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# Investigation of Some Antibiotic Residue Levels in Honey Produced in Ardahan Province

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**Abstract**
Beekeeping has been a major agricultural activity since ancient times. The importance of beekeeping is still present in our country, just like in other parts of the world. Honey, beeswax, pollen, propolis, royal jelly, and bee venom are the products produced by beekeeping. Honey is particularly important for human nutrition among these. It is crucial that the honey is free of antibiotics and other residues for this reason. The purpose of this study is to determine the level of residues of certain antibiotics (tetracycline and sulfonamide group) in honey produced in Ardahan province. As part of the research, honey samples from beekeepers in Ardahan and its counties (Göle, Çıldır, Hanak, Damal, and Posof) were collected in August 2023. 90 honey samples were collected from 15 different apiaries operating in each settlement. The residue level of tetracycline and sulfonamide group antibiotics in the honey samples collected directly from the producers was determined using the ELISA method. In this study, tetracycline and sulfonamide group antibiotic residues were not found in any of the 6 centers sampled, namely Ardahan, Göle, Çıldır, Hanak, Damal, and Posof. The research concluded that the honey that is produced in Ardahan province is safe for human health. It is thought that it would be useful to provide training to beekeepers on the subject in order to maintain healthy honey (free of antibiotics and other residues) production in Ardahan province.

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## 1. Introduction

Beekeeping is a significant agricultural activity that has been practiced since ancient times. As in the rest of the world, beekeeping maintains its importance in our country. Our country's rich plant flora and diversity play a major role in the management of beekeeping (Ekici and Yazıcı, 2018; Öztürk, 2021). Beekeeping produces honey, beeswax, pollen, propolis, royal jelly, and bee venom. Bee creates honey from flower nectar, which is a natural product that is widely consumed throughout the world (Demirhan and Demirhan, 2022). This is why it has a special place and importance in human life (Kadirhan et al., 2019). Honey is a natural and nutritious food that is consumed by humans (Ağaoğlu et al., 2020). In addition, honey has been used in the treatment of many diseases, particularly those affecting the digestive and respiratory systems, since ancient times (Mahmud et al., 2015; Ekici and Yazıcı, 2018). The estimated annual production of honey worldwide is around 1.4 million tons (Aydemir Atasever and Yüksel, 2022). Asian countries contribute a significant amount of this production (about 40 %). Our country follows China in terms of world honey production and produces significant amounts of honey (Mahmoudi et al., 2014; Aydemir Atasever and Yüksel, 2022).

In the structure of honey, there are various vitamins, minerals, carbohydrates, amino acids, enzymes, and proteins (Kutlu et al., 2017). Because of the nutrients it contains, it is used in the production of creams used in the food and cosmetics industries (Ağaoğlu et al., 2020). Honey, which is obtained in a natural environment, can be contaminated with a range of harmful substances found in the environment (air, water, and soil) and plants (Mahmoudi et al., 2014). These harmful substances are transported to the hives by honeybees. Furthermore, the use of chemicals by beekeepers has a significant impact on the contamination of honey (Mahmoudi et al., 2014; Aydemir Atasever and Yüksel, 2022). It is reported that the most important environmental pollutants affecting bee products are pesticides, aflatoxins, heavy metals, bacteria, and radioactivity (Mahmoudi et al., 2014; Özkan et al., 2015). Bee diseases are a major factor that affects beekeeping negatively. Serious economic losses can occur as a result of bee diseases. The most significant diseases that affect beekeeping are American foulbrood, European foulbrood, and varroa (Aydemir Atasever and Yüksel, 2022).

The residue in honey is caused by the use of unnecessary and insufficient doses of antibiotics in the fight against these diseases (Aydemir Atasever and Yüksel, 2022). Humans may experience health problems if they consume honey that contains antibiotic residue (Kutlu et al., 2017; Ağaoğlu et al., 2020). Some of the important health problems that can be seen include anaphylactic shock, allergic reactions, bacterial resistance, intestinal flora disruption, nervous and hematopoietic system diseases. It is reported that there is a possibility of carcinogenic and teratogenic effects (Apić et al., 2015; Kutlu et al., 2017; Ağaoğlu et al., 2020; Aydemir Atasever and Yüksel, 2022). The consumption of honey that contains antibiotic residues, particularly by children, the elderly, and sick individuals, can result in serious health problems (Kumar et al., 2020). As a result of these adverse effects, the use of antibiotics in apiculture is prohibited in our country as well as in European countries. The honey's maximum residue limits have not been determined due to this reason (Apić et al., 2015; Özkan et al., 2015; Ağaoğlu et al., 2020; Demirhan and Demirhan, 2022).

