ABSTRACT

Beekkeeping, performed in many parts of the world, has a very large place in the world trade market with bee products such as wax, bee venom, propolis and royal jelly, especially honey production. However, honey bee diseases are quite common and restricted the production of bee products. One of the most important of these diseases, Nosema, is caused by spores in intestinal epithelium cells of the honeybee. Nosema apis and Nosema ceranae are the factors of this disease and also common in our country. These two species can be distinguished from each other by molecular diagnostic methods. In this study, materials collected from 152 apiaries located in 13 districts of Muğla province and 62 water sources close to these apiaries. The spores were counted using Thoma lame under light microscope. DNA isolation was carried out from spore positive samples. 218MITOC FOR-REV and 321APIS FOR-REV primers were used to figure out the N. apis and N. ceranae species. After DNA sequence analysis of the obtained amplifications, it was determined that all samples formed 3 haplotypes according to studied sequences for the first time. In Muğla region, the presence of only N. ceranae as a disease agent was determined and the prevalence of the disease was detected at a rate of 71.53±6.02%. Moreover, blast analysis showed that the N. ceranae sequence detected high similarity (94-100 %) with the previously reported in Lebanon, France, Morocco and Thailand samples.

Keywords: N. apis, N. ceranae, molecular detection, Haplotype, Muğla, Turkey

ÖZET

Dünya’nın pek çok yerinde hayvansal üretime faaliyeti olarak yapılan arıcılık, başta bal üretimi olmak üzere bal mumu, arı zehri, propolis, arı sütü gibi arı ürünleri ile de dünya ticaret pazarında oldukça geniş bir yere sahiptir. Ancak, ancıklıkta elde edilecek verimi kısıtlayan bal arısı hastalıkları oldukça yaygınlaşmış durumdadır. Bu hastalıkların en önemlileri arasında Nosema, bal arısı olup.
Nosema hastalığı, ergin baların sterilitetini, karaciğerde organizmaların apoptozis etkileri ve hemoglobinin birçoğunun kayboldüğü gibi etkileri ile, bal arıların tohumlama ve büyüme potansiyeli ne kadar azalır. Arı üretime ve tohumlamaya zarar veren Nosema hastalığı genellikle arı tohumlarının tohumlanmaması durumunda belirgini artırır. Nosema hastalığı, karaciğerde organizmaların apoptozis etkileri ve hemoglobinin birçoğunun kayboldüğü gibi etkileri ile, bal arıların tohumlama ve büyüme potansiyeli ne kadar azalır. Arı üretime ve tohumlamaya zarar veren Nosema hastalığı genellikle arı tohumlarının tohumlanmaması durumunda belirgini artırır.

Amaç: Bal arıları ekonomik ve biyolojik yönünden oldukça önemlidir. Bal arıları bal, propolis, arı sütü gibi ürünlerle ekonomi boyunca önemli bir rol oynamaktadır. Arıcılık, bal, polen, bal mumu gibi ürünler sayesinde ekonomiye büyük bir katkı sağlar. Arı ürünleri sayesinde ekonomiye büyük bir katkı sağlar. Arı ürünleri sayesinde ekonomiye büyük bir katkı sağlar.

Yöntem: Bal arıları ekonomik ve biyolojik yönünden oldukça önemlidir. Bal arıları bal, propolis, arı sütü gibi ürünlerle ekonomi boyunca önemli bir rol oynamaktadır. Arıcılık, bal, polen, bal mumu gibi ürünler sayesinde ekonomiye büyük bir katkı sağlar. Arı ürünleri sayesinde ekonomiye büyük bir katkı sağlar.

Anahtar kelimeler: Kova, Arı, Nosema, Tespit, Bilgisayar destekli laboratuvar, Bal arıları.
INTRODUCTION

Honeybees (Apis mellifera L.) provide not only bee products such as honey, propolis, royal jelly, pollen, bee wax, bee venom to be placed in the market by ensuring the production of World trade (Özbek 2002) but also pollinating the wild flora and industrial crops (Van Engelsdorf and Meixner 2010). However, bee diseases and pests affect beekeeping activities and the quality of the products obtained from beekeeping. Bee diseases and pests spread all over the world in a short time because of the trading of the bees, bee products and beekeeping materials between countries (Öztürk 2001). Also, migratory beekeeping activities are an important factor in the rapid spread of honey bee diseases and pests within the country (Gülpınar 2005). Because of these reasons, honey bee diseases and pests are one of the most important factors that slowing the progress of beekeeping in our country and decrease the efficiency of production (Doğaroğlu 1999).

Nosemosis is a disease caused by microsporidia called Nosema apis and Nosema ceranae, which are quite common in adult honeybees. N. apis was first described by Zander (1909) and has a worldwide distribution (Matheson1996). N. ceranae was reported in 1996 in the Asian honey bee, Apis cerana (Fries et al.1996). Later, Higes et al. (2006) mentioned that A. mellifera in Europe was infected by N. ceranae.

