

Prevalence of Nosemosis and Varroosis in Honeybees (*Apis mellifera* L., 1758) in Bolu RegionBolu Yöresi Bal Arılarında (*Apis mellifera* L., 1758) Nosemosis ve Varroosis'in YaygınlığıMustafa Yaman¹ , Tuğba Sağlam Güvendik² 

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Abstract: Bolu province has a potential to progress in beekeeping due to its rich flora and vegetation. However, the amount of honey production per beehive in the province of Bolu is 8-10 kg, which is below the average (14.4 kg) of Turkey. Honey bee diseases and parasites are among the most important factors that retard the development of beekeeping and limit production efficiency in Turkey. In this study, it was aimed to evaluate the presence of varroosis and noseamosis in honey bee samples in different districts of Bolu. The presence of noseamosis and varroosis diseases in bee colonies in Bolu province are documented for the first time. Nosemosis was the most common disease. 190 of the 3030 examined bee samples were infected by *Nosema* spp. Average of the noseamosis infection was 6.3%. On the other hand, *Varroa* infestation was observed in the six of nine localities examined in Bolu province. 118 of the 3753 examined bee samples were infected by *Varroa* mites. Total infection ranged from 0.6 to 19.2%. This study has shown a prevailing higher percentage of infested with *Varroa* mites and *Nosema* spp. in Bolu region and revealed that the beekeepers in the Bolu region should be informed in detail about the precautions to be taken in the monitoring and controlling varroosis and noseamosis.

Keywords: Honeybee, Nosemosis, Varroosis, Bolu, Turkey.

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Öz: Bolu ili zengin flora ve bitki örtüsü nedeniyle arıcılıkta gelişme potansiyeline sahiptir. Ancak Bolu ilinde kovan başına bal üretim miktarı 8-10 kg olup Türkiye ortalamasının (14,4 kg) altındadır. Bal arısı hastalıkları ve parazitleri Türkiye'de arıcılığın gelişmesini geciktiren ve üretim verimini sınırlayan en önemli faktörlerin başında gelmektedir. Bu nedenle bu çalışmada, Bolu ilinin farklı ilçelerinde bal arısı örneklerinde varroosis ve noseamosis varlığının değerlendirilmesi amaçlandı. Sonuç olarak, Bolu ilindeki arı kolonilerinde noseamosis ve varroosis hastalıklarının varlığı ilk kez belgelendi. Nosemosis en yaygın hastalık olarak bulundu. İncelenen 3030 arı örneğinden 190'ı *Nosema* spp. ile enfekte idi. Nosemosis enfeksiyonunun ortalaması %6,3 olarak bulundu. Diğer taraftan, Bolu ilinde incelenen dokuz lokalitenin altısında *Varroa* enfestasyonu gözlemlendi. İncelenen 3753 arı örneğinden 118'i *Varroa* akarı ile enfekte bulundu. Toplam enfeksiyon % 0,6 ile % 19,2 arasında değişiklik gösterdi. Çalışmamız, Bolu yöresi arıcılarının varroosis ve noseamosis'in izlenmesi ve kontrolünde alınması gereken önlemler konusunda detaylı olarak bilgilendirilmesi gerektiğini ortaya koymaktadır.

Anahtar Kelimeler: Bal arısı, Nosemosis, Varroosis, Bolu, Türkiye.

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INTRODUCTION

Turkey has a vibrant flora because of its geographical location and climate diversity. Despite the richness of the flora in Turkey, the desired increase in honey production cannot be achieved. While the average honey production per hive in the world is 20.1 kg, this rate is 14.4 kg in Turkey. However, the amount of honey production per beehive in the province of Bolu is 8-10 kg, which is below the average (14.4 kg) of Turkey. Despite having sufficient colonies in honey production, honey bee diseases and parasites are among the most essential factors that retard the development of beekeeping and limit production efficiency in Turkey. Pathogens and parasites commonly found in bee colonies have been identified as bacterial, viral, fungal, protist and mite origin. Two of them, nosemosis and varroosis cause significant decreases in honey production. Nosemosis disease is of fungal origin and is caused by two different species, *Nosema apis* and *Nosema ceranae*, which are included in the genus *Nosema* in Microspora (Fries, 1988; Paxton, 2010; Yaman et al., 2015). As a parasitic disease, varroosis is caused by Varroa mites feeding on adults and larvae of honey bees. These disease agents are widely known in Turkey and cause weakened immune systems, decreased body weight, and a shortened lifespan. Finally, in advancing infections, the colony disappears and thus serious economic losses occur in beekeeping (Kumova, 2003; Yücel, 2005; Güler, 2017). Therefore, both diseases are known as the most important reasons for the low yield in honey production in Turkey.