Beekeepers use antibiotics of the tetracycline group to treat and protect bee diseases. (Especially American foulbrood and European foulbrood) (Apić et al., 2015; Hosseinpour et al., 2021; Aydemir Atasever and Yüksel, 2022; Şahin et al., 2024). Oxytetracycline is commonly used for this purpose (Mahmoudi et al., 2014). Drugs in this group (such as tetracycline, chlortetracycline, oxytetracycline, and doxycycline) have a broad spectrum. They have bacteriostatic effects on gram-positive, gram-negative, aerobic, and anaerobic bacteria. They inhibit

protein synthesis in susceptible bacteria (Ağaoğlu et al., 2020). Other anti-bacterial medicines used in apiculture are sulfonamides. There are numerous drug derivatives in this group. Some of them include sulfadiazine, sulfamethazine, sulfadoxine, sulfabenzamide, sulfachlorophidazine, sulfathiazole, sulfamethoxazole, sulfadimethoxine, and sulfizoksazole. The drugs in this group have in bacteriostatic effect. They demonstrate their effects by replacing PABA (para amino benzoic acid), which is involved in the synthesis of folic acid, necessary for the growth and development of bacteria (Özkan et al., 2015; Kutlu et al., 2017).

According to Turkish Statistical Institute 2022 data, the production of honey in Turkey is 118,297.464 tons. In Ardahan province, there are 658 registered beekeeping enterprises. It is reported that the quantity of honey produced in the province of Ardahan is 244.963 tons (Anonymous, 2023). These data indicate that beekeeping in Ardahan is an important agricultural activity that contributes to the honey production of our country. Caucasian breed bee (*Apis mellifera caucasica* L.*)* breeding is carried out in Ardahan province (Cengiz and Yazıcı, 2018). It is also known as the genetic center of this breed. *Pseudomonas aeruginosa* bacterium was found in dead bees and honeycomb samples in a study conducted in Ardahan province (Kadirhan et al., 2019). In other research, it is stated that foulbrood, nosema, and lime disease are the most common diseases in Ardahan province (Cengiz and Yazıcı, 2018). The presence of bacterial diseases of bees in the province of Ardahan strengthens the possibility for beekeepers to use antibacterial drugs. The purpose of this research was to examine the residue levels of tetracycline and sulfonamide group antibiotics in honey produced in Ardahan and its surrounding counties (Göle, Çıldır, Hanak, Damal, and Posof).

## 2. Materials and Methods

The research material consisted of honey samples obtained by beekeeping producers in Ardahan and its counties (Göle, Çıldır, Hanak, Damal, and Posof) in August 2023. The samples were taken from 15 different beekeepers operating in each settlement center. As a result, 90 honey samples were collected from 6 settlement centers. After harvesting, the samples that were collected directly from beekeepers (about 15 g) were placed in sterile disposable falcon tubes (50 ml) and the lid was tightly closed. The honey samples were taken from each hive to ensure homogeneity. The samples were then brought to the laboratory. Until they were tested in the laboratory, they were stored at room temperature to protect them from humidity and light. The level of antibiotic residue in the honey samples was determined using the ELISA method. Tetracycline (Cat. No: CSB-E12090f, CusaBio, U.S.A) and Sulfonamide (Cat. No: CSB-E12094f, CusaBio, U.S.A) analysis kits were utilized for this purpose. The samples were prepared and analyzed according to the instructions given by the manufacturer. At the end of the analysis, the standards and the optical density of the samples were read on the ELISA reader (BioTek ELx800, USA) at 450 nm. The optical densities obtained from samples were compared to those obtained from standard solutions, and the residues of tetracycline and sulfonamide were calculated.

## 3. Results and Discussion

In Table 1 below, tetracycline residue levels were presented in the honey samples obtained from Ardahan and its counties.

**Table 1.** Residues of tetracycline in the honey (ppb)

| Region | The number of samples (n) | Tetracycline [a] Group |
|---|---|---|
| Ardahan | 15 | 0 ± 0 # |
| Göle | 15 | 0 ± 0 # |
| Çıldır | 15 | 0 ± 0 # |
| Hanak | 15 | 0 ± 0 # |
| Damal | 15 | 0 ± 0 # |
| Posof | 15 | 0 ± 0 # |

a : Tetracycline, Minocycline, Rolitetracycline, Aureomycin, Demeclocycline, Terramycin, and Doxycycline
#: It was not detected within the test susceptibility limits (#: <0.05 ppb).