Shortly after, the existence of N. ceranae has been confirmed in the America and Asia (Chauzat et al. 2007, Cox-Foster et al. 2007, Klee et al. 2007, Huang et al. 2007, Paxton et al. 2007, Chen et al. 2008, Sarlo et al. 2008, Williams et al. 2008). Scientists has also been suggested that N. ceranae may replace with N. apis at the same time period (Klee et al. 2007, Martin-Hernandez et al. 2007, Paxton et al. 2007, Higes et al. 2009, Yoshiyama and Kimura 2011). It has been reported by scientists in many countries that N. ceranae has spread all over the world. (Klee et al. 2007, Fries 2010, Higes et al. 2010, Ivgin Tunca et al. 2016, Mohammadian et al. 2019, Shumkova et al. 2020). It was reported that N. apis cause infection in the middle intestinal epithelium of adult bees (Fries et al. 2006, Huang et al. 2007), while N. ceranae infects other tissues and impairs intestinal tissue integrity (Chen et al. 2009, Gisder et al. 2010, Dussaubat et al. 2012).

Nosema spores depend on their hosts to meet the ATP requirement and use transporters to draw energy from the host cell (Paldi et al. 2010). It has been shown that the routine regeneration in the intestines is not possible because of the suppression of the genes that sustain homeostasis in colonies infected with N. ceranae and early death occurs (Dussaubat et al. 2012). Latest, Higes et. al. (2020) examined tissue tropism of N. apis and N. ceranae in worker honey bees as well. It has been shown that the expression of the gene encoding vitellogenin (Vg), a glycolipoprotein produced and stored in the honey bees’ fat body, is significantly reduced in bees infected with N. ceranae (Antunez et al. 2009, Goblirsch et al. 2013, Garrido et al. 2016, Badaoui et al. 2017). Recent studies have shown that N. ceranae C-type nosemosis has been reported to be the most common bee pathogen and has a major impact on global colony losses (Higes et al. 2007, 2010, 2013, Paxton et al. 2007, Cox-Foster et al. 2007, Fries 2010).

The first detection of Nosema disease in Turkey were reported in 1952 (Uygur and Girişkin 2008) and the presence and effects of Nosema disease were first identified by Kutlu (1988) in the Eastern Mediterranean (Adana) and the southwestern Aegean (Muğla). In the following years, molecular studies were carried out the existence of N. apis and N. ceranae by 3 different researchers in the same year. (Utuk et al. 2010, Muz et al. 2010, Whitaker et al. 2010). Later studies, N. ceranae has shown to be more common in our country (Ivgin Tunca et al. 2016, Saribiyik and Özkırım 2018).

In 2020, Tokarev et al (2020) informed that “the family Nosematidae is redefined and includes the genera Nosema and Vairimorpha comprising a monophyletic lineage of Microsporidia”. However, Grupe and Quandt, (2020) also informed this new classification but they used as Nosema in their article in order to evaluate their study with previous literatures. In the current study, it is mentioned as Nosema in order to make a healthy comparison with the existing literatures.

The aim of this study is to determine the presence of Nosema in Muğla province in South West Anatolia. According to Beekeeping Registration System (ACS), there are 80.675 registered beekeepers and bee hives 8.12836 million in 2019 in Turkey. The registered local bee colonies in Muğla are around 1.2 million. During the pine honey production season, the number of bee hives reaches 3-3.5 million colonies with migratory beekeepers from other provinces. 90% of Turkey's pine honey is produced in Muğla. Therefore, Muğla is a very crowded and...
important area for beekeeping activities. Microscopic and molecular diagnosis of Nosema spores were done from worker bee samples collected in different apiaries in 13 districts of Muğla and water samples taken from water sources which were close to apiaries. Thus, the presence and condition of Nosemosis in Muğla, which is one of the important centers for the honey bee sector, was determined by this study.

MATERIAL AND METHODS

Honey bee samples were collected from 152 different apiaries located in 13 districts of Muğla province in the spring and autumn period separately from the local and migratory beekeeping. An average of twenty hives were determined randomly from each apiary. Average five bees were taken from each hive and a total of one hundred bee samples were put into alcoholic tubes from the entrance of these selected hives from in each apiary (Table 1). At least 50 ml of water samples were taken from the 62 water sources (12 of them stable water resources inside the apiaries, rest of them water channels, streams and fountains) used by the bees near the apiaries (Table 2). Field studies were completed in May, April (Spring period) and in October-November (Autumn period) in 2017. Homogenates from honey bee samples were prepared according to the World Organization for Animal Health (OIE) application manual (2008). Detection, counting and calculation of the spores were done with thoma lame under 400x magnified light microscope using OIE terrestrial manual (2008).

Kruskal-Wallis test (SPSS 24/IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) and ANOM analysis (MINITAB 18) were applied in order to determine the differences between sampling locations according to spor numbers.

DNA isolations were made in samples having highest number of spores taken from an average of 3 apiaries with from 13 districts. Commercial isolation kit (PureLink Genomic DNA Mini Kit) was used for the isolation study. 218MITOC For-Rev and 321APIS For-Rev primers were used replicate the relevant gene regions.

