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RESEARCH ARTICLE

Investigation of *Nosema apis* and *Nosema ceranae* in bees by Multiplex PCR in Kırıkkale Region

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Kırıkkale Yöresi Arılarında *Nosema apis* ve *Nosema ceranae*'nin Multiplex PCR ile Araştırılması

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Öz

Amaç: Bu çalışmanın amacı, Kırıkkale yöresindeki bal arılarında Nosemosis varlığının ve yaygınlığının Multiplex PCR ile belirlenmesidir.

Gereç ve Yöntem: Kırıkkale İl Merkezi, Bahşili, Balışeyh, Keskin, Sulakyurt ve Yahşihan ilçelerinde bulunan 52 adet arılıktan numuneler alınmıştır. Her 5 koloniden toplanan 10 arının göğüs ve karın kısımları diseke edilerek porselen havanda steril fizyolojik su ile ezildi. Nosema sporları içeren numuneler Safranin ile boyandı ve aynı numunelere tür teşhisi için multipleks PCR uygulandı.

Bulgular: *Nosema* spp. sporları, 52 arılığın 12'sinde (%23,07) mikroskobik olarak gözlenmiştir. Pozitif örnekler arasında en yüksek oran %57,14 ile Balışeyh ilçesinde bulundu. Balışeyh ilçesini %33,33 ile Delice ilçesi ve %23,07 ile Kırıkkale/Merkez ilçesi izlemektedir. Bahşili, Keskin, Sulakyurt ve Yahşihan ilçelerinden alınan örneklerde Nosema sporlarına rastlanmadı. Genel olarak ilçelerin enfeksiyon oranları Merkez (%5,76), Balışeyh (%7,69), Delice (%9,61) olarak belirlendi. Tüm pozitif örnekler multipleks PCR ile *N. ceranae* olarak tespit edildi.

Öneri: Bu Kırıkkale yöresi bal arılarında Nosemosis varlığı, yaygınlığı ve etiyolojik ajanı olan *N. ceranae*'nin belirlendiği ilk çalışmadır. Bu çalışmada elde edilen veriler doğrultusunda bölgedeki arı kayıplarında Nosemosisin mutlaka göz önünde bulundurulması ve arıcıların konu hakkında bilinçlendirilmesi gerektiği kanaatine varılmıştır.

Anahtar kelimeler: *Apis mellifera*, Multiplex PCR, *N. apis*, *N. ceranae*, Kırıkkale

Abstract

Aim: The aim of this study was conducted to determine the presence and prevalence of Nosemosis in honeybees in Kırıkkale region by Multiplex PCR.

Materials and Methods: Samples were collected from 52 apiaries in Kırıkkale City Center, Bahşili, Balışeyh, Keskin, Sulakyurt and Yahşihan districts. The thorax and abdomen of ten bees collected from every 5 colonies were dissected and crushed with sterile physiological water in a porcelain mortar. The samples with Nosema spores were stained with Saffron and the multiplex PCR was applied to detect specimens to the same samples.

Results: *Nosema* spp. spores were observed in 12 out of 52 apiaries (23.07%) microscopically. Among the positive samples, Balışeyh county was found with the highest rate of 57.14%. The township district is followed by Delice district with 33.33% and Kırıkkale /Center district with a ratio of 23.07%. Nosema spores were not found in samples taken from Bahşili, Keskin, Sulakyurt and Yahşihan districts. In general, the infection rates of the districts were determined as Central (5.76%), Balışeyh (7.69%), and Delice (9.61%). All the positive samples were detected as *N. ceranae* by multiplex PCR.

Conclusion: This is the first study to determine the presence and prevalence of Nosemosis in honey bees in the Kırıkkale region and its etiological agent, *N. ceranae*. In line with the data obtained in this study, it was concluded that Nosemosis should be taken into consideration in bee losses in the region and beekeepers should be made aware of the issue.

Keywords: *Apis mellifera*, Multiplex PCR, *N. apis*, *N. ceranae*, Kırıkkale.