In Table 1 above, it is seen that the tetracycline group antibiotic residue could not be detected in any of the 90 honey samples taken from 6 regions (Ardahan, Göle, Çıldır, Hanak, Damal, and Posof). In Table 2 below, the level of sulfonamide residue was presented in the honey samples obtained from Ardahan and its counties.

**Table 2.** Residues of sulfonamide in the honey (ppb)

| Region | The number of samples (n) | Sulfonamide [b] Group |
|---|---|---|
| Ardahan | 15 | 0 ± 0 * |
| Göle | 15 | 0 ± 0 * |
| Çıldır | 15 | 0 ± 0 * |
| Hanak | 15 | 0 ± 0 * |
| Damal | 15 | 0 ± 0 * |
| Posof | 15 | 0 ± 0 * |

[b] : Sulfamerazine, Sulfadiazine, Sulfamonomethoxine, Sulfametoxydiazine, Sulfamethazine, Sulfisomidine, Sulfadimethoxypyrimidine, Sulfadimoxine, Sulphamethoxazole, Sulfadoxine, Phthalylsulfathiazole, Sulfamethizole, Sulfaisoxazole, Sulfathiazole, Sulfaquinoxaline, Sulfamethoxypyridazine, Sulfaclozine, Sulfachloropyridazine, and Sulfabenzamide

*: It was not detected within the test susceptibility limits (*: <1 ppb).

Table 2 above shows that no sulfonamide group antibiotic residue was detected in any of the 90 honey samples collected from Ardahan and its counties (Göle, Çıldır, Hanak, Damal, and Posof). Beekeeping activity remains important in the agricultural sector in our country. Beekeeping's products (honey, propolis, royal jelly, beeswax, pollen, and bee venom) are a significant source of income. Pollination by bees has great benefits for agricultural products. As such, apiculture is of great importance to humans. Honey, which is a good source of nutrients for humans, is included in bee products. It has been used in the treatment of certain diseases since ancient times, in addition to its nutritional properties (Kadirhan et al., 2019). The digestive tract infections caused by *Shigella sonnei* in humans are among these diseases (Al-Masaudi et al., 2020). It is also reported for in vitro efficacy against dermatophytes such as *Trichophyton tonsurans*, *Microsporum canis*, *Aspergillus niger*, and *Candida albicans* (Mahmud et al., 2015).

Bee diseases adversely affect beekeeping and cause economic damage (Kadirhan et al., 2019). The diseases, particularly American foulbrood caused by *Streptococcus pluton* and European foulbrood caused by *Paenibacilus (Bacillus) larvae*, are both contagious and dangerous (Mahmoudi et al., 2014; Çakar and Gürel, 2019; Hosseinpour et al., 2021). The use of antibiotics for treating and preventing these diseases can result in residue problems in honey. Such residues diminish the quality of honey and its market share (Mahmoudi et al., 2014). Additionally, when consumed as food, they can be harmful to human health. The risk increases due to the long half-life of antibiotic residues (Apić et al., 2015; Özkan et al., 2015; Kutlu et al., 2017).

Some of the antibiotics used in bees are tetracycline and sulfonamides (Mahmoudi et al., 2014; Özkan et al., 2015; Kutlu et al., 2017). Unconscious use of these drugs can lead to them being passed to humans through honey. Their presence can lead to bacterial resistance and other harmful effects in humans (Apić et al., 2015). *Crystalluria* and kidney dysfunction have been reported in humans due to sulfonamide derivatives. Besides adverse effects on the kidneys, allergic reactions, serum diseases, and mucosal lesions have been reported in sensitive individuals (Korkmaz et al., 2017). Pregnant women and children under 8 years of age should not take tetracycline group drugs as they can cause permanent color changes in their teeth (Ağaoğlu et al., 2020). The lack of antibiotic residues in honey is therefore needed to protect human health.