Each PCR reaction mixture contained 10 ng DNA, 0.5 U Taq DNA polymerase, 10XPCR buffer, 2.5 M MgCl₂, 0.4 µM 218MITOC F/R, 0.5 µM 321APIS F/R primers and deionized water. Final concentration volume was 20 µl. PCR conditions included initial denaturation at 95°C for 2 min and 35 cycles were performed in 45 seconds at 95°C, 45 seconds at 59.3°C, and 1 minute at 72°C and finally 7 minutes at 72°C. PCR products were controlled in 2% agarose gel (Fries et al. 2013, Martín-Hernández et al. 2007). A total of 35 PCR products were sent to sequencing but 30 of them gave evaluating results. 30 sequence results were analyzed using MEGA 6.0 (Tamura et al. 2013), phylogenetic tree image obtained in SplitsTree (Huson and Bryant, 2006), and Network (Bandelt et al. 1999), haplotype determination was made using the program and sequence similarity analysis was performed via BLAST database.

RESULTS

The different densities of the spores were found in almost all of the samples from different apiaries (Figure 1).

Figure 1. Nosema spores from bee samples at 400x microscope

In general, Nosema spores were found in all samples (100%) from Dalaman. This was followed by Yatağan with 92%, Milas with 90%, Ulua with 84%, Ortaca with 83%, Marmaris with 80%, Köyceğiz with 75%, Bodrum with 70%, Menteşe with 69%, Fethiye with 54%, Seydikemer with 30% and Kavaklıdere with 29% (Table 1). As a result, Nosema spores caused Nosema disease by 71.53±6.02% were found throughout Muğla. The highest number of spores was observed from Yatağan samples and the lowest one was observed for samples from
Seydikemer honeybee samples (Table 1). A total of 62 water samples were examined and an average of 5.16 ± 0.64 water samples were analyzed at each location. Nosema spores were not found in the water samples taken from Menteşe, Marmaris, Dağça, Bodrum, Seydikemer, Ortaca, Köyceğiz and Fethiye (Table 1). However, the spores were determined in one sample from Kavaklıdere, Dalaman and Yatağan, and in 2 samples taken from Milas and there were no water samples from Ula.

Table 1. The number of apiaries where bee samples were collected, Nosema positive apiary number, the rate of the positive apiaries, and Nosema spore numbers, the number of water samples, spore positive water samples, and ratio of positives.

| Location     | Number of sampled Apiaries | Number of spore positive apiaries | Ratio(%) | Spore numbers | Numbers of water samples | Positive water | Ratio(%) |
|--------------|----------------------------|-----------------------------------|----------|---------------|--------------------------|----------------|----------|
| Menteşe      | 13                         | 9                                 | 69.23    | 2.1X10⁶       | 3                        | 0              | 0%       |
| Marmaris     | 10                         | 8                                 | 80.00    | 0.7 X10⁶      | 4                        | 0              | 0%       |
| Milas        | 10                         | 9                                 | 90.00    | 0.8X10⁶       | 7                        | 2              | 28.57%   |
| Dağça        | 12                         | 7                                 | 58.33    | 1X10⁶         | 7                        | 0              | 0%       |
| Dalaman      | 13                         | 13                                | 100.00   | 2.2X10⁶       | 5                        | 1              | 20%      |
| Bodrum       | 10                         | 7                                 | 70.00    | 1X10⁶         | 8                        | 0              | 0%       |
| Seydikemer   | 13                         | 4                                 | 30.77    | 0.3X10⁶       | 9                        | 0              | 0%       |
| Ortaca       | 6                          | 5                                 | 83.33    | 1.9X10⁶       | 6                        | 0              | 0%       |
| Köyceğiz     | 4                          | 3                                 | 75.00    | 1.3X10⁶       | 2                        | 0              | 0%       |
| Ula          | 19                         | 16                                | 84.21    | 1X10⁶         | No sample                |                |          |
| Fethiye      | 22                         | 15                                | 68.18    | 0.5X10⁶       | 3                        | 0              | 0%       |
| Yatağan      | 13                         | 12                                | 92.31    | 9.3X10⁶       | 5                        | 1              | 20%      |
| Kavaklıdere  | 7                          | 2                                 | 28.57    | 2.6X10⁶       | 3                        | 1              | 33.33%   |

Normal distribution tests (Kolmogorov-Smirnov and Shapiro-Wilk) were applied to decide which tests can be performed before starting the statistical analysis of the data obtained from the spore numbers. When all data were evaluated together, it was determined that the data did not show normal distribution (P <0.05; 0.01). The same results were found when normal distribution tests were performed for the measurements made on the basis of the districts where samples were collected. Since the data sets do not show normal distribution, the nonparametric alternative of ANOVA, Kruskal-Wallis Test (SPSS 24) was applied. According to the result of Kruskal-Wallis analysis, there are statistical differences between districts in terms of the number of spores (P <0.01) (Figure 2).