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Introduction

Nosemosis is seen in adult honey bees, a microsporidian disease caused by *Nosema apis* (*N. apis*) and *Nosema ceranae* (*N. ceranae*). Microsporidia are obligatory intracellular organisms (Fries 2010). *Nosema apis* was found in the European honeybee, *Apis mellifera* (*A. mellifera*), while *N. ceranae* was specific for the Asian honeybee, *Apis cerana*. However, it has been reported in recent years that *N. ceranae* is the most common species in *A. mellifera* worldwide (Paxton et al 2007). By using PCR and RFLP methods, It is reported that *N. ceranae* was observed in hypopharyngeal glands, brains, guts, malpighian tubules, and fat bodies except for muscle (Chen et al 2009, Gisder et al 2010), while *N. apis* was detected only in the midguts of infected honey bees. Therefore, *N. ceranae* is quite a pathogen species. Nosemosis causes significant economic losses in which honey and the other bee products. This disease shortens the life span of bees at the same time (Tlak Gajger et al 2010). Recently, honey bee colonies in the Colony Collapse Disorder (CCD) cause the causative agent is stated to be *N. ceranae* (Higes et al 2007, Martín-Hernández et al 2007). *Nosema apis* spores primarily transmissions between bees by an oral-fecal route within hives, during contaminated water consumption, food by trophallaxis exchange, or clean contaminated comb. *Nosema ceranae* transmissions with a similar route to *N. apis* and the other a route may be between hives by drifting worker bees, especially drones. Drifting behavior causes colony contamination when return to the wrong hive after leaving their parent colony (Free 1958). Traver and Fell (2011) reported that the drifting behaviour of drones could cause the spread of *N. ceranae* to other hives in an apiary.

Our study aimed to determine the Nosema infection in honey bees in Kırıkkale and to determine which Nosema species were present if any.

Material and Methods

The samples were taken from bee colonies with different pathological problems like depopulation, leanness, or high colony mortality. Samples, especially during the first spring after wintering inspection of colonies, were collected from dead bees. Each common sample has one apiary.

Sample collection

Honeybees were collected between September-October 2014 and March-June 2015 from 52 apiaries in Kırıkkale City Center, Bahşili, Ballışeyh, Delice, Keskin, Sulakyurt, and Yahşihan districts where colony losses were obtained as anamnesis result. The samples were brought to the Laboratory of Parasitology Department of the Veterinary Faculty of Kırıkkale University for microscopic examination. All bee samples were kept at 5 minutes, -20 °C for death.

Microscopic examination

The thorax and abdomen of ten honey bees *(A. mellifera)* collected from every 5 colonies were dissected and were macerated in 3 ml sterile physiological water. A few drops of the suspension were placed on a slide under a cover slip and examined microscopically at X40 magnification under the light microscope. In the case of positivity, 1 ml of suspension was filtered and centrifuged for 5 min. at 3000 rpm and supernatants were removed. The samples with Nosema spores were stained with Saffron (Figure 1). The remaining spores were stored at -20 °C until DNA extraction (Martín-Hernández et al 2007).

DNA extraction

After removal of the supernatant, DNA extraction was performed from the sediment at the bottom using a tissue kit (Fujifilm QuickGene Genomic DNA Kit S (DT-S) KURABO, Japan) by a semi-automated QuickGene-Mini80 Nucleic acid isolation device (Autogen / FujiFilm Corporation, Tokyo, Japan). The extracts were stored at –20 °C until the multiplex PCR (Martín-Hernández et al 2007) .

Polymerase Chain Reaction (PCR)

The specific primer pairs of the 16S rRNA gene of N. *ceranae* and *N. apis* were used in PCR, which was designed for multiplex PCR by Martin-Hernandez et al (2007), and the characteristics of the primers are presented in Table 1. PCR was carried out in a final volume of 50 µl, containing 26.15 µl of DNase- and RNase-free sterile distilled water, 5 µl of 10X PCR buffer (Thermo Scientific, Lithuania), 5 µl of 25 mM MgCl2, 0.6 µl of 10 mM dNTP mix, 2 µl of each

Table 1. Primers used in Multiplex PCR and their specialty (Martin-Hernandez et al 2007).