The fact that antibiotic residues have harmful effects on human health has prompted research on this subject worldwide and in our country. Antibiotic residues were found in 5 out of the 193 honey samples obtained in

Serbia (2.59 %) (Apić et al., 2015). An Indian study reported that oxytetracycline was detected in 15.3 % of 150 honey samples. In the same study, it is stated that 21.4 % of the samples contaminated with oxytetracycline are uncertified branded, 14.3 % are unbranded, and 10.2 % are certified branded honeys (Kumar et al., 2020). Tetracycline residues (mean 77.86 µg $kg^{-1}$) were found in all 9 honey samples offered for sale in the United States, and it is stated that sulfonamide residue could not be detected in any of the samples (Sarkar et al., 2023). In Iran, 19 (14.07 %) honey samples were reported to contain tetracycline, while 20 (14.81 %) had sulfonamide residues (Mahmoudi et al., 2014).

The presence of antibiotics in honey has been investigated in many regions of our country. Of the 59 pine honey samples collected from the Aegean region (16 from Aydın, 36 from Muğla, and 7 from Balıkesir), it is reported that 35 contain tetracycline (6-42 ppb) residues and 31 contain sulfonamide (3-32 ppb) residues (Korkmaz et al., 2017). It is stated that 80 samples of honey sold in markets in Ankara province contain 7 different antibiotic residues. In the positive samples, there were residues of dihydrostreptomycin (58.75 %), streptomycin (22.5 %), erythromycin (13.75 %), sulfadimidine (10%), and enrofloxacin (2.5 %). In the same study, tetracycline and doxycycline were only found in one sample (Demirhan and Demirhan, 2022). Antibiotic residues were investigated in 60 samples, 30 of which were open and 30 of which were packaged, in Sivas region. It has been reported that 73.3 % of packaged honeys and 60 % of open honeys contain tetracycline residues (Ağaoğlu et al., 2020). In another study conducted on honey, it is stated that tetracycline residues at the level of 2.1-47.08 ppb (mean 9.33 ppb) were found in 37 (46.8 %) of 79 samples collected from Erzurum province (Aydemir Atasever and Yüksel, 2022). In another study, it was reported that 52 % of the 180 honey samples collected from Ardahan province contained sulfonamide residues (Özkan et al., 2015). Tetracycline and sulfonamide residues were not found in honey collected from 20 different regions in Bitlis (Hizan) province (Kutlu et al., 2017) and 10 honey samples were collected from Muş province (Kutlu and Bengü, 2020). A study has demonstrated that tetracycline applied to hives can result in drug residues in honey in untreated hives (Martel et al., 2006).

In this study, tetracycline (Table 1) and sulfonamide (Table 2) group antibiotic residues could not be detected in a total of 90 honey samples, 15 from each of 6 different centers (Ardahan, Göle, Çıldır, Hanak, Damal, and Posof). The results obtained from this research are similar to the research results reported by Kutlu et al. (2017) and Kutlu and Bengü (2020). The fact that antibiotic residue cannot be detected in the honey can be explained by the fact that the producers in Ardahan province do not use antibiotics in bees. The results obtained from this study are not similar to the results of the research by Apic et al. (2015), Kumar et al. (2020), Sarkar et al. (2023), Mahmoudi et al. (2014), Korkmaz et al. (2017), Demirhan and Demirhan (2022), Ağaoğlu et al. (2020), Aydemir Atasever and Yüksel (2022), and Özkan et al. (2015) reporting antibiotic residues in honey. It is thought that the reasons for this may be the occurrence of different bee diseases in each region, the knowledge levels of beekeepers, and the habits of using antibiotics.

## 4. Conclusion

In conclusion, antibiotic residues of tetracycline and sulfonamide groups could not be detected in the honey produced in Ardahan area. This situation is crucial for the province of Ardahan, which is considered the genetic center of the Caucasian bee race. The honey that is produced in the Ardahan region is sold not only in the region but also in other regions. Therefore, the fact that it contains no antibiotic residues is beneficial to human health. It is believed that these findings will have a positive impact on the market share of Ardahan honey. It is very important to maintain healthy honey production (without antibiotics and other residues) in Ardahan area. The protection of honey against various pollutants is essential for human health. It is suggested to take some

measures for this. Regular checks on the sales of veterinary drugs and antibiotic residue levels in honey are necessary for this purpose. Furthermore, individuals who are engaged in beekeeping need to be educated on the subject. It is expected that these measures will contribute to national and international food security by promoting organic and healthy honey production.

**Ethical Approval**

The Ministry of Agriculture and Forestry (Ardahan Provincial Directorate of Agriculture and Forestry - dated 19.06.2023 and E-29486769-325.99-10277578) approved this research before it started.

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