One-Way ANOM test was performed using Minitab 18 program to determine which districts differ in terms of the number of spore (Figure 3). In the graph, the number of spores obtained from Yatağan location (red box) is higher than other districts and have created a statistically significant difference.
Figure 2: Box-plot for the distribution of spores' value in the samples collected areas. Circle and asterix indicated samples having extreme number of spores. The numbers above the circles and asterix are the sample order. The vertical axis shows the spore numbers and the names of locations where samples were collected on the horizontal axis are given.

Figure 3: One-Way ANOM Test (1: Dalaman, 2: Fethiye, 3: Yatağan, 4: Menteşe, 5: Kavaklıdere, 6: Datça, 7: Ula, 8: Seydikemer, 9: Marmaris, 10: Köyceğiz, 11: Ortaca, 12: Bodrum, 13: Milas) Red lines indicate upper and lower boundaries. The blue dots indicate the locations that do not differ, and the red box indicates the location that differs from the general average in terms of the number of spores.
Molecular Analysis Results

Molecular analysis has shown that the observed spores belong to *N. ceranae* and *N. apis* spores were not found in this study. The sequence result of only 30 of the 35 samples, which sent to the DNA sequence, could be evaluated. As a result of the BLAST analysis, the samples showed 94-100% similarity with the *Nosema ceranae* sequences determined in Lebanon, France, Thailand and Morocco samples.

Hit sequence sequences were aligned on the MEGA 6 (Tamura et al. 2013) program. As a result of the Network (Bandelt et al. 1999) analysis, it was determined that all samples formed by 3 haplotypes according to studied sequences for the first time in Turkey. Haplotype analysis was performed to determine common genomic sequences shared by all individuals in the studied populations. The widest frequency was obtained in haplotype 1. The out group was in haplotype 1 and the out group referans sequence was from NCBI gene data bank (GenBank: Accession LC510254.1). According to the data obtained from the sequence results, haplotype 1 was detected from 21 sequenced samples from 13 location in Muğla and one sequence data from out group. Haplotype 2 was detected only in 8 samples belonging to Datça, Yatağan, Ula, Ortaca, Milas and Menteşe locations. Haplotype 3 was detected in only one sample belonging to the Ula location (Figure 4). A philogram constructed in SplitsTree (Huson and Bryant, 2006) was drawn to visualize the genetic similarities or differences identified in the populations studied (Figure 4). According to the phylogenetic tree, the Ula and out group (GenBank: Accession LC510254.1) were in different branch, the other groups from Muğla were located in other main branch.

*Figure 4:* Dendogram from sequenced data for *N. ceranae* and the haplotypes obtained as a result of Network analysis of genes belonging to *N. ceranae*
DISCUSSION

Molecular detection of the animal and human pathogens is known to be more sensitive than microscopic analysis (Fayer et al. 2003, Giersch et al. 2009, Kahler and Thurston-Enriquez 2007, Valencakova et al. 2011). The most reliable way to distinguish and diagnose the *N. ceranae* and *N. apis* is using molecular methods (Gatehouse and Malone 1998, Sagastume et al. 2010, Tay et al. 2005). Nosema disease is effective all over the world as well as in our country. From time-to-time Nosematosis cause significant colony losses. In the current study, Nosema spores were detected from worker honeybees and water sources found in or near the apiaries. Statistical differences between Nosema spores were found to be significant among the sampled locations in Muğla. In the study, it is thought that number of Nosema spores increase in the hive due to heavy rainfall and humidity in the spring. Previous studies have also reported that the linear relationship between Nosema spore density and humidity is statistically significant (Büyük 2016, Tosun 2012, Gisder et al. 2010, Martín-Hernández et al. 2009). Traver and Fell (2012) also mentioned that *N. ceranae* spores have been reported to occur at high levels in spring and low levels in fall and winter. In the study, a very small proportion of the collected water samples were contaminated by nosome spores according to the water analysis. Water samples were collected from apiaries (the bee samples were taken in the same apiary) or near the apiaries. The few number of spores were found in the water samples due to the fact that the water sources were mostly them are flowing water (stream, water channel, fountain, etc.). But nematodes and protozoa species have been observed rather than the spores. In addition, the pollution was observed in the water containers placed in the apiaries in order to meet the water supply fro the bees. Because the water containers were not cleaned and changed frequently enough. At this point, it should be taken into account that bees benefiting from stable water resources may be exposed to other diseases due to dirty containers and water. In the current study, *N. apis* were not found both molecular and microscopic analyses in worker bees and water samples. *N. ceranea* spores were the only spores in both sample types.