Primers	Primer sequence (5' - 3')	Amplification target	Length of amplicon (bp)
218MITOC-FOR	CGGCGACGATGTGATATGAAAATATTAA	16S rRNA	218-219
218MITOC-REV	CCCGGTCATTCTCAAACAAAAAACCG	(N. ceranae)	
321APIS-FOR	GGGGGCATGTCTTTGACGTACTATGTA	16S rRNA	321
321APIS-REV	GGGGGGCGTTTAAAATGTGAAACAACTATG	(N. apis)	

primer (40 pmol), 5 µl of template DNA, and 0.25 µl of Taq DNA polymerase (5U/µl) (Thermo Scientific, Lithuania). The PCR conditions were as follows: 2 min at 95 °C (initial denaturation), 35 cycles of 1 min at 95 °C, 1 min at 50 °C, and 1 min at 72 °C, and finally 5 min at 72 °C (final extension). The PCR products were separated on agarose gels (1.5%) and 1X TBE (Tris-borate- EDTA), stained with 5 µl ethidium bromide (Sigma, Germany), and visualized and photographed on a UV transilluminator (Nyxtechnik Illuminyx, USA) (Figure 2) (Martín-Hernández et al 2007).

Results

Microscopic examination revealed that 12 out of 52 (23.07%) apiaries were *Nosema* spp. spores positive. Among the positive samples, Balışeyh province was found with the highest rate of 57.14%. The district of Balışeyh is followed by the town of Delice with a rate of 33.33% and the Kırıkkale/ Central district with a rate of 23.07%. Nosema spores were not found in the samples taken from Bahşili, Keskin, Sulakyurt and Yahşihan districts. In general, the infection rates of the districts were determined as Central (5.76%), Balışeyh (7.69%), and Delice (9.61%). *Nosema* spp. which in staining with 1% Saffron (Figure 1) is red.

Multiplex PCR results

Specimens were identified by multiplex PCR in 12 specimens with a positive microscopic appearance. *Nosema apis* with a product size of 321 bp, product size 218-219 bp N. ceranae according to the multiplex PCR result, it was determined that the causative agent of Nosemosis was *N. ceranae*, which is more dangerous than that of *N. apis* (Figure 2).

Discussion

Nosemosis agents, which are microsporidia entomopathogens in honey bees, are *N. apis* and *N. ceranae. Nosema apis* European honey bees (*A. mellifera*), and *N. ceranae* is the parasite of Asian (*A. cerana*) and European honey bees. Both parasites are responsible for cross-infection between host species. Recently, *N. ceranae* has also been detected in European honey bee populations in Europe, South and North America, and Asia. It is not known what kind of disorders *N. cerenae* causes in *A. mellifera* bees. The two species look similar, but it is known that *N. ceranae* is very sensitive to low temperatures and can reproduce even at high temperatures (OIE 2013).

In recent years, serious colony losses have been observed in honey bees in the world, and it has been emphasized that viruses, *N. ceranae*, and *Varroa destructor*, loss of habitat, insufficient floral resources, and chemical use cause serious colony loss syndrome (CLS) in bees (Paxton 2010, Botías et al 2013, Muz and Muz 2017).

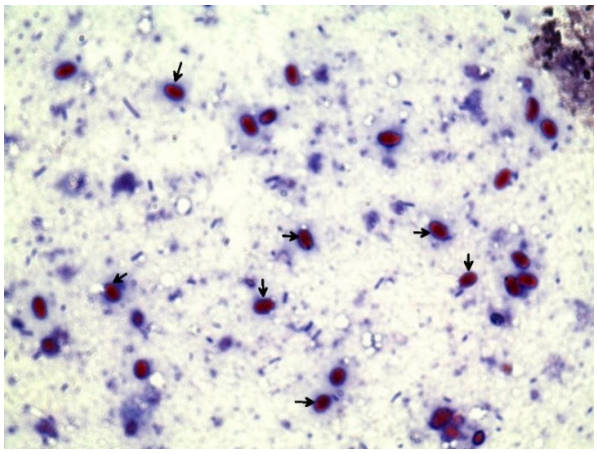

Figure 1. Staining with Saffron 100X (Original)

