean of 10 studies)			68±5		
Glucose			100		

The carbohydrates are the main constituents, comprising about 95% of the honey dry weight. Organic acids, proteins, amino acids, minerals, polyphenols, vitamins and aroma compounds are the other compounds.

Carbohydrates: Monosaccharides fructose and glucose are the main sugars. Additionally, about 25 different oligosacharides have been detected. The principal oligosaccharides in blossom honey are the disaccharides sucrose, maltose, trehalose and turanose, also, panose, 1-kestose, 6-kestose and palatinose are the nutritionally relevant ones. If ıt is compared, blossom honey honeydew honey contains higher amounts of the oligosaccharides melezitose and raffinose. In the process of digestion after honey intake the principal carbohydrates fructose and glucose are transported into the blood in a quick way and can be used for energy requirements by the body.

Proteins, enzymes and amino acids. There are approximately 0. 5% proteins, mainly enzymes and free amino acids in honey. The contribution of that fraction to human protein intake is marginal. The three main honey enzymes are diastase (amylase), decomposing starch or glycogen into smaller sugar units, invertase (sucrase, α-glucosidase), decomposing sucrose into fructose and glucose, also, glucose oxidase, producing hydrogen peroxide and gluconic acid from glucose.

Vitamins, minerals and trace compounds. The amount of vitamins and minerals is

small and the recommended daily honey intake (RDI) of the different trace substances is marginal. It is known that different unifloral honeys contain different amounts of minerals and trace elements.

Aroma compounds, taste-building compounds and polyphenols. There is a wide range of honeys varying to tastes and colours, depending on their botanical origin. The main taste-building compounds are the sugars. Generally, honey with a high fructose content (e.g. acacia) are sweeter then those with high glucose concentration (e.g. rape). The honey aroma depends also on the quantity and type of acids and amino acids present. Extensive research on aroma compounds has been carried out in the past decades and more than 500 different volatile compounds were found in different types of honey. Indeed, most aroma building compounds differs in the different types of honey depending on its botanical origin.

Contaminants and toxic compounds. Honey can be contaminated like any other natural food by the environment, e.g. by heavy metals, pesticides, antibiotics etc. Generally, the contamination levels found in Europe is not a health hazard. The contamination by antibiotics are the main problem in recent years, used against the bee brood diseases, but nowadays this problem seems to be under control. In the European Union antibiotics are not allowed for that purpose, and thus honey which contains antibiotics is also not permitted to be traded on the market. A few plants used by bees are known to produce nectar containing toxic substances. Two main toxin groups relevant in nectar are Diterpenoids and Pyrrazolidine alkaloids.

II.2. General Features of Honey

II.2.1. Physical Features

Physical features are basic criterias for simple measurements and information on honey composition and for honey classification. Honey colouring, rheological and hygroscopic properties, taste and aroma, optical rotation properties, electrical conductivity, specific gravity and refractive index are among the physical features that can be measured.

Electrical conductivity from physical properties, honey varies according to the content of mineral matter and is used to distinguish between flower honeys and secretion honeys.

In monofloral (unifloral) honey, the proportion of the original nectar from which honey is obtained is at least 51%, or the pollen ratio of single plant species is over 45% (6). Honey can be named using the name of the plant from which it is obtained (eg acacia, chestnut, manuka, etc.). Monofluoral honeys have their own unique and distinctive flavours attached to the original source of nectar. Pollen of single plant spice in polyfluoronal honey is not dominant. As with the Anzer Honey, the naming is usually done according to the geographical region in which it is obtained.

Honey color is evaluated according to the Pfund scale. According to Turkish Food Codex Honey Communiqué, hony can vary from water white to dark amber colour. The colours of nectar and pollen, which are the source of honey, the sugar reactions in honey, and the being old or new of honey effect the colour. Honey color comes from compounds such as polyphenols, flavonoids, terpenes and carotenoids, which can absorb visible light in the visible range. In addition, Maillard reaction products such as melanoidin contribute to the whitening of the honey. The Maillard reaction or nonenzymatic browning occurs between amino groups of amino acids and reducing sugars, and is a common side effect of honeypowder. During the preservation, the browning of honey is a factor that negatively affects consumer choice. Long-term storage, especially at high temperatures, causes the honey to be darker colour.

Viscosity, according to the content of honey; depends on (the disaccharides give more viscosity), the amount of small crystals and air bubbles it contains and the moisture content. Honey viscosity is taken into account when designing and using equipment used in honey processing. It is known that high heat treatment applications reduce the viscosity. However, it has been reported that viscosity is increased when the high heat treatment applied honeys are brought back to room temperature and waited.

The most important physical quality criterion is that honey is crystallized. Crystallization is a natural process and there is no difference in nutritional value between crystallized honey and liquid honey. Crystallization occurs as the glucose in the honey is separated from the water and the glucose molecules collide with other particles in the honey to form small crystals. Natural crystallization is undesirerable situation by consumers as it causes deterioration in honey textures. In addition, the weak liquid phase separated from sugar can start fermentation. Industrial crystallization applications are carried out using Dyce

method to prevent these problems. In this method, about 10% to 20% of well crystallized honey is mixed into the liquid honey to start the crystallization process and left at 14 ° C. When made in a crystallization controlled manner, creamy honey in buttery consistency and smooth texture is obtained. Particles and air bubbles remaining in the honey during filtration are factors that increase the crystallization. Glucose / water ratio and fructose / glucose ratio affect crystallization. Honeys with Glucose / water with a value of 1.70 or less are not crystallized, whereas honeys with a value of 2.10 are crystallized very quickly. While fructose / glucose is between 1. 0 and 1. 2, crystallization is rapid, while when this ratio is 1.3 or more crystallization is delayed. It has been reported that high-temperature short-time treatments delay the crystallization that may occur in the honey, but this depends on the glucose, fructose and water ratios of honey. Tosi and ark stated that a honey sample with a fructose / glucose ratio of 1. 03 and a glucose / humidity ratio of 1. 89 crystallized at 8 ° C at 4 ° C without heat treatment, were crystallized at 16 weeks when they were kept at 80 ° C for 60 seconds and kept at this temperature for 30 seconds and then stored at 4 ° C. Heat treatment is applied to liquefy the crystallized honey. The Turkish Food Codex has stated that heated honeys above 45 ° C can be used as baking products. But in the world honeys are heated in various forms and grades:

a) Heating in a water bath

b) Heating with air

c) Heating with electric beds

d) Pasteurization: Heating at 70-78 ° C and cooling immediately

e) Use of ultrasonic waves

F) Heating in microwave ovens

g) Heating in infrared ovens (1).

II.2.2. Chemical Features:

In general, about 80% of honey comes from different sugars (35% glucose, 40% fructose, 5% sucrose) and 17% water. The remaining part 3% consists of up to 180 different substances such as enzymes, amino acids, gluconic acid, phenol compounds, lactones, minerals and various vitamins. There are also valuable minerals such as iron, copper, potassium, calcium, magnesium, phosphorus, silicon, aluminum, chromium, nickel and

cobalt in honey. The distinctive features of honey are determined by many small components originating from nectar and honey. Most of these components that provide honey's special flavour and biological features are resistant to heat.

