Bee Studies 13(2), 39-42
http://doi.org/10.51458/BSTD.2021.16

RESEARCH PAPER

A Seasonal Perspective of Nosemosis; the Presence of *Nosema* spp. in Honeybee Colonies During Summer Months in Ankara, Turkey

Ahmet Mahmut Alperen[1,*], Sedat Sevin[2], Nafiye Koç[3]

[1] Ankara University, Veterinary Medicine, Ankara, Turkey
[2] Ankara University, Veterinary Medicine Department of Pharmacology and Toxicology, Ankara, Turkey
[3] Ankara University, Veterinary Medicine Department of Department of Parasitology, Ankara, Turkey

Article History
Received 17 November 2021
Accepted 12 December 2021
First Online 28 December 2021

***Corresponding Author**
Tel.: +905075420231
E-mail: ahmetalperen86@gmail.com

Keywords
Apis mellifera L.
Nosema spp.
Seasonal effect
Summer
Ankara

Abstract

Nosemosis is known as a serious disease of adult honey bees, *Apis mellifera* L. (Hymenoptera: Apidae) caused by *Nosema apis* and *N. ceranae* which are obligate intracellular microsporidian parasites. The parasites infect the epithelial cells of honey bee ventriculum and lead to critical changes in midgut mucosa that cause digestive and metabolic disorders. Accordingly, the infestation causes the death of adult honey bees and leads to great economic losses for beekeeping industry worldwide. Seasonal patterns of nosemosis are consistent and mostly observed in the spring or autumn months, with the highest spore counts and viability. The aim of this work was to evaluate the seasonality of nosemosis in the light of previous literature and specifically investigate the presence of *Nosema* spp. during the summer season including June, July, and August from various locations in Ankara. Honey bee individuals were collected from 80 apiaries located in 14 different areas in Ankara. The samples were analyzed from pools of ten adult honey bees per population using digestion methods. Before analysis, the anesthesia was induced by cold (30 sec, -80 ℃) on the bees. According to obtained data, 12 out of 80 (15%) sampled apiaries were infected with *Nosema* spp. spores. Infected apiaries were mainly located in the central and north parts of Ankara. Results show nosemosis might be detrimental to honey bee colonies and its productivity in the summer months. Therefore, the treatment might be needed when infections of *Nosema* spp. reach to an infectious level even in summer.

Introduction

Microsporidia are eukaryotic, obligate intracellular parasites that invade vertebrates and invertebrates. They are spore-forming organisms and classified as fungi. Spores are the infective stage of microsporidian parasites and keep them surviving outside of the host (Adl et al., 2005; Higes et al., 2006). The western honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), is mostly infected by two microsporidia species, *Nosema ceranae* (Fries et al., 1996) and *Nosema apis* (Zander, 1909) (Microsporidia: *Nosema*tidae), causing *Nosema* disease. Although *N. ceranae* and *N. apis* both infect honey bees, *N. ceranae* dominates its distribution geographically in the world (Fries et al., 2006; Higes et al., 2013b).

Nosemosis is known as one of the most significant diseases of adult honey bees. The infections possess decreasing honey production, foraging behavior, and pollination productivity (Higes et al., 2006). In severe cases, it can cause bee mortality and even colony collaps (Cox-Foster et al., 2007; Higes et al., 2008; Martín-Hernández et al., 2007).

Nosema spp. are transmitted orally with contaminated nectar, pollen, honey, and bee feces (Smith, 2012). When the spores of *N. ceranae* reach the infected level, infections lead to cytopathological changes in the midgut epithelial cells by degenerating columnar cells, disrupting microvilli, damaging goblet cells. Additionally, *N. ceranae* inhibits programmed cell death, apoptosis by inducing the genes involved in apoptosis in order to prevent cell death to limit pathogen growth (Ceylan et al., 2019; Higes et al, 2013a; Kurze et al., 2018; Martín-Hernández et al., 2017).

Since the first description of *N. ceranae* in 1996 (Fries et al., 1996), the prevalence, distribution, and seasonality have been determined and showed variability of seasonal patterns in the world. However, seasonal patterns of Nosemosis are consistent and mostly observed in the spring or autumn months, with the highest spore counts and viability (Brenna et al., 2012; Gisder et al., 2010; Traver and Fell, 2011). On the other hand, a study from Spain reported that *N. ceranae* raised the number of winter hive losses (Martín-Hernández et al., 2007). Rarely, consistent high levels of summer infection prevelance were demonstrated (Pernal et al., 2010). Many significant gaps remain in our understanding of the variations in seasonal patterns of Nosemosis.

This study investigates the seasonal pattern of *Nosema* spp. infection in the light of literature and focuses on the presence of Nosemosis in honey bee colonies during the summer months in Ankara, Turkey.