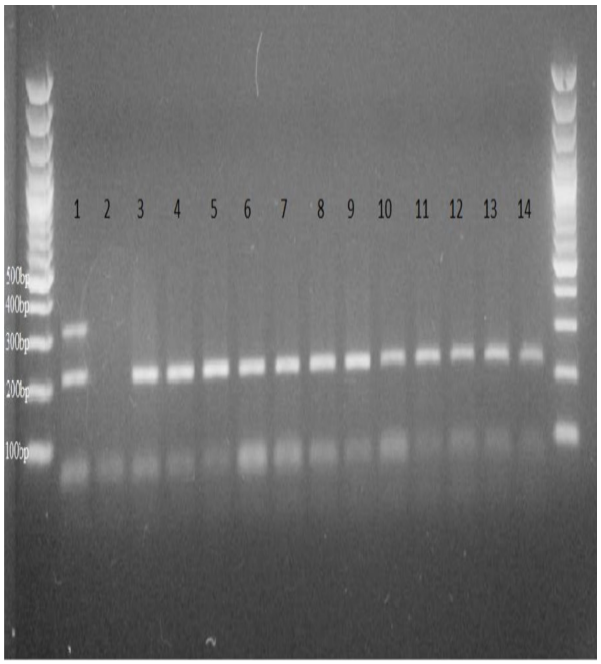

Figure 2. M: Marker (100 bp DNA ladder, Thermo Scientific Gene Ruler 100 bp Plus, Lithuania), 1: Positive controls of *N. apis* and *N. ceranae*, 2: Negative control (sterile ultra purity water), 3: 3-14 samples with *N. ceranae* positive

The fact that Nosemosis is considered to be a serious problem in the other countries because CCD has hit the beekeeping sector in the other countries and led to numerous studies on the specific species separation at the molecular level in the samples identified in almost every country in this area. The epidemiology of parasites is created and the importance of treatment, protection, and control is emphasized. From these studies, in the past decade's analysis of old bee specimens in Finland, only *N. apis* infection was seen, whereas the subsequent samples showed mixed infection of *N. apis* and *N. ceranae* or only *N. ceranae* infections (Paxton et al 2007). If *N. ceranae* tends to replace *N. apis*, this suggests the possibility that a large number of microsporidian parasites may occur in honey bees (Fries 2010). One hundred thirty-five of 150 specimens

from different localities in Croatia-Zagreb were found to be positive by microscopic examination, and multiplex PCR, and 100% of them were diagnosed as *N. ceranae* (Tlak Gajger et al 2010). This confirms the advantages of molecular methods. The benefits of molecular methods are increased sensitivity, specificity, and perhaps most importantly, the ability to identify all stages of development of *Nosema* spp. (Martín-Hernández et al 2007). In the research conducted in many countries has been generally seen that most *N. ceranae* species. In contrast, *N. apis* in Slovakia was 14.3% and *N. ceranae* was 8.5% (Staroň et al 2012).

In Turkey, the molecular biological diagnosis of *N.ceranae* and *N. apis* was made for first time by Muz (2009). Later, three studies were conducted in 2010 (Muz et al 2010, Ütük et al 2010, Whitaker et al 2010). Whitaker et al (2010) detected for first time intra-species differences from these studies. These investigators, *N. ceranae* and *N. apis* species in the samples have identified 16S SSU 208 bp by examining the differences in intra-species and explained that there is no difference. The number of molecular studies of this disease in Turkey because of the spread of the agent all over our country and the serious losses in honey bees, researchers are increasingly attracting attention to this problem and improving the awareness of conservation and control methods in beekeepers (Muz et al 2010, Ütük et al 2010, Whitaker et al 2010, Muz et al 2012, Tosun 2012, Ütük et al 2016, Büyük et al 2017, Muz and Muz 2017, Oğuz et al 2017). CCD is also experienced in our country, similar studies were also carried out in Turkey, as in other countries, the dominant species was observed in data obtained in *N. ceranae* (Şimşek 2005, Muz et al 2012, Tosun 2012, Yaman et al 2015, Ütük et al 2016, Büyük et al 2017, Muz and Muz 2017, Oğuz et al 2017). In this study, only *N. ceranae* was found to support data from other studies. *Nosema apis* infection in the honey bees *A. mellifera* worldwide is replaced by *N. ceranae*. This confirms the idea that this infection could be spread by bees brought from different places to make bee trade and hobby beekeeping.