The content of water in honey is the most important criterion for evaluating honey's age and shelf life. The obtaining of the filtered honey from unsealed honeycombs, climatic conditions during harvest and adverse preservation conditions increase the water content (57,79). According to the TGK Honey Communiqué, Codex Alimentarius Commission (Codex Alimentarius Comission-CAC) and Directive 2001/110 / EC of the European Union, the water content of honey should be less than 20%.

The sweetness of honey is felt to be less than that of foods containing sugar in similar amounts, due to the substances in the acid character it contains. The free acidity is additive to flavour, provides resistance against microorganisms, increase chemical reactions, antibacterial and antioxidant features and gives some information about the source of honey. Honey acidity is caused by various organic acids, especially gluconic acid, and inorganic ions such as phosphate and chloride. The increase in free acids is considered to be a sign of fermentation. Because honey sugars and alcohols are converted into acids by the yeast in honey.

Although the honey protein source is not identified as a nutrient, the amino acids in the honey are important for the origin of honey. Examples of amino acids include proline, lysine, phenylalanine, gamma-amino butyric acid (GABA), beta-alanine, arginine, glutamine, serine, glutamic acid and aspartic acid. The amino acid proline, the most abundant in bald, is the precursor of hydroxyproline in the structure of collagen and elastin. Prolin is an amino acid present in plants in various amounts (222 mg / kg in acacia, 956 mg / kg in thyme) so the amount of prolin is used as a criterion for separating honey from nectar obtained from sugar beet-fed bees and honey. The amount of proline recommended by the TGK for honey is a minimum of 180 mg per kg of honey.

Enzyme content is a for honey quality criteria. The invertase enzyme sucrose glucose and fructose, responsible for the conversion of nectar to honey, the diastase enzyme converts the starch to small sugars, B-glucosidase, glycogen glucose and maltosylase (8, 79); Glucose oxidase enzyme, glucose gluconic acid and hydrogen peroxidase, and catalase hydrogen peroxide convert to oxygen and water.

Diastase number and HMF level are chemical quality criteria used for about 75 years to determine honey quality. The most practical way to distinguish fresh honeys from honeys that have been over-heat treated or stored for a long time is to measure diastase and HMF activities. HMF (hydroxymethylfurfural) is a product, as a result of heat treatment applied to food during cooking or sterilization, a non-enzymatic browning reaction (Maillard reaction) of reducing sugars with amino acids, or formed as a result of dehydration in the acid catalysis of hexoses. In many foodstuffs containing carbohydrates, the amount is limited by regulations to prevent excessive heat application. Due to the presence of simple sugars (glucose and fructose) in the high concentration in the contents and many acids, honey provides very favorable conditions for HMF formation. The degree and duration of heat treatment, the storage conditions (such as exposure to light), and the use of metal containers in the enclosure can lead to an increase in the amount of HMF in the honey. It is suggested that initial heat application accelerates the formation of acidity and triggers an increase in the amount of HMF throughout the storage period.

II.2.3. Biological Features of Honey

Antimicrobial, antiviral and antiparasitic activity: Honey has been used by many cultures for medical purposes since ancient times. It is understood from the records of Dioscorides that it has been used for at least 2000 years in the treatment of infectious wounds. Today there is a growing interest in using honey for therapeutic purposes. For therapeutic purposes; ulcers, skin infections as a result wound and burn injuries and bed sores. There are studies that report honey has not only inhibitory features against bacterias but also viruses, fungi and parasites. Concentration of 10% of the honey granulosus was found to have a lethal effect from the third minute onwards against Ecinococcus. Rubella virus has been reported to be inactivated by honey in vitro conditions. The first laboratory and clinical studies on honey antibacterial activity were carried out on manuka bred from plants of *Leptospermum scoparium* ve *Leptospermum erico*ides grown in Australia and New Zealand. Manuka honey has been reported to exhibit antibacterial activity against approximately 60 different bacterial species including aerob, anaerob, gram negative and positives. Honey; high osmolarity due to sugar concentration, low water activity, low pH, hydrogen peroxide production are effective on antibacterial activity. The most important

antibacterial compound in honey is hydrogen peroxide, which is formed as a result of the of the glucose oxidase enzyme in the hypopharyngeal glands of bees oxidize the glucose of honey.

It has been shown that the catalase enzyme resulting from the pollen of some plants continues to have an antibacterial effect on the ball in which hydrogen peroxide is inactivated. Polyphenols, phenolic acids (caffeic acid, ferulic acid) and their derivatives (methyl syringate), non-dissociative organic acids such as aromatic acids and flavonoids, gluconic acid, and recently, antibacterial activity of honey in Maillard reaction products are effective on antibacteril effect of honey. Lysosomes and volatile compounds are also thought to play a role in bacterial inhibition. These compounds are called non-peroxide compounds. The antibacterial activity of manuka honey is due to non-peroxide compounds. The antibacterial activity in dark colored ball was found to be higher than the light colored honeys, this result is related to the antibacterial activity of phenolic compounds found more in dark honeys. Bacteria and fungi in which honey has antibacterial and antifungal effects have been reported in various studies. Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Enterobacter cloaca, were found to be antibacterial bacteria and among the fungi, Candida xerosis, Candida albicans, Candida tropicalis and Rhodotorula rubra were found to be present. Moreover, it has been reported that the related effects of karakovan honey are higher than comb honey and filtering honey.

The Anti-Inflammatory Effect of Honey: In the treatment of infected wounds, besides antibacterial features of honey, stimulation of lymphocytic and phagocytic activity by honey is also effective. It has been reported that a variety of honeys stimulate the secretion of macrophages from the cytokine TNF-α inducing wound healing, reported that the amount of thromboxane B2 decreased by 35% from the inflammatory substances in the plasma 3 hours after ingesting 70 g of honey. It is stated that while honey reduces inflammation and swelling in wounds, it also increases granulation and epithelization.

Antimutagenic Activities of Honey: It has been reported that oral taken honey activates the immunity system and protects against cancer and metastasis. The mutation of Trp-p-1 (3-Amino-1,4-dimethyl-5H-pyridol [4.3-b] indole) which is a heterocyclic amine in vitro conditions was inhibited by all of the compounds obtained from seven different sources.

In rats and mouses, honey (1 g / kg for rats, 2 g / kg for rats) given orally for 10 days before inoculation of experimentally generated tumors showed significant antimetastatic effect.

Antioxidant Feature of Honey: The antioxidant feature of honey depends on seasonal and environmental factors to the vegetable source where nectar is collected. Substances that give honey antioxidant features, Polyphenols such as flavonoids (apigenin, pinobanksin, pinosembrin, kaempferol, galangin, luteolin, hesperetin etc.) and phenolic acids (caffeic, ferulic, ellagic, chlorogenic acid etc.), Riboflavin, -tocopherol, ascorbic acid, salicylic acid, sulfhydryl groups, carotenoid derivatives, glucose oxidase, catalase, peroxidase, organic acids (gluconic, citric, malic acid), Maillard reaction products, amino acids and proteins. It has been reported that the antioxidant activity of artificial honey is much lower than that of natural honey. Honey prevents unwanted oxidation events in foods such as enzymatic browning of fruits and vegetables or lipid oxidation of the meat. Flavonoids, terpenes, isoprene units and long chain phenolic acids form chromophores that absorb visible photons. The total phenolic compounds, which increase the antioxidant activity of honey, there are researches that show higher amounts of dark-colored honeys such as buckwheat honeys, pine-like secretion honeys, or chestnut honey. It was reported that more flavonoids were detected in the flower honey than secretion honey.