The distribution and effects of Nosema disease in Adana and Muğla provinces was carried out by Kutlu (1988) and 15600 worker honey bee samples collected from 312 apiaries were studied as a result of microscopic analyses. Their results showed that the disease level was determined as 31.3% in Muğla, 29.8% in Adana, 29.6% in Dalaman, 28.6% in Aydınl, 25.7% in Datça, 25.0% in Milas, 23.8% in Fethiye, 23.3% in Kıyıceğiz and 20.5% in Marmaris. In present study, the ratio of disease in all Muğla region is 71.53%. A 2.5-fold increase in the percentage of disease is observed from Kutlu’s study in 1988 to 2017 in which our study was conducted. *N. ceranae* has a more severe effect than *N. apis*. The study was conducted in 1988 on the basis of Nosema apis, whereas today *N. ceranae* has an impact on the whole region. This situation shows that the effects of Nosema disease in Muğla region are more serious.

In other study for Muğla region, Nosema was effective in winter and spring periods, and also the disease was the most intense in the Thrace region and Muğla (Başar 1990). Another study investigated the density of *N. apis* on 7820 honeybees between in August 1988–June 1989, they found that Nosema infection prevalence was highest in April–November (Keskın et al. 1996). At different time periods, Nosema spores were determined by morphological method in Muğla (Şimşek 2007, Şimşek et al. 2010). According to different studies, *N. ceranae* was found from the bee samples in Muğla (Whitaker et al., 2010; Utuk et al. 2016; Ivgin Tunca et al. 2016) Sarıbiyik and Özkırım (2018) collected 51 samples from Muğla province in 2 periods including spring and August in Muğla province. In 102, they found *N. ceranea* in 20, *N. apis* in 13, and both spores in 69 samples.

Since molecular techniques were not so widespread in the past, Nosema disease was shown as *N. apis*. On the other hand, *N. ceranae* was thought to infect only *Apis ceranae* until twenty years ago. The later studies revealed that *N. ceranae* also infects western European honey bees. In a recent study in Thailand to understand the biology of *N. ceranae*, the genetic diversity in different hosts (*A. mellifera, A. ceranea*) was investigated using both PCR and genome-based methods, and that *N. ceranae* populations shared many SNPs with other global populations and it was observed to be clonal. However, on the contrary of previous studies, it has been determined that these populations carry many SNPs that are not found elsewhere, and these
populations have evolved in their current geographic location for some time (Peters et. al. 2019).

In current study, Nosema spores have not been detected in flowing water resources as a result of water analysis but it does not mean that there are no spores. This study shows that N. ceranae is widespread in Muğla province. At the same time, the molecular haplotype of N. ceranae gene regions from mediterranean samples were determined for the first time in Turkey. It will be possible to examine the determining role of haplotypes on the wintering ability and reproductive performance of bees with current data.

As a result, beekeeping contributes the economy of the countries directly and indirectly. Bee diseases play effective role in the quality of bee products and the sustainability of colonies. Therefore, periodic monitoring of bee diseases and investigation of their effects is important in terms of sustainable beekeeping activity.

**REFERENCES**

Antúnez, K., Martin-Hernandez, R., Prieto, L., Meana, A., Zunino, P., Higes, M., (2009). Immune Suppression in the Honey Bee (Apis mellifera) Following Infection by Nosema ceranae (Microsporidia), Environ. Microbiol., doi:10.1111/j.1462-2920.2009.01953x.

Badaoui F., Amar A., Hassou L., Zoglat A., Okou CG. (2017). Dimensionality Reduction And Class Prediction Algorithm With Application To Microarray Big Data, Journal of Big Data, 4: 32. https://doi.org/10.1186/s40537-017-0093-4.

Bandelt, HJ., Forster, P., Röhl, A., (1999). Median-Joining Networks For Inferring Intraspecific Phylogenies, Molecular Biology and Evolution, 16:37-48.

Başar, E. (1990). Ülkemizdeki Bal Arılarında (Apis mellifera) Acarapis woodi ve Nosema apis Parazitlerinin Araştırılması, Yüksek Lisans Tezi, Hacettepe Üniversitesi, Fen Bilimleri Enstitüsü.

Büyük, M., (2016). Kırşehir İlindeki Arılarda Nosema Hastalığının Belirlenmesi, Yüksek lisans Tezi Kırşehir Ahi Evran Üniversitesi, Fen Bilimleri Enstitüsü, Zootekni Anabilim Dalı.

Chauzat, MP., Higes, M., Martín-Hernández, R., Meana, A., Cougoule, N., Faucon, JP. (2007). Presence of Nosema ceranae in French Honey Bee Colonies, J. Invertebr. Pathol. 97, 127–128.

Chen, Y., Evans, JD., Smith, IB., Pettis, JS. (2008). Nosema ceranae is a Long-Present And Wide-Spread Microsporidian Infection of the European Honey Bee (Apis mellifera) in the United States, J. Invertebr. Pathol. 97, 186–188.

Chen YP, Evans JD, Murphy C, Gutell R, Zuker M, Gundensen- Rindal D, Pettis JS (2009). Morphological, Molecular, And Phylogenetic Characterization of Nosema ceranae, a Microsporidian Parasite Isolated From The European Honey Bee, Apis mellifera, J Eukaryot Microbiol 56:142–147.