Materials and Methods

Collection of Bee Samples

Samples were requested from beekeepers in the different locations of Ankara, which are registered in the Bee Registration System. Adult bee samples collected in the study were taken from a total of 80 hives from 23 beekeepers who agreed to participate in the study from Ankara districts (Ayaş, Bala, Beypazarı, Çankaya, Çubuk, Gölbaşı, Güdül, Kalecik, Kazan, Kızılcahamam, Nallıhan, Polatlı, Haymana, Yenimahalle). Sample was taken once from the hives in which beekeepers thought there was a disease and deaths were seen in June, July and August months. In order to detect the presence of nosemosis, 50 bee samples per hive were taken from the outermost frame of the hive.

Detection and Counting of *Nosema* spores

Counting was performed with a Neubauer slide for the detection of *Nosema*. For the preparation of samples for *Nosema* counting, 10 bees were randomly taken from the groups after inducing the anesthesia by cold (30 sec, -80 ℃) (Tutun et al., 2020). The abdomens of the bees were separated from their bodies with the help of forceps and collected in a container. Abdominal pieces collected in the container were crushed to allow the intestinal contents to come out. A homogeneous mixture was obtained as a result of crushing using 1 mL of distilled water per bee. Then, the body parts were separated by filtering with 3 layers of gauze patch. Furthermore, the mixture was placed in 15 mL centrifuge tubes and centrifuged at 5000-6000 rpm for 10 min. The supernatant was removed from the tube and counting was performed by adding 1 mL of distilled water per bee (Güzerin, 2013; Terrestrial Manual of the OIE, 2018).

Safranin (1%) and Giemsa (5%) stained smears were prepared for a more detailed examination. For both methods, the smears were firstly fixed with 100% methanol. Then, they were stained with Safranin and Giemsa, 30 and 45 min., respectively. Stained smears were rinsed with water, air-dried, and examined under a light microscope with 100x objective and immersion oil.

Results

Within the scope of this research, bee samples were collected from different locations in Ankara to determine the level of nosemosis infestation in the summer. *Nosema* agent was found to be positive in 12 out of 80 hives in total, and the infestation rate was determined to be 15% in the collected samples. Nosemosis positive samples were obtained from Çubuk, Gölbaşı, Kalecik, Kazan, Kızılcahamam and Yenimahalle districts (Figure 1).

Discussion

Although Nosemosis caused by *N. cerenae* and *N. apis* is known as a serious disease of adult honey bees worldwide, some gaps still remain in our understanding

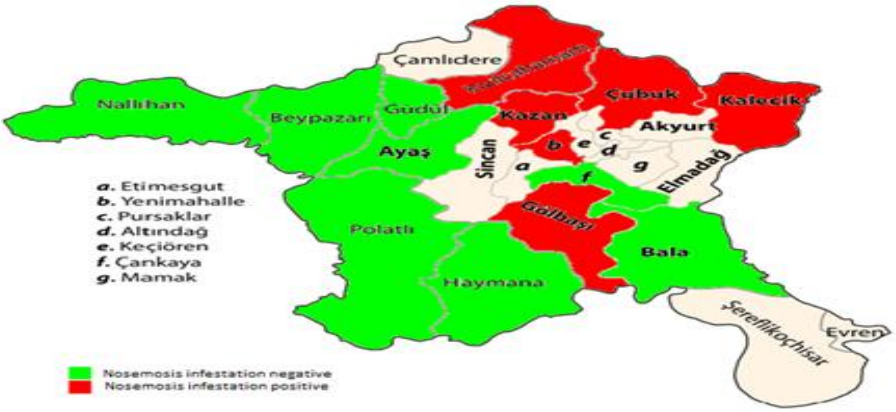

Figure 1: Map of sampling locations of Honey bee populations from beekeeping areas in Ankara. The colors, green, red indicate the positive and negative samples, respectively.

of their biology, particularly its seasonal features. Therefore, the current study aimed to provide information about the seasonal features of nosemosis, focusing on summer status in Ankara, Turkey. The occurrence of *Nosema* infection was first described in 1986 in Turkey (Tutkun & Inci, 1992), and, so far, the infestation rate has been reported in different ratios such as 26.4%, 60%, 45.8% in 1990, 2005, 2016 respectively (Kutlu & Kaftanoğlu, 1990; Aydın et al., 2001; Özkırım et al., 2019). One of the studies conducted in Ankara showed the existence of nosemosis and 93.75% prevalence of *N. ceranae* and 6.25% *N. apis* infection (Utuk et al., 2016).

The results of previous studies showed that seasons influence the abundance of nosemosis in hives. A high spore abundance was detected in spring and autumn (Brenna et al