It has been reported that honey has high antioxidant activity connected with vitamin and enzyme content in raw and fresh honey. These materials are very sensitive to heat treatment, light and adverse storage conditions. In Doğan and Kolankaya studies, they found that the increase in vascular permability that rats produce by using ethanol in mids can be prevented by the Anzer Honey. Researchers say that the result may be caused by high ascorbic acid content that Anzer honey. The Anzer Honey meets the reported vitamin C requirement of 60-100 mg per day for a 70 kg person with ascorbate content of 62.67 mg / g (1).

Prebiotic Effec of Honey. It is emphasized that honey must be consumed 50-80 g daily to be able to exhibit positive effects (17). Honey has 25 different oligosaccharides such as panose, melezitose and raffinose as well as basic monosaccharides such as glucose and fructose from energy-providing carbohydrates (17). These oligosaccharides show a similar effect to fructooligosaccharides (FOS) and glucooligosaccharides (GOS) and increase the development of intestinal bifidobacteria and have a prebiotic effect. It has been reported that

bifidobacteria used in the production of honey fermented dairy products have improved the development and helped to maintain bacterial counts up to 21 days in the storage at 4 ° C. In another study, it was reported that in honey, cestose and nystose fructooligosaccharides were present in honey and stimulated the development of Bifidobacterium longum. It is emphasized that honey has stop effect diarrhea, which helps to regulate the intestinal flora. It is reported that besides the studies that honey has high carbohydrate content causing tooth decay, this effect is lower than the sucrose known as tea sugar. It has been proven in various studies that microorganisms that cause tooth decay are inhibited by the antibacterial effect of honey. Grobler and ark. they have studied the erosion that honey and fruit juice brings on the tooth mine by electron microscopy. The erosion was observed in the toothpaste after10 minutes from consumption of fruit juice but the erosion was reported to be very weak even after 30 minutes from consumption of honey. Researchers say that calcium, phosphorus, fluoride and other colloidal compounds in honey help to protect the toothpaste from erosion.

Fermentation in Honey. It is natural to have fungi and yeasts originating from nectar and pollen in honey. The water activity of honey was measured between 0.593 and 0.637 and this value inhibits the development of almost all microorganisms. However, when the moisture content of honey exceeds 20%, osmophilic yeasts and fungi are present at a level of $2x10^3$ -3x104 cfu / g. Osmophilic yeasts, fructose, and glucose can bring carbon dioxide, ethanol, and volatile or non-volatile acids to form acetic acid with oxygen. It is called fermentation, which is characterized by a sour taste with high levels of yeast, glycerol, butanediol and ethanol. Ozmophilic yeasts and fungi were found to be completely inhibited in the honey held at this temperature for 30 seconds at 80 ° C for 60 seconds. In order to inhibit osmophilic yeasts in the world, pasteurization applications such as 7.5 minutes at 63 ° C and 1 minute at 69 ° C are being applied.

II.3. Effects of Honey on Health.

Oral health: There is much debate about whether honey is harmful to teeth. Some reports state a cariogenic effect of honey or a much less cariogenic effect than sucrose. Honey ingestion inhibits the growth of bacteria, causing caries and might induce a carioprotective effect because of its antibacterial activity. It was shown that Manuka honey is a a very potent

antimicrobial honey. It has a positive effect against dental plaque development and gingivitis and also can be used instead of refined sugar in the manufacture of candy. Electron microscope studies show that the ingestion of honey causes no erosion of tooth enamel as observed after drinking fruit juice. Tooth erosion was observed, ten minutes after consumption of fruit juice, while 30 minutes after honey 12 ingestion the erosion was very weak This effect can be explained by the calcium, phosphorous and fluoride levels of honey and other colloidal honey componentsalso have a role. Summarising the different findings, we can conclude that honey is probably not as cariogenic as other sugars and in some cases it can be carioprotective. It is advised that cleaning the teeth after consumption of honey is safe for health of teeth.

Gastroenterology. Honey has a big potential of the causing agent of peptic ulcers and gastritis, *Helicobacter pylori*. In experiments on rats honey acted against gastric ulcers induced by indomethacin and alcohol. Ther is not prostaglandin production in honey, but it has a stimulatory effect on the sensory nerves in the stomach that respond to capsaicin. For the second mechanism of action, postulating that this effect is due to the antioxidant properties of honey. Honey intake in rats prevented indomethacin-induced gastric lesions in rats by reducing the ulcer index, microvascular permeability, and myeloperoxidase activity of the stomach. Also, honey was found to maintain the level of non-protein sulfhydryl compounds (e.g. glutathione) in gastric tissue lead to diseases like ulceration. Ingestion of dandelion honey decrease gastric juice acidity by 56%. The gastric emptying of saccharides after ingestion of honey was slower than that after ingestion glucose and fructose mixture. Other effects of honey on human digestion have been related to oligosaccharides. These honey constituents have prebiotic effects, similar to that of fructo-oligosaccharides. The most active oligosaccharide was the oligosaccharide panose. The oligosaccharides lead to an increase of bifidobacteria and lactobacilli and also they exert the prebiotic effect in a synergistic mode of action. According to an invitro study on five bifidobacteria strains honey has a growth 13 promoting effect like fructose and glucose oligosaccharides. Unifloral honeys of sour-wood, alfalfa and sage origin encouraged the growth of five human intestinal bifidobacteria. In a different important study honey increased both in vivo (small and big intestines of rats) and in vitro the building of *Lactobacillus acidophilus* and Lactobacillus

plantarum, but sucrose had no effect. In clinical studies with infants and children honey shortens the duration of bacterial diarrhoea and did not extend the duration of non-bacterial diarrhoea. In some cases, consumption of relatively large amounts of honey (50 to 100 g) can cause to a mild laxative effect in individuals with insufficient absorption of honey fructose.

II.4. National-International Regulations on Honey

The properties that should be carried in the stages of production, preparation, processing, storage, transport and supply to the market in our country in accordance with the honeycomb technique and hygienic must be determined so that The Turkish Food Codex Honey Communiqué (COMMUNICATION NO: 2012/58, No: 28366) has been published by the Ministry of Food, Agriculture and Livestock on 27.07.2012. This Communiqué covers honey produced by Apis mellifera, a honey bee. In addition, this Communiqué has been prepared on the basis of the Turkish Food Codex Regulation published in the Official Gazette dated 29/12/2011 and numbered 28157 3. In this communiqué, information about the content and quality of honey, as well as the rules that must be complied with for all processes from manufacture to sale are given.