Cox-Foster, DL., Conlan, S., Holmes, EC., Palacios, G., Evans, JD., Moran, NA., Lipkin, WI.
(2007). A Metagenomic Survey of Microbes in Honey Bee Colony Collapse Disorder, Science, 318, 283–287.

Doğaroğlu, M. (1999). Modern Arcılık Teknikleri, Anadolu Matbaa ve Ambalaj San. Tic. Ltd. Sti., İstanbul.

Dussaubat, C., Brunet, JL., Higes, M., Colbourne, JK., Lopez, J., Choi, JH., Martín-Hernández, R., Botias, C., Moritz, RF., Le Conte, Y., Alaux, C., (2012). Gut Pathology And Responses To The Microsporidium, Nosema ceranae in the Honey Bee Apis mellifera, PLoS One, 7(5): e37017.

Fayer, R., Santín, M., Trout, JM., (2003). First Detection Of Microsporidia in Dairy Calves in North America, Parasitol Res 90:383–386.

Fries, I. (2010). Nosema ceranae in European Honey Bees, Journal of Invertebrate Pathology 103, 73-79.

Fries, I., Chauzat, MP., Chen, YP., Doublet, V., Genersch, E., Gisder, S., Higes, M., Mcmahon, DP., Martín-Hernández, R., Natsopoulou, M., Paxton, RJ., Tanner, G., Webster, TC., Williams, GR. (2013). Standard Methods for Nosema research. In: The COLOSS BEEBOOK: Volume II: Standard methods for Apis mellifera Pest and Pathogen Research, Dietemann V, Ellis J.D. & Neumann P, eds. Journal of Apicultural Research, 52 (1):http://dx.doi.org/10.3896/IBRA.1.52.1.1.14

Fries, I., Feng, F., Da Silva, A., Slemenda, SB., Pieniazek, NJ. (1996). Nosema ceranae (Microspora, Nosematidae), Morphological And Molecular Characterization Of A Microsporidian Parasite Of The Asian Honeybee Apis cerana (Hymenoptera, Apidae), European Journal of Protistology 32, 356-365.

Fries, I., Martin, R., Meana, A., García-Palencia, P., Higes, M. (2006). Natural Infections of Nosema ceranae in European Honeybees, Journal of Apicultural Research 45, 230-233.

Garrido, PM., Pornini, MP., Antúnez, K., Branchiccella, B., Martinez-Noel, GMA., Zunino, P., Salerno, G., Eguaras, MJ., Leno, E. (2016). Sublethal Effects Of Acaricides and Nosema ceranae Infection On Immune Related Gene Expression in honeybees, Garrido et al. Vet Res 47:51DOI 10.1186/s13567-016-0335-z

Gatehouse, HS., Malone LA., (1998). The Ribosomal RNA Gene Region of Nosema apis (Microspora): DNA Sequence for Small and Large Subunit rRNA Genes and Evidence of a Large Tandem Repeat Unit Size, J Invertebr Pathol. Mar;71(2):97-105.

GenBank: ACCESSION No LC510254.1 AUTHORS Takashima, S., Ohari, Y. and Itagaki, T. TITLE The prevalence of newly found Nosema species and Nosema ceranae from honeybees, Apis cerana japonica and Apis mellifera in Tohoku region of Japan.

Giersch, T., Berg, T., Galea, F., Hornitzky, M. (2009). Nosema ceranae Infects Honeybees (Apis mellifera) and Contaminates Honey in Australia, Apidologie 40, 117-123.

Gisder, S., Hedtke, K., Möckel, N., Frielitz, MC., Linde, A., Genersch, E. (2010). Five Year Cohort Study Of Nosema spp. in Germany: Does Climates Hape Virulence And As Sertiveness Of Nosema ceranae?, Applied and Environmental Microbiology76,3032–3038.

Grupe, A.C. II., Quandt, C.A. (2020) A growing pandemic: a review of Nosema parasites in globally distributed domesticated and native bees. PLOS Pathogens 16(6): e1008580. https://doi.org/10.1371/journal.ppat.1008580

Goblirsch, M., Huang, ZY., Spivak, M., (2013). Physiological and Behavioral Changes in Honey Bees (Apis mellifera) Induced by Nosema ceranae Infection, https://doi.org/10.1371/journal.pone.0058165

Gülpınar, V. (2005). Bal Arısı Hastalıkları ve Zararlıları, Teknik Arıcılık. 87: 2-7.

Higes M, García-Palencia P, Martín-Hernández R, Aranzazu M. (2007). Experimental Infection of Apis mellifera Honey Bees with Nosema ceranae (Microsporidia), J Invertebr Pathol, 94(3): 211-217.

Higes, M., Martín-Hernández, R., Garrido-Bailón, E., González-Porto, AV., García-Palencia, P., Meana, A., et al. (2009). Honeybee Colony Collapse due to Nosema ceranae in Professional Apiaries, Environmental Microbiology Reports1,110–113.

Higes, M., Martín-Hernández, R., Meana, A. (2006). Nosema ceranae, A New Microsporidian Parasite in HoneyBees in Europe, Journal of Invertebrate Pathology 92, 93–95.
Higes, M., Martín-Hernández, R., Meana, A. (2010). *Nosema ceranae* in Europe: An Emergent Type C nosemosis, Apidologie 41,375–392.