According to TUIK data, Bolu province ranks 72nd in honey production in Turkey with 116.78 tons of honey, although it has 629 beekeeping establishments and 22.000 colonies (TUIK, 2021). Although the province of Bolu has a rich flora, the amount of honey production per beehive in the province of Bolu is 8-10 kg, which is below the average (14.4 kg) of Turkey. Pathogens and parasites are among the leading causes of low productivity in beekeeping, particularly in the province and generally in Turkey. However, studies on bee diseases in the Bolu region are scarce. In this study, the presence of nosemosis and varroosis diseases in bee colonies in Bolu province are documented for the first time.

MATERIAL AND METHOD

Bee Samples

The minimum number of apiaries to be sampled in Bolu province were determined using the following formula according to the literatures (1) (Águila and González-Ramírez, 2014; Can and Yağın, 2015; Zerek, 2021).

$$n = \frac{Nt^2p(1-p)}{d^2(N-1) + t^2p(1-p)} \quad (1)$$

In the formula, N is the number of hives of active beekeepers in Bolu. The prevalence value p was taken into account as 0.5, since its range was given quite widely in the literature. While d^2 is the square of 0.1, which is the margin of error in the sample, t^2 is the square of 1.64, the corresponding figure in the table. According to the calculation of the minimum number of beekeepers to be taken as samples, total 3.753 adult bee samples were collected from 32 beekeepers in 9 localities, including Bolu centre and 8 districts (Dörtdivan, Gerece, Göynük, Kibriscık, Mengen, Mudurnu, Seben and Yeniçağa).

Investigation for Nosemosis

A total of 3.030 adult bee samples, 1.830 dead and 1.200 living bees were dissected in Ringer's solution and wet smears were prepared. Host fat body, Malpighian tubules, gut epithelium, and hemolymph were examined for the presence of pathogens under a light microscope at $\times 400$ – 1000 magnification (Yaman, 2020). When an infection with the pathogen was observed, a part of the material was used for the preparation of Giemsa-stained smears. For this, the slides were air-dried and fixed with methanol, then stained with a freshly prepared 5% solution of Giemsa stain and reexamined under the microscope by using the oil immersion lens. The spores detected by the light microscopy were measured and photographed using a microscope with a digital camera and Soft Imaging System.

Investigation for Varroosis

A total of 3.753 adult bee samples, 2.634 dead and 1.119 living bees were examined for varroosis. The dead and living bees were flooded with 70% alcohol and shaken on a shaker for 30 minutes. After shaking, it waited for 10-15 minutes for the bee samples and other particles to fall out. Then, it was filtered with filter paper and bee and varroa samples were transferred to a new filter paper on a bright plate, where the mites can be easily identified and counted under stereo microscope (Kuvancı et al., 2013). The diagnostic method for *Varroa destructor* was based on morphological identification by alcohol wash method according to Oliver (2020). The percentage of a mite infestation level was calculated by the following formula: mite infestation (%) = (Number of varroa mites/number of adult bees) × 100 (Salkova and Gurgulova, 2022).

RESULTS AND DISCUSSION

In this study, the presence of noseiosis disease, which is one of the most critical diseases in honey bees, and varroa mites, known as the most common external parasite, in bee colonies in Bolu centre and 8 counties were investigated for the first time. During the study, both noseiosis and varroosis were observed in all apiaries investigated Bolu Province. Noseiosis was the most common disease. Noseiosis was observed in all examined localities. 190 of the 3030 examined bee samples were infected by *Nosema* spp (Figure 1). Total infection ranged from 0.5 to 11.7%. The average infection was 6.3% (Table 1). However, while noseiosis disease was detected in all of the dead bee samples examined in 9 localities sampled during the study, the disease was detected in live bee samples collected from only 6 regions (Bolu/Merkez, Yeniçağa, Dörtdivan, Gerede, Seben and Mengen). The disease was observed with the lowest rate (0.7%) in Göynük and with the highest rate (11.7%) in Bolu centre. In total, the noseiosis infection (7.76%) in dead bees was relatively higher than that rate (4%) in living bees. Furthermore, the infection reached a considerably high level (18.7%) in dead bees in Bolu province.