Also for substances that should not be present in its content according to the provisions Turkish Food Codex Classification of Pharmacological Active Ingredients to be found in Animal Husbandry in the Regulation on Maximum Residual Limits published in the Official Gazette dated April 5, 2012 and numbered 28282, it has been stated that it must be mobilized. To prevent economic losses that may cause to drug residues and protection of consumer health some organizations carried out important activities. 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. While Basic regulations regulate the use of hormonal, thyrostatic, and β-agonists in animals, creating legal grounds for prohibiting substances used for growth support. An instruction on the approved medicines has been recently updated and provided a list of

permitted medicines and the maximum residue limits (MRL) related to them. All of these instructions 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. Law No. 3285. regulated The violation of the residue levels and the legal infrastructure. When prohibited substance is found in the inspections of the farm, the related matter is confiscated. Also legal proceedings are carried out on the concerned persons. When legal breakthroughs are not anticipated in live animals sent to the slaughterhouse, the animal is prevented from being slaughtered, furthermore the animal is under official supervision during this time. In accordance with Law 5179, If the prohibited substance or residue over MRL level are found on the cuts or animal products and fish, there wıll be some sentences such as product is withdrawning the product from the market confiscating and money and jail sentences. . Furthermore, there are two different legal arrangements in the EU. . The first instuction sets out the Union and National Reference Laboratories to work on veterinary drugs and the second instuction 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 has 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. . Besides, Turkey since 2000; the National Residue Monitoring Programs started to be implemented about honey, fish, poultry and eggs, and the results were properly reported to the EU (4).

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

Because of 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, veterinary medicines cause the objectionable environment and food contamination, futhermore this 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 applied 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 also 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.5. Antibiotics Types

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

Within the framework of the national residue request scheme, there are some antibacterial medicines such as quinoline group and sufanomide group drugs are analyzed in poultry meat products, milk and fish. German researcher Paul Ehrlich used Chemotherapy term in the late 19th century. Chemotherapy is a therapeutics to prevent to growth or kill the bacteria, internal and external parasites, viruses, protozoa. These are present in the body without harming or or little damage to host due to numerous factors that can lead to disease in the body, such as helminths, protozoa, fungi, bacteria, viruses, insects, etc. For the first time in 1871, Pasteur used for the antibiotic phenomenon which expresses that the various microorganisms synthesize and deliver to the culture medium obstructs or kills the development of other disease-causing agents. We can define the term of Antibiotic 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 lead to more or less displeasing effects in the patient. Drug

allergy, interferes with other drugs, cause to increased severity and frequency of adverse effects, lead to disruption of normal mycloflora balance in the patient, leading to the emergence of resistance in antibiotic bacteria, cause to suppression of the immune system in the patient, causes the immune system to be suppressed or reduce 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 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. Some of these are Fluoroquinolones, tetracylines, aminoglycosides, β-lactams, macrolides and amphenicol (8).

Beta-Lactam Antibiotics

Beta-lactam antibiotics are a large group of antibiotics with antibacterial activity, chemical structure and pharmacokinetic features. Presence of a beta-lactam ring in all its structure, the mechanisms of effect and the ways of resistance against them are the common features of this group of members. 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. Consequently, bacteria that can not synthesize the cell wall lysis and die. Beta-lactam antibiotics are bactericidal (10).

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

Penicillins

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

A. Fleming gave the first information on penicillin 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. A. Fleming recorded this bacteria as antibiotic. The mushroom forms a substance that inhibits the reproduction of staphylococci and secrete, 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). Batchclor, Doyle, Meyler and Robinson consist of the main core of the group penicillin (8). Penicillin is largely derived from Penicillium notatum and Penicillium chrysogenum fungi. More than 40 penicillin derivatives are prepared until today. While Some of them were obtained from natural environment, some of them were biosynthetically formed by incorporating pre-materials into the culture medium and some have been reported to be semisynthetic by attaching different groups to 6-PA (11).

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

When the penicillins are absorbed, they are usually distributed in the body's extracellular fluid. Due to 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 without changing. 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 also it 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 in a semi-synthetical way with 1 mole of chlorine away from the chlortetracer. Tetracyclines are amphoteric substances, that is, salts with acids and bases. Tetracyclines are very dissoluble 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 tetracyclines are the most abundant metabolites in urine, tissues, and stools. It has been reported that all tetracyclines are 5-10 times higher in density than plasma in bile (11). 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. Thus, some of the tetracyclines coming into the intestines were reabsorbed after then entero-hepatic circulation was involved and effective concentrations pass to milk. Compared to the sort of compound, the amount of passing to milk can change but it is present in the milk at 5%. However 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. They also pass to the prostate, joint and eye fluids and milk and eggs to a significant extent. It has been reported that it may lead to developmental disorders and deformations also coloring in fetus, bones and primar tooth (11).

Tetracyclines lead to stomach intestinal irritation at different stages also 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 including no calcium, magnesium and aluminum (11).). Tetracyclines which has a broad spectrum antibiotic, used in the treatment of various diseases and in veterinary cautery due to its high antimicrobial efficacy. It is used therapeutically in various types of infectious diseases in a large part of bacterial patients such as respiratory system diseases.

Aminoglycosides

Aminoglycoside antibiotics play an very important role in the treatment of infections in the clinic. Aminosugars are present in the structure of all aminoglycosides but for the spectinomycin. These antibiotics are divided into two main groups according to the presence of streptidine or 2-deoxystreptamine. Streptomycin is the only compound with clinical significance in the streptidin-containing group. The group containing 2-deoxystreptamine can be divided into two in itself. These are 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. However 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. Penicillin, was found in the result of coincidence, but 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 cause 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 some diseases such as 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. Furthermore, 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 commonly used in the treatment of infectious diseases in humans (8).

Tablo II.4: Maximum Residual Limits of Veterinary Wastes (MRL) (µg / kg) (14).

Antibiotic Name	MRL		Antibiotic Name	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 was first used in 1949 and is a cheap antibiotic. It is the first option in the treatment of enteric fever and many other infections, particularly in developing countries. It is still one of the alternative therapies in the treatment of highly resistant bacterial infections and 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 extensive, affecting many microorganisms including bacteria, spirochetes, ricketsia, chlamydia and mycoplasmas. It is generally sensitive 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 sensitive to chloramphenicol. Clinically, *H.influenzae* resistance is around 0. 6%. In Canada, more than 99. 2% of isolates are sensitive 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) and also effective against anaerobic bacteria (15).

II.3. Antibiotic Use In Food Valuable Animals

Antibiotics are commonly 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 these purposes. However, due to their unfavorable forms and unlawful uses, the residues comes out in meat, milk, eggs, honey and other edible tissues

of animals. Antibiotic residue may lead to allergic reactions in humans, it also causes serious conditions such as increased antibiotic resistance in pathogenic bacteria and may lead to dangerous health problems. Besides, residues may lead to lower quality of fermented food. Because of the all these dangerous and serious problems, the identification of drug residues in foodstuffs is very important issue for consumers. Today, many advanced and quantitative analytical methods are used to detect antibiotic residues in various 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, but also plays a role in protecting consumers' belief in branding and statutory auditing in the field of food. There is always a risk of residual, created in the foods we consume. This is not only development of resistant bacteria strain but also the risk of transfer to pathogen bacterias. It is a worrying situation for consumers. It is also an unavoidable 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. Actullay, approximately 80% of the animals currently used in food production, some part of their lives or many times are treated with medication.