Higes, M., Meana, A., Bartolomé, C., Botiás, C., Martín-Hernández, R. (2013). *Nosema ceranae* (Microsporidia), a Controversial 21st Century Honeybee Pathogen, Environmental Microbiology Reports 5,17–29. doi:10.1111/1758-2229.12024.

Mariano Higes, Pilar García-Palencia, Almudena Urbeta, Antonio Nanetti, Raquel Martín-Hernández (2020). *Nosema apis* and *Nosema ceranae* Tissue Tropism in Worker Honey Bees (*Apis mellifera*) Veterinary Pathology 57(1) 132-138. https://doi.org/10.1177/0300985819864302

Huang, WF., Jiang, JH., Chen, YW., Wang, CH. (2006). Does Infection by *Nosema ceranae* Cause "Colony Collapse Disorder" in Honeybees (*Apis mellifera*), *Apidologie, 38*, 30-37.

Huson, DH., Bryant H. (2006) Application of Phylogenetic Networks in Evolutionary Studies, Mol. Biol. Evol., 23(2):254-267.

İvgin Tunca, R., Oskay, D., Gösterit, A., Tekin, K., (2016). Does *Nosema ceranae* Wipe Out Nosema apis in Turkey? (Short Communication), Iranian Journal of Parasitology, 11(2):259-264.

Kahler, AM., Thurston-Enriquez, JA., (2007). Human Pathogenic Microsporidia Detection in Agricultural Samples: Method Development And Assessment, Parasitol Res 100:529–538.

Keskin, N., Basar, E., Saraçbaşı, T., (1996). Türkiye’nin Bazı Yörelerindeki Bal Arılardında (*Apis mellifera*) Nosema Hastalığı, Hacettepe Fen ve Mühendislik Bilimleri Dergisi, 17, 25-35.

Klee J, Besana A, Genersch E, Gisder S, Nanetti A, Tam DQ, et al. (2007). Wide Spread Dispersal Of The Microsporidium *Nosema ceranae*, An Emergent Pathogen Of The Western Honeybee, *Apis mellifera*, Journal of Invertebrate Pathology, 96,1–10.

Kutlu, MA. (1988). Ergin Balarısı (*Apis mellifera L.*) Hastalığı *Nosema apis*’ın Dağılımı ve Enfeksiyon Oranı Üzerine Bir Araşturma, Yüksek Lisans Tezi, Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Adana, 45 s.

Martín-Hernández, R., Meana, A., García-Palencia, P., Marin, P., Botiás, C., Garrido-Bailon, E., et al. (2009). Effect of Temperature on The Biotic Potential of Honeybee Microsporidia, Applied and Environmental Microbiology, 75, 2554–2557.

Martín-Hernández, R., Meana, A., Prieto, L., Martinez- Salvador, A., Garrido- Bailon, E., Higes, M. (2007). Outcome of Colonization of *Apis mellifera* by *Nosema ceranae*, Applied and Environmental Microbiology, 73, 6331-6338.

Matheson, A. (1996). World Bee Health Update, Bee World, 77: 45–51.

Mohammadani, B., Bokaie, S., Moharrami, M., Nabian, S., Forši, M. (2019). Prevalence of Honeybee Colony Collapse Disorder and Its Relation To *Nosema* spp. and Climate In Apiares Of Ira’, Journal of Veterinary Research, 74(1):11-18 10.22059/JVR.2017.235690.2649

Muz, MN., Girisgin, AO., Muz, D., Aydin, L. (2010). Molecular Detection Of *Nosema ceranae* and *Nosema apis* Infections in Turkish Apiaries With Collapsed Colonies, Journal of Apicultural Research, Vol. 49 (4), 342.

Özbek, H., (2002). Anlar ve Doğa, Uludağ A. Özbek, H., (2002). Anlar ve Doğa, Uludağ A., 2 (3): 22-25.

Öztürk, Al. (2001). Balarısı Hastalıkları, Muğla’da Tarım, 1(5): 57-59.

Paldi N., Glick, E., Oliva, M., Zilberberg, Y., Aubin, L., Pettis, J., Chen, Y., Evans, JD., (2010). Effective Gene Silencing in a Microsporidian Parasite Associated with Honeybee (*Apis mellifera*) Colony Declines, Applied and Environmental Microbiology, 76(17): 5960–5964.

Paxton, RJ. (2010). Does Infection by *Nosema ceranae* Cause “Colony Collapse Disorder” in Honeybees (*Apis mellifera*), Journal of Apicultural Research 49, 80-84.

Paxton, RJ., Klee, J., Korpela, S., Fries, I. (2007). *Nosema ceranae* Has Infected *Apis mellifera* in Europe Since At Least 1998 and May Be More Virulent Than *Nosema apis*. Apidologie, 38,558-565.