Kırıkkale province is located at 39.846821 north latitude and 33.525251 east longitude. In Kırıkkale province where the continental climate is dominant, winters are cold and summers are hot and dry. There are very few beekeepers in our city, usually, stationary beekeeping is carried out. Beekeepers mostly do not consist of professional beekeepers. Most of our beekeepers are retired individuals who have between 50 and 100 colony bees and beekeeping is an additional income or hobby. The knowledge of beekeepers in the region is not sufficient for the diagnosis and treatment of Nosemosis. This situation increases the cross-contamination between the apiaries. This study has been conducted for the first time in Kırıkkale because of the absence of any data about this infection in honey bees. The infection rate of 23.07% with the microscopic examination was consistent with that of Kırşehir (21.56%) (Büyük et al 2017). It is thought that this is because Kırşehir is close to Kırıkkale and it has the same climate. Among the positive samples, Balışeyh district was determined with the highest concentration of 57.14%. The district of Balışeyh is followed by the town of Delice with a rate of 33.33% and the Kırıkkale/Merkez district with a rate of 23.07%. When the province was taken into consideration, the infection rate of the districts was determined as Central (5.76%), Balışeyh (7.69%), and Delice (9.61%). The reason Delice's infection is intensive, showing a great interest in beekeeping a large portion of the population, it is an appropriate district's flora, a passing hobby from father to son, and is due to the continuation of additional sources of income. PCR-Restriction Fragment Length Polymorphism Analysis (PCR-RFLP) is the best method in terms of type distinction and it gives 100% accurate results according to the multiplex PCR method, it provides ease of diagnosis in multiple samples, but this method takes a too long time and is expensive. If 100% correct results are required, the preferred method is PCR-RFLP (Stevanovic et al 2011). Klee et al (2007) have developed a method to rapidly differentiate the partial SSU rRNA gene region with PCR-RFLP and make a distinction between *N. apis* and *N. ceranae* and the accuracy of this method has been confirmed by the sequencing of 29 isolates from around the world. In several studies in Turkey, 16S rRNA was used as the target gene region (Muz et al 2010, Ütük et al 2010, Tosun 2012, Utuk et al 2016, Büyük et al 2017, Muz and Muz 2017, Oğuz et al 2017). In this study, the species were separated by using the same gene region and as a result, *N. ceranae* was detected in all 12 samples. It was concluded that the multiplex PCR method in single or mixed infections, single-stage, easy and economical is more advantageous than PCR-RFLP (Ütük et al 2010). In addition, Ütük et al (2010) primary pair and multiplex PCR method designed by Martin-Hernández et al (2007) stated that OIE (World Animal Health Organization) is a recommended method for the diagnosis of Nosemosis.

Conclusion

Nosema ceranae infection was dominant in temperate climates compared to warmer regions in both America and Asia. However, *N. apis* may be more common in cold climates. However, it is not clear whether this temperature difference is effective in spreading from north to south. It was even explained that *N. ceranae* could eliminate European honey bee *A. mellifera* (Fries 2010). The infection rate was reported to be the highest in June and July (Tosun 2012). These studies were performed during the spring season when diarrheal events and deaths were most common. The presence of only *N. ceranae* in positive samples confirms the opinion of Fries (2010) and Tosun (2012) when the air temperature is observed to increase in months. In Turkey, the dominant species in other studies on this subject, it has been found *N. ceranae*. The fact that this may be due to the

effect of global warming in our country and the fact that our bees are against the risk of extinction is revealed. This situation is not only for our country but also for all countries of the world. This study was carried out for the first time in Kırıkkale to determine the presence, distribution, and type of Nosemosis. It is thought that it will contribute to future studies in our country.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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References

Botías C, Martin-Hernández R, Barrios L, Meana A, et al., 2013. *Nosema* spp. infection and its negative effects on honey bees (*Apis mellifera iberiensis*) at the colony level. Vet Res, 44, 25.

Büyük M, Tunca İvgin R, Taşkın T, 2017. Kırşehir ilindeki arılıklarda nosema hastalığının belirlenmesi. TURJAF, 5(1), 1-5.

Chen YP, Evans JD, Murphy C, Gutell R, et al., 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.

Free JB, 1958. The drifting of honey-bees. J Agric Sci, 51, 294–306.

Fries I, 2010. *Nosema ceranae* in European honey bees (*Apis mellifera*). J Invertebr Pathol, 103, 573–579.

Gisder S, Hedtke K, Möckel N, Frielitz MC, et al., 2010. Five-year cohort study of *Nosema* spp. in Germany: does climate shape virulence and assertiveness of *Nosema ceranae*? Appl Environ Microbiol, 76, 3032-3038.

Higes M, Garcia-Palencia P, Martin-Hernandez R, Meana A, 2007. Experimental infection of *Apis mellifera* honeybees with *Nosema ceranae* (Microsporidia). J Invertebr Pathol, 94(3), 211-217.

Klee J, Besama AM, Genersch E, Gisder S, et al., 2007. Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, Apis mellifera. J Invertebr Pathol, 96, 1-10.