It is reported that 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 extend 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 so as to remove the stress effects of environmental changes, vaccination and other management practices, and to increase growth. Over 40,000 antibiotics have been discovered, and about 80 of them have been used in veterinary-agriculture and fisheries. It 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 intense production often put animal rights and health, and human health, into the second plan. B-lactam (penicillins and cephalosporins), tetracycline group, chloramphenicol, macrolides, spectinomycin, lincosamide, sulfonamide, nitrofuran, nitroimidazole, trimethoprim, polymyxin, quinolone, and macrocyclic (ansamycin, glycopeptides and aminoglycosides) groups are most frequently used antibiotics in veterinary medicine.

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

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 system.
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 also pass on animal products such as milk, eggs and honey. When animals are given a high dose of drug, remaining in foodstuffs occurs, and also 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. Public health is also negatively affected by consumption of nutrients such as milk, eggs and honey from animals exposed to uncontrolled administration (1).

II.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 and so the use of screening assays is essential (16).

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

II.4.2. Automatic and Manual ELISA (Linked Immunosorbent Assay) Methods

The most common practice is the use of Enzyme-Linked Immunosorbent Assay (ELISA) systems. This technology is extremely sensitive because of the use of antibodies developed according to the target molecule. Due to its high orderability, the results of the analysis are reliable. It is possible to analyze numerous samples for different drug residues when they contain easy sample preparation procedures (16). With 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, though there are different forms. These are direct, indirect and sandwich ELISA. Several ELISA tests have been developed to define pathogenic microorganisms and toxins. Many ELISA kits nowadays in use have a high standard and automatically increase speed and efficiency for their work and reduce human error (17).

II.4.3. Lateral Migration Immunoassay Method

The other 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 different samples of pathogens such as E. coli 0157, Salmonella, Listeria and Avian influenza (17).

II.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. Nowadays several diagnostic systems (ELISA) have been combined with the immuno-magnetic coating system. Thus, the incubation period was shortened and the sensitivity increased (17).

II.3.4.5. Rapid 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 susceptible 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. After 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. In the end, the test tubes are subjected to a final incubation and color changes(18).

II.4.6. High Performance Liquid Chromatography (HPLC)

Chromatographic methods can be described as separation techniques. This technique involves mass transfer between the stationary phase and the mobile phase. Liquid chromatography is one of these methods. This method was found at the beginning of the 1900's, neverthless it has been the subject of extensive research for the development of methods for residue analysis since 1960's. HPLC is a 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 different areas. Chemical separation, purification, identification and concentration determination are its primary uses (19).

III. MATERIAL AND METHODS

III.1. Materyal

For our work we collected 20 pieces of packaged, 20 pieces of honey samples sold in the open. Open samples were collected from various beekeeping cities of Turkey (Erzurum, Siirt, Malatya, Kars, Gümüşhane, Çanakkale, Artvin, Erzincan, Giresun, Trabzon, Rize). Packaged samples were obtained from the market. Received samples were delivered to the laboratory in sterile conditions on the chill 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. Tetracyclin levels of all samples were then quantitatively determined by elisa method. One of the most common antibiotics in food-value foods is the tetracycline group antibiotics.

III.2.1. Antibiotic Assay with MeRA Test Kit

MeRA is a microbiological test containing Geobacillus stearothermophilus spores for the detection of antimicrobial substance 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 includes a rapid pre-incubation step that allows growth and multiplication of G. stearothermophilus. Following this step, the interaction between the vegetative form of G. stearothermophilus and the 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).

Preperation of MeRA Test

1) 2 grams of honey samples to be tested were taken and 6 ml distilled water was transferred to a 10 ml test tube (honey: water ratio 1: 3) (Figure 1).

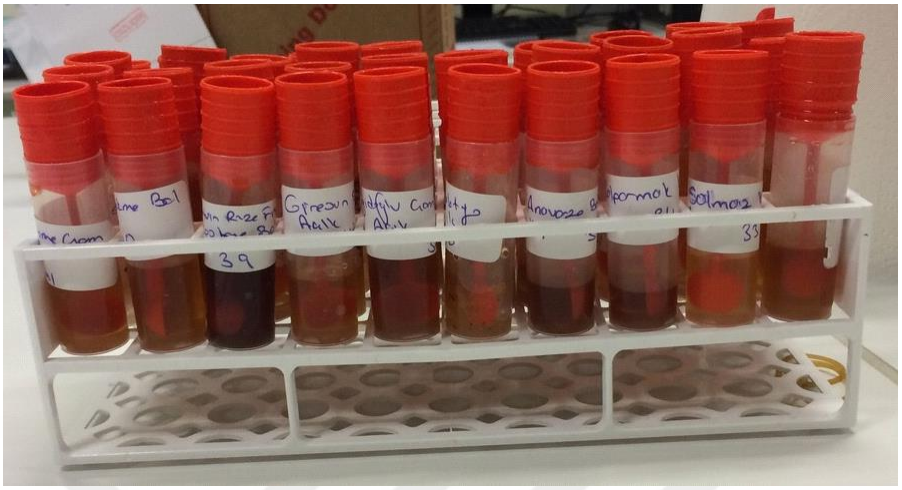

Figure III.1: Apperance of Honey Samples

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 medium solution.

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

6) After incubating the incubated solution at 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 to effect the antimicrobial agent (if present) in the test sample.

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

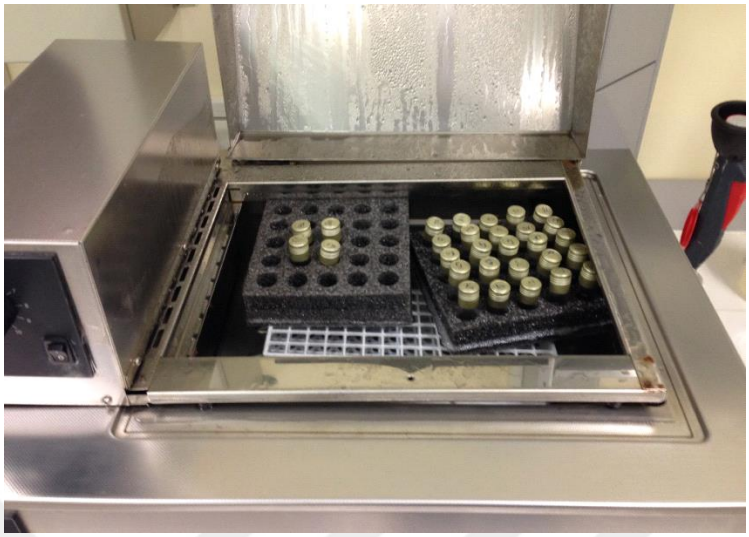

Figure III.2: Incubation of Honey Samples in Water Bath

8) If there is no discoloration after observing the color change in the tube (Blue-Green Color): The concentration of antimicrobial agent in the sample is accepted above the detection limits. If there is color change (Yellow Color): No antimicrobial agent is detected in the sample or its concentration is below the detection limits (Picture 3) (18).