Peters, M.J., Suwannapong, G., Pelin, A., Corradi, N., (2019). Genetic and Genome Analyses Reveal Genetically Distinct Populations of the Bee Pathogen *Nosema ceranae* from Thailand. Microbial Ecology 77(4):877-889. doi: 10.1007/s00248-018-1268-z.
Sagastume, S., del Ágila, C., Martín-Hernández, R., Higes, M., Henríques-Gil, N., (2010). Polymorphism And Recombination For rDNA In The Putatively Asexual Microsporidian Nosema ceranae, A Pathogen Of Honey Bees, Environmental Microbiology, 13(1): 84-95. http://dx.doi.org/10.1111/j.1462-2920.2010.02311.x.

Sanbıyık, C., Özkırm, A. (2018), The Transition Ratio of Nosema spp. Spores From Colonies to Honey Versus Honey to Colonies, Journal of Agricultural Science; Vol. 11, No. 1; 2019, 72-80.

Sarlo, E., Medici, SK., Braunstein, M., Eguaras, M., (2008). Presencia Y Distribución De Nosema ceranae En La Región Sudeste De La Provincia De Buenos Aires, In: Actas del Segundo Congreso Argentino de Apicultura, Mar del Plata, Argentina, Agosto 2008, p. 26.

Şimşek, D., Keskin, N., Aktaş, S., (2009) Türkiye, Arıcılık Endüstrisinde Önemli Bir Yere Sahip Olan Muğla'da Nosemosis Üzerine Bir Araştırma, Mellifera, 9, 2-87.

Tay, WT., O’mahoney, EM., Paxton, RJ., (2005). Complete rRNA Gene Sequences Reveal That The Microsporidium Nosema bombi Infects Diverse Bumble Bee (Bombus spp.) Hosts and Contains Multiple Polymorphic Sites, Journal of Eukaryotic Microbiology, 52(6): 505-513. http://dx.doi.org/10.1111/j.550-7408.2005.00057.x.

Tokarev, Y.S., Huang, W. F., Solter, L.F., Malyshev, J. M., Becnel, J. J., Vossbrinck, C. R., (2020) A formal redefinition of the genera Nosema and Vairimorpha (Microsporida: Nosematidae) and reassignment of species based on molecular phylogenetics, Journal of Invertebrate Pathology 169: 107279. doi.org/10.1016/j.jip.2019.107279.

Tosun, O. (2012), Bal Arılarında (Apis mellifera L., 1758) Nosemosis (Nosematosis) Hastalığının Doğu Karadeniz Bölgesinde Bulunan Arı Kolonilerindeki Varlığı, Dağlımlı ve Hastalık Etkenlerinin Karakterizasyonu, Doktora Tezi, Karadeniz Teknik Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji Anabilim Dalı, Trabzon, 112 s.

Traver, BE., Fell, RD. (2012). Prevalence and Infection Intensity of Nosema in Honey Bee (Apis mellifera L.) Colonies In Virginia, J. Invertebr. Pathol., 107, 43–49.

Uygur, ŞÖ., Girişgin, AO. (2008). Bal Arısı Hastalığı ve Zararlıları, Uludağ Arıcılık Dergisi, 8, 4,130-142.

Ütük, A.E., Piskin, F.Ç., Kurt, M. 2010. Türkiye’de Nosema ceranae’nin ilk moleküler tanısı. Ankara Üniversitesi Veterinerlik Fakültesi Dergisi, 57: 275-278.

Ütük, AE., Piskin, F.Ç., Girişgin, AO., Özgür, S., Aydın, L., (2016). Microscopic and Molecular Detection of Nosema spp. In Honeybees of Turkey, Apidologie 47: 267-271.

Van Engelsdorp, D., Meixner, MD., (2010). A Historical Review of Managed Honey Bee Populations in Europe and the United States and the Factors that May Affect Them, Journal of Invertebrate Pathology, 580-595.

Valencakova, A., Balent, P., Ravaszova, P., Horak, A., Obornik, M., Halanova, M., Malcekova, B., Novotny, F., Goldova, M., (2011). Molecular Identification and Genotyping Of Microsporidia in Selected Hosts, Parasitol Res. doi:10.1007/s00436-011-2543-9.

Whitaker, J., Szalanski, AL., Kence, M. (2010). Molecular Detection of Nosema ceranae and N. apis from Turkish Honey Bees, Apidologie doi:10.1051/apido/2010045.

Williams, GR., Shafer, ABA., Rogers, REL., Shutler, D., Stewart, DT. (2008). First detection of Nosema ceranae, A Microsporidian Parasite of European Honeybees (Apismellifera), in
Canada and Central USA., Journal of Invertebrate Pathology, 97, 189–192.

Yoshiyama, M., Kimura, K. (2011). Distribution of *Nosema ceranae* in the European Honey Bee, *Apis mellifera* in Japan, Journal of Invertebrate Pathology, 106, 263–267.

Zander, E. (1909). Tierische Parasiten als Krankheitserreger bei der Biene. Münchener Bienenztg, 31, 196–204.