Noseiosis disease is of fungal origin and is caused by two different species, *Nosema apis* and *Nosema ceranae*, which are included in the genus *Nosema* in Microspora (Fries, 1988; Paxton, 2010; Yaman et al., 2015). It has been documented that noseiosis causes sudden colony losses in many countries and in Turkey. In the recent studies, occurrence of noseiosis have been investigated in different regions of Turkey. In the studies on the occurrence and distribution of noseiosis in Turkey, the disease was found as 26% in Bursa region (Aydın et al., 2001), 15.74% in Kars region (Topçu and Aslan, 2004), 8.7% in Elazığ region (Şimşek, 2005), 6.5% in the Thrace region (Doğaroğlu and Sıralı, 2005), 100% in Muğla region (Şimşek, 2007), 10% and 21% in the Hatay region (Muz et al., 2012, Zerek, 2022), 44% in the Ordu region (Yaman et al., 2015) and 43.18% in Siirt and Şanlıurfa (Tel et al., 2021). Noseiosis varied from 25 to 85% infections in Ordu province (Yaman et al., 2015). When compared the infection rates of noseiosis in Muğla (Şimşek, 2007), the Ordu region (Yaman et al., 2015) and Siirt and Şanlıurfa regions (Tel et al., 2021), the infection in Bolu region is considerable low. Climatic conditions of the Bolu region may be the cause of low infections. When the disease potentials of *Nosema apis* and *Nosema ceranae*, the two most common pathogens of honey bees all over the world, are evaluated together with climatic conditions, the effect of *N. ceranae* varies considerably according to changing environmental conditions. At high temperatures, *N. ceranae* develops better in the honey bee, *Apis mellifera* than *N. apis* and causes disease at a higher rate (Martin-Hernandez et al., 2007). Studies on the distribution and environmental resistance of *N. ceranae* show how it differs from *N. apis*. It has been determined that *N. ceranae* spores are not as resistant as *N. apis* spores (Fries, 2010). The effect of *N. ceranae* varies considerably according to changing environmental conditions. Therefore, the noseiosis agent in Bolu regions should be identified at the species level.

On the other hand, Varroa density in bee colonies in Bolu region was also investigated in this study. Varroa infestation was observed in the six of nine localities examined in Bolu province. 118 of the 3753 examined bee samples were infected by *Varroa* mites (Figure 2). Total infection ranged from 0.6 to 19.2%. Average of the total infection was 3.1% (Table 2). In total, there is no difference in the infestation rates between dead (3.5%) and living (3.75%) samples. However, the highest infestation rate (25%) was observed in the living samples in Mudurnu and the lowest (0.7%) in Kırışık. There was no mite infestation in Gerede, Seben and Dörtdivan.

The ectoparasite mite, *V. destructor* has caused severe damage to honeybee colonies, worldwide in recent years (Salkova and Gurgulova, 2022). Therefore, its occurrence in different countries and Turkey has been investigated. In the recent studies, occurrence of varroa mites have been investigated in different regions of Turkey. In the studies on the occurrence and distribution of varroosis in Turkey, the parasite was found as 10.5–15.1% in Aegean region (İlikler and Yüzbaş, 1980), 13.32% in Cukurova Region (Kumova, 2001), 89% in Blacksea region including Sinop, Samsun, Ordu, Giresun, Trabzon, Rize ve Artvin locations (Yaşar et al., 2002), 2.9–15.9% in the İstanbul region (Akkaya and Vuruşaner, 1996), 1.2–13.5 in Ankara region (Kar et al., 2006), 23.8% in Düzce region (Kekeçoğlu et al., 2013) and 5% in Ordu region (Kuvancı et al., 2013). When compared the infection rates in all investigated regions of Turkey, in our studies, we found a very high percentage of bee colonies infested with *V. destructor* mite in Mudurnu district.

As a result of the study, we found a considerable high percentage of bee colonies infested with both *V. destructor* and *Nosema* spp. Bolu province ranks 72nd in honey production in Turkey (TUIK, 2021). Although the province of Bolu has a rich flora, the amount of honey production per beehive in the province of Bolu is 8-10 kg, which is below the average (14.4 kg) of Turkey. One of the main reasons for the low yield are nosemosis and varroosis, the most prevalent and damaging infection diseases of honeybees, which cause large losses in beekeeping (Salkova and Gurgulova, 2022). For this reason, this study aimed to evaluate the presence of varroosis and nosemosis in honey bee samples in different districts of Bolu.