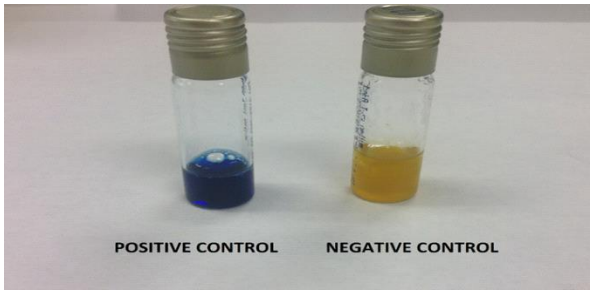

Figure III.3.: Pozitif Control ve Negatif Control

III.2.2. Elisa Test:

Antimicrobial residue levels of tetracycline were measured by ELISA method at the same time.

III.2.2.1. Tetracycline Elisa Test Method

Preparation of the samples: 4 g of honey samples were transferred to 50 ml falcon tubes and

20 ml of 50 mM succinic acid was added. Then the falcon tubes were shaken in a 15 min shaker incubator at room temperature. It was then centrifuged 15 min at 4000 g for 15 min. After centrifugation the 1/10 of supernatant was diluted (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 because of they are concentrated. Each standard (50 µl) was diluted with 450 µl sample buffer 1. The standards are prepared on the working day as it should be fresh.

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 to complete the operation (Figure 5).

Figure III.4: Kit of Elisa Test

1. Test wells were placed in the plate as many as the sample and standard.
2. Pipeted into 50 µl wells with standard and sample respectively.
3. 50 µl of anti-tetracycline antibody was pipetted into each well. It was then incubated for 1 hour at room temperature.
4. In an automatic elisa washer, 250 µl wash buffer was washed 3 times in each wash.
5. 100 µl of conjugate were added to each buffer with the help of a multi-channel pipette, shaked, and incubated at room temperature for 15 min (Figure 6).
6. In an automatic elisa washer, 250 µl wash buffer was washed 3 times in each wash.
7. Susbstrat / chromogen was added 100 µl of to each medium, shaked, and incubated at room temperature for 15 min.
8. 100 µl stop solution was added and read by Elisa reader by using a 450 nm filter.
9. A standard curve graph was drawn using the Rida Soft Win program (Figure 7).

Absorbance sample / zero values of tetracycline values in ppb were calculated using the standard absorbancex100 formula (20).

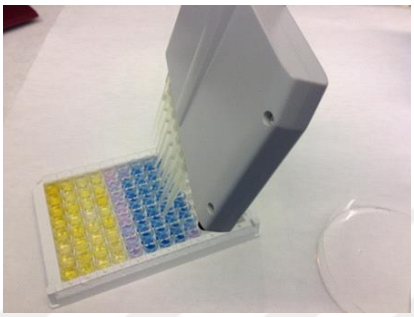

Figure III.5: Work of Elisa Test

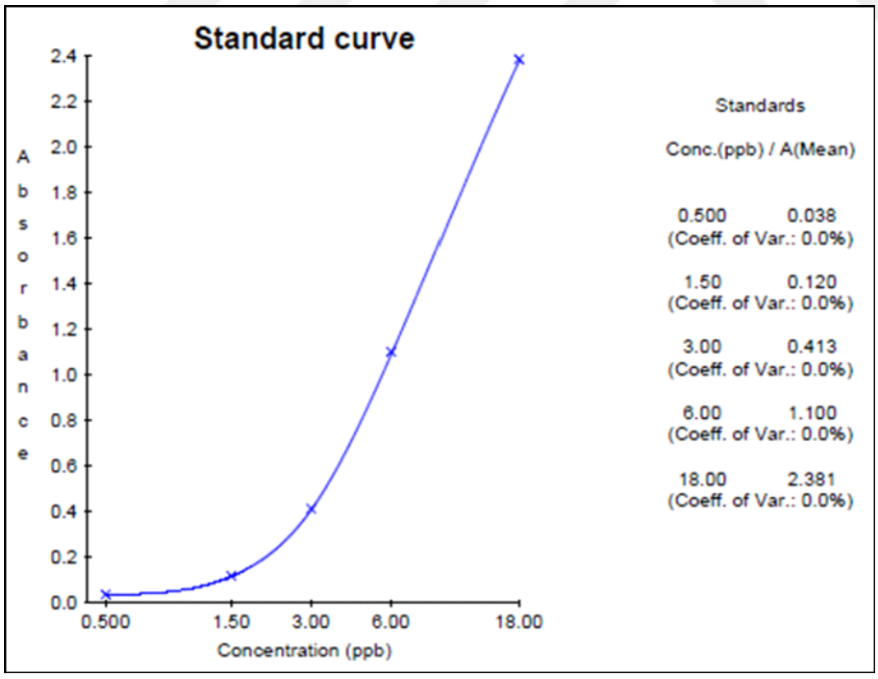

Figure III.6: Standart curve graph of Tetracycline Elisa Test

IV. RESULTS

IV.1. MeRA Test Results

It was aimed to qualitatively determine the results with this test. This kit is designed to be optimized for meat products. It has been suggested by the manufacturer to use for antibiotic residue identification in honey samples. Analyzes of 40 samples were made and the positivity was found on 19 samples of samples were sold open 16 samples of packaged samples (Table 4).

Table IV.1: MeRA Test Results

Unpacked Sample No	Results	Packed Sample No	Results
1	Pozitif	1	Negatif
2	Pozitif	2	Pozitif
3	Pozitif	3	Pozitif
4	Pozitif	4	Pozitif
5	Pozitif	5	Negatif
6	Pozitif	6	Negatif
7	Pozitif	7	Pozitif
8	Pozitif	8	Pozitif
9	Pozitif	9	Negatif
10	Pozitif	10	Pozitif
11	Pozitif	11	Pozitif
12	Negatif	12	Pozitif
13	Pozitif	13	Pozitif
14	Pozitif	14	Pozitif
15	Pozitif	15	Pozitif