Martín-Hernández R, Meana A, Prieto L, Salvador AM, et al., 2007. Outcome of colonization of *Apis mellifera* by *Nosema ceranae*. Appl Environ Microbiol, 73(20), 6331-6338.

Muz MN, 2009. *Nosema ceranae* ve *Nosema apis*'in PCR ve RFLP ile tespiti. 16. Ulusal Parazitoloji Kongresi, 1-7 Kasım 2009, Adana.

Muz MN, Girişgin AO, Muz D, Aydın L, 2010. Molecular detection of *Nosema ceranae* and *Nosema apis* infections in Turkish apiaries with collapsed colonies. J Apicultural Res, 49, 342-344.

Muz MN, Solmaz H, Yaman M, Karakavuk M, 2012. Kış salkımı erken bozulan arı kolonilerinde paraziter ve bakteriyel patojenler. YYU Vet Fak Derg, 23(3), 147–150.

Muz D, Muz MN, 2017. Tekirdağ'da "Koloni Kaybı Sendromu" benzeri kayıp görülen arılıklarda bazı patojenlerinin araştırılması. Kocatepe Vet J, 10(1), 21-28.

OIE Terrestrial Manual, 2013. Nosemosis of Honey Bees, pp: 1-6.

Oğuz B, Karapınar Z, Dinçer E, Değer MS, 2017. Molecular detection of *Nosema* spp. and black queen-cell virus in honeybees in Van province, Turkey. Turk J Vet Anim Sci, 41, 221-227.

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.

Paxton RJ, 2010. Does infection by *Nosema ceranae* cause "Colony Collapse Disorder" in honey bees (Apis mellifera)? J Apic Res, 49(1), 80-84.

Staroň M, Jurovčíková J, Čermáková T, Staroňová D, 2012. A scientific note on incidence of *Nosema apis* and *Nosema ceranae* in Slovakia during the years 2009 and 2010. Slovak J Anim. Sci, 4(1), 36-38.

Stevanovic J, Stanimirovic Z, Genersch E, Kovacevic SR, et al., 2011. Dominance of *Nosema ceranae* in honey bees in the Balkan countries in the absence of symptoms of colony collapse disorder. Apidologie, 42, 49–58.

Şimşek H, 2005. Elazığ yöresi bal arılarında bazı parazit ve mantar hastalıklarının araştırılması. Ankara Üniv Vet Fak Derg, 52, 123-126.

Tlak Gajger I, Vugreg O, Grilec D, Petrinec Z, 2010. Prevalence and distribution of *Nosema ceranae* in Croatian honeybee colonies. Vet Med, 55(9), 457–462.

Tosun O, 2012. Bal arılarında (*Apis mellifera* L., 1758) Nosemosis (Nosemosis) hastalığının Doğu Karadeniz Bölgesi'nde bulunan arı kolonilerindeki varlığı, dağılımı ve hastalık etkenlerinin karakterizasyonu Doktora Tezi,

Karadeniz Teknik Üniversitesi Fen Bilimleri Enstitüsü, Trabzon.

Traver BE, Fell RD, 2011. *Nosema ceranae* in drone honey bees (*Apis mellifera*). J Invertebr Pathol, 107, 234-236.

Utuk AE, Piskin FC, Girisgin AO, Selcuk O, et al., 2016. Microscopic and molecular detection of *Nosema* spp. in honeybees of Turkey. Apidologie, 47, 267-271.

Ütük AE, Pişkin FÇ, Kurt M, 2010. Türkiye'de *Nosema ceranae*'nın ilk moleküler tanısı. Ankara Univ Vet Fak Derg, 57, 275-278.

Whitaker J, Szalanski AL, Kence M, 2010. Molecular detection of *N. ceranae* and *N. apis* from Turkish honey bees. Apidologie, 42(2), 174-180.

Yaman M, Yarılgaç EŞ, Güner BG, Ertürk Ö, 2015. Ordu yöresi bal arılarında Nosemosis hastalığının varlığı ve yayılışı. Türkiye Parazitol Derg, 39, 47-51.

Author Contributions

Motivation/Concept: MA; Design: MA; Control/Supervision: MA; Processing: MA; Data Collection and/or Processing: ACY; Analysis and/or Interpretation: MA; Literature Review: MA; Writing the Article: MA; Critical Review: MA

Ethical Approval

Kırıkkale University Experimental Research and Application Center, Animal Experiments Ethics Committee 27.02.2014, 14/51 Number Ethics Committee Decision.