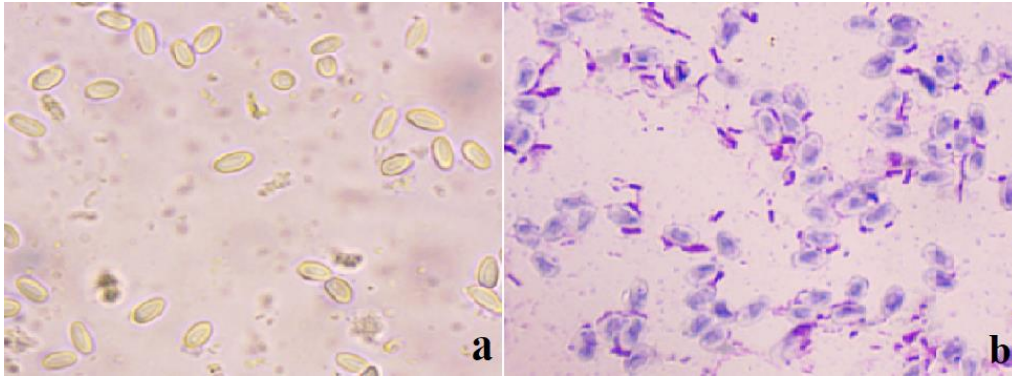


Figure 1. *Nosema* spp. fresh (a) and Giemsa stained (b) spores (1000X).
Şekil 1. *Nosema* spp.'nin taze (a) ve Giemsa ile boyanmış (b) sporları (1000X).



Figure 2. *Varroa* mites from adult bees in Bolu region.
Şekil 2. Bolu yöresindeki ergin arılardan *Varroa* akarları

Table 1. Occurrence of Nosemosis in Bolu province, Turkey.

Çizelge 1. Bolu ilinde Nosemosis varlığı

Locality	Living bee samples			Dead bee samples			Total		
	Exam. bee	Nosema	%	Exam. bee	Nosema	%	Exam. bee	Nosema	%
Centre	335	9	2.68	432	81	18.7	767	90	11.7
Yeniçağa	194	24	12.4	326	18	5.52	520	42	8.1
Dörtdivan	49	1	2.04	50	2	4	99	3	3.03
Gerede	148	8	5.40	212	11	5.2	360	19	5.28
Seben	68	1	1.47	212	15	7.1	280	16	5.71
Kıbrısçık	144	0	0	289	2	0.7	433	2	0.5
Mengen	103	5	4.85	71	7	9.85	174	12	6.9
Göynük	107	0	0	168	2	1.2	275	2	0.7
Mudurnu	52	0	0	70	4	5.71	122	4	3.3
Total	1.200	48	4.0	1.830	142	7.76	3.030	190	6.3

Table 2. Occurrence of Varroosis in Bolu province, Turkey.

Çizelge 2. Bolu ilinde Varroosis varlığı

Locality	Living bee samples			Dead bee samples			Total		
	Exam. bee	Varroosis	%	Exam. bee	Varroosis	%	Exam. bee	Varroosis	%
Centre	562	8	1.4	758	25	3.3	1.320	33	2.5
Yeniçağa	165	0	0	860	60	7	1.025	60	5.9
Gerede	80	0	0	241	0	0	321	0	0
Seben	62	0	0	102	0	0	164	0	0
Kıbrısçık	129	0	0	402	3	0.7	531	3	0.6
Mengen	52	10	19.23	-	-	-	52	10	19.3
Göynük	41	1	2.4	201	3	1.5	242	4	1.7
Mudurnu	28	7	25	70	1	1.4	98	8	8.2
Total	1.119	26	2.3	2.634	92	3.5	3.753	118	3,1

CONCLUSION

In conclusion, this study has shown a prevailing higher percentage of infested with *Varroa* mite and *Nosema* spp. in Bolu region. The reason for these high undesirable diseases may be that the beekeepers are not aware of these two disease factors and they do not have enough knowledge and experience in control treatments the diseases. Our study reveals that the beekeepers in the Bolu region should be informed in detail about the precautions to be taken in the monitoring and controlling varroosis and nosemosis.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DECLARATION OF AUTHOR CONTRIBUTION

Mustafa Yaman and Tuğba Sağlam Güvendik: Design of the study, statistical analysis, evaluation of the study, writing of the manuscript, performing of the field and the laboratory studies

Tuğba Sağlam Güvendik and Mustafa Yaman: Carrying out of the experiment, performing of the laboratory studies

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