16	Pozitif	16	Pozitif
17	Pozitif	17	Pozitif
18	Pozitif	18	Pozitif
19	Pozitif	19	Pozitif
20	Pozitif	20	Pozitif

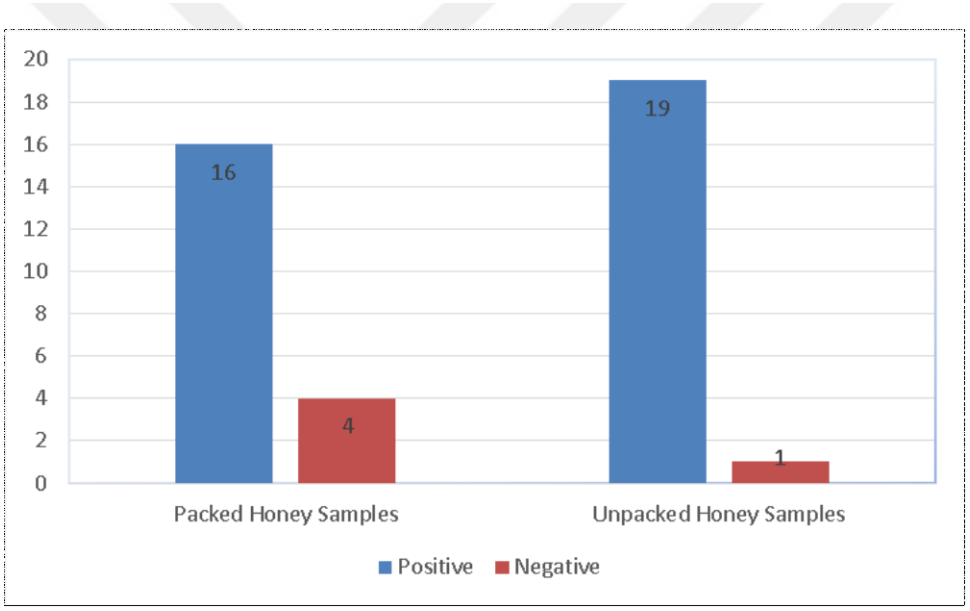

Figure IV.1: Positive - Negative Distrubition of Mera Test Results

IV.2. Elisa Test Results

As a result of the elisa study performed with tetracycline (r-bioPharm), 13 of the samples were found to be positively positive and 11 of the packaged honey samples were not positive (Table 5).

Table IV.2: Tetracycline Elisa Test Results

Tetracycline Elisa Test Results					
Unpacked Sample No	**Elisa Count (ppb)**	**Results**	**Packed Sample No**	**Elisa Count (ppb)**	**Results**
1	4,3	Negative	1	30,47	Negative
2	> 180	Positive	2	>180	Positive
3	100	Positive	3	>180	Positive
4	5,47	Negative	4	>180	Positive
5	5,54	Negative	5	53,87	Negative
6	4,3	Negative	6	30,22	Negative
7	> 180	Positive	7	161,84	Positive
8	> 180	Positive	8	52,26	Negative
9	4,6	Negative	9	28,32	Negative
10	> 180	Positive	10	39,42	Negative
11	> 180	Positive	11	>180	Positive
12	4,6	Negative	12	74,56	Negative
13	> 180	Positive	13	114,83	Positive
14	> 180	Positive	14	>180	Positive
15	> 180	Positive	15	>180	Positive
16	> 180	Positive	16	>180	Positive
17	78,48	Negative	17	>180	Positive
18	> 180	Positive	18	21,2	Negative
19	> 180	Positive	19	67,75	Negative
20	> 180	Positive	20	>180	Positive

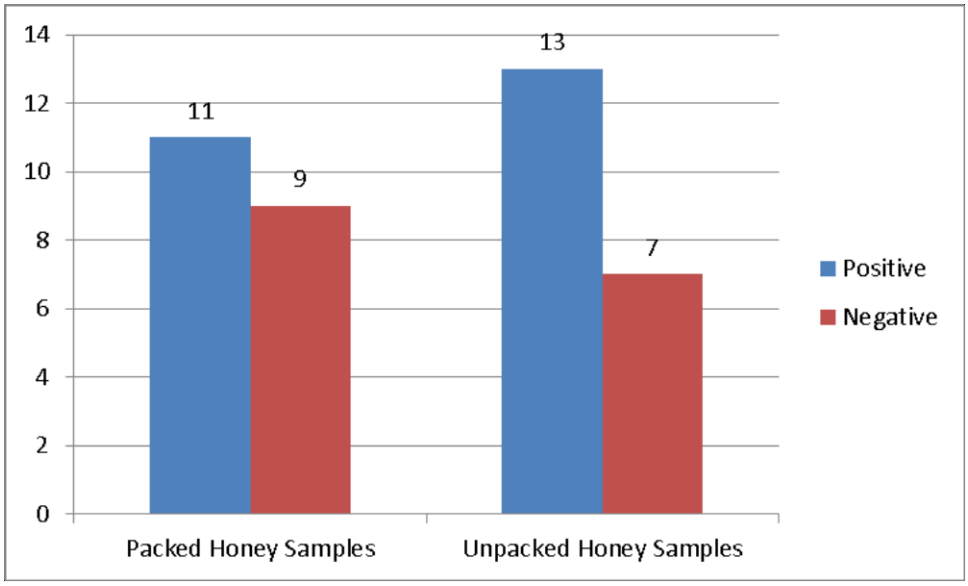

Figure IV.2.: Positive - Negative Distrubition of Mera Test Results

IV.2. Elisa Test Results

As a result of the elisa study performed with tetracycline (r-bioPharm), 13 of the samples were found to be positively positive and 11 of the packaged honey samples were not positive (Table IV.3.)

Table IV.2: Tetracycline Elisa Test Results

Tetracycline Elisa Test Results					
Unpacked Sample No	**Elisa Count (ppb)**	**Results**	**Packed Sample No**	**Elisa Count (ppb)**	**Results**
1	4,3	Negative	1	30,47	Negative
2	> 180	Positive	2	>180	Positive
3	100	Positive	3	>180	Positive
4	5,47	Negative	4	>180	Positive
5	5,54	Negative	5	53,87	Negative
6	4,3	Negative	6	30,22	Negative

7	> 180	Positive	7	161,84	Positive
8	> 180	Positive	8	52,26	Negative
9	4,6	Negative	9	28,32	Negative
10	> 180	Positive	10	39,42	Negative
11	> 180	Positive	11	>180	Positive
12	4,6	Negative	12	74,56	Negative
13	> 180	Positive	13	114,83	Positive
14	> 180	Positive	14	>180	Positive
15	> 180	Positive	15	>180	Positive
16	> 180	Positive	16	>180	Positive
17	78,48	Negative	17	>180	Positive
18	> 180	Positive	18	21,2	Negative
19	> 180	Positive	19	67,75	Negative
20	> 180	Positive	20	>180	Positive

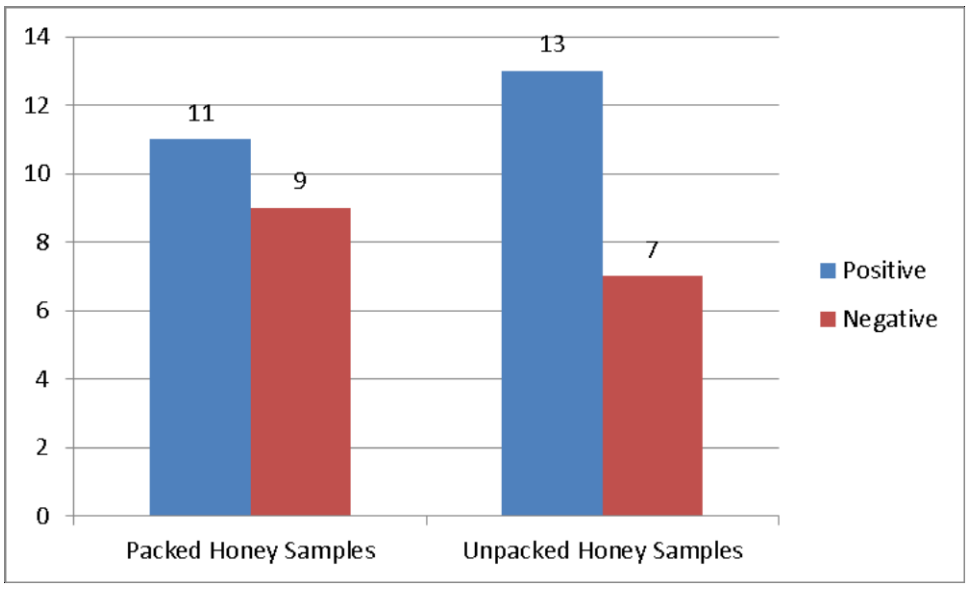

Figure IV.3.: Positive - Negative Distrubition of Elisa Test Results.

V.DISCUSSION AND CONCLUSION

In today's livestock applications, the use of veterinary medicines is of utmost importance for 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 for the prevention of diseases and acceleration of development in animals. However, it is considered to be a residue if the food value exceeds the permissible limits of nutrients such as meat, milk, eggs, honey (26, 27).

In the last 50 years, 1 million tons of antibacterial substances have been used in the world, of which about 50% are veterinary and agricultural (12, 29). This rate suggests that we need to deal with antibiotic residues in foods. The reasons for this are stated as excessive drug delivery from the doses prescribed to the animals, and in particular the delivery of the drug to the animals without the adherence to 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 (Tetracycline, oxytetracycline and chlortetracycline) group has been reported to be widely used in animal breeding, due to the broad spectrum of antibiotics and low toxic effects. For this reason, the presence of tetracycline residues in many nutrients is important (32-35).

When the results of the MeRA test kit were examined in our study, positives were found in 19 of the open honey samples; and 16 in packaged honey samples. In the study done with Elisa, positives were found in 13 of the open honey samples; 11 packaged samples. The difference in number between MeRA test and ELISA can be explained by examining a large number of antibiotics in the MeRA test kit. With Elisa, only tetracycline levels were examined.. Because samples negative (12 from open samples, 1, 5, 6 and 9 from packaged samples) were negatively detected with elisa. However, the positivity rate was 60% for Elisa and 87.5% for MeRA test. The rate of positivity was found to be higher because only the tetracycline was not observed with the MeRA test kit.

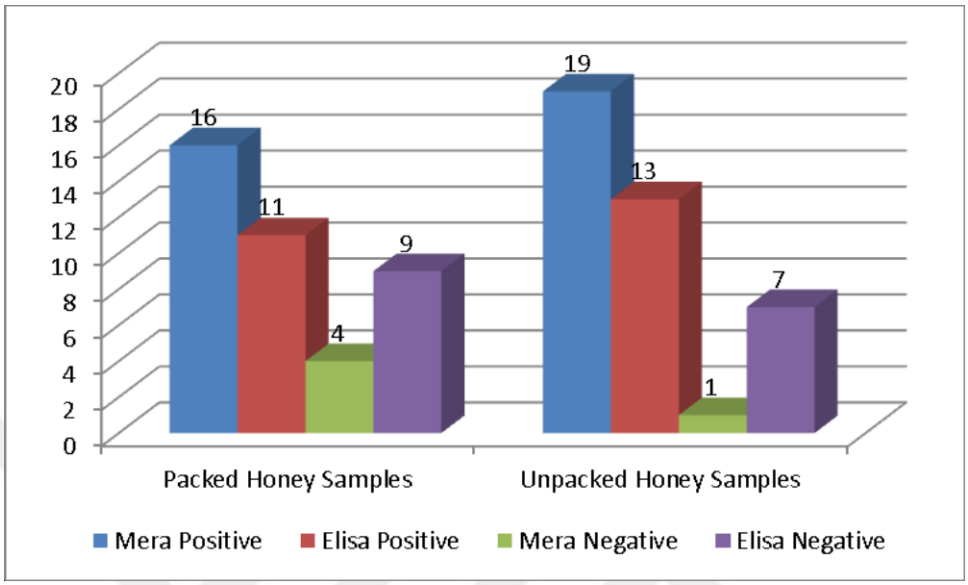

Figure V.1.: Comparison of Mera test and Elisa test results.

Studies on antibiotic residues in honey both in our country and abroad have also found high positivity in our study. In a study made in Romania in 2011, tetracyclin levels were found to be above the limits of all thirty samples studied (37). In 2014, a study made with 135 honey samples in Iran reported 14. 7% positivity with elisa method (38).

When the levels of tetracyclin in our study were quantitatively examined; It is seen that it is between 4,3 ->; 180 ppb. In a study made in Romania in 2011, positivity was higher in our study than in our study, Tetracycline levels ranged between 180-199 ppb (37).

A study was done by a private honey production company in Istanbul named as ‘’Residue, trick and origin detection in honey according to region R&D activities‘’. According to study some beekeepers have been using drug against to bee diseases and this drug especially used in the poultry sector contains tetracycline and also sulfadimidine. This extremely misapplication causes the tetracycline group to increase the antibiotic residues in our country. According to this study, the average of the results of 1425 samples were analyzed in the first half of 2006 is below 10 ppb. 10 - 15% of the beekeepers use antibiotics containing tetracycline and the amount of residue in the honeys is extremely high (>200

ppb.) In general, 90% of honey contains. tetracycline at lower rates such as 13.65 ppb. In the analyzes, no trace of tetracycline was found in 75% of honeys (39).

In the same study, it was reported that some of the beekeepers continued to use antibiotics unconsciously in some diseases. When the residue problem is examined according to the regions, it is reported that sulfa and tetra group antibiotic residues concentrate in Muş, Bingöl, Şemdinli, Yüksekova regions and Marmaris and Muğla regions. It is known that mobile beekeeping is common in these regions. Regional beekeepers need to pay attention to drugs when they are used against the rot fungus diseases, preferring methods other than drug use to cure this disease is of great importance in terms of solution of the problem. It is estimated that some drugs known to be used especially in poultry (chicken, turkey etc.) cause residual problems of use by beekeepers. In this regard, it is strongly recommended that beekeepers pay attention to the fact that the drugs they use are legally allowed to be used in beekeeping (39-40).

Beekeeping industry in our country needs to pay attention to honey production because of the increased control and sensitivity of the European Union. Otherwise, we may have difficulties in selling from our country to Europe. For this reason, some of our beekeepers should stop immediately use of antibiotics with the aim of protecting bee health. In addition to causing the residual problem, this application weakens the immune system of the bees and causes the disease-causing bacteria to become resistant to antibiotics (39).

As a result, it is seen that the use of antibiotics is still uncontrolled in honey production in our country. For quality honey production, more care must be taken. Compared with Elisa, the MeRA test was found to be a cheap, fast and practical test for Tetracycline residues. However, this study needs to be expanded by increasing the number of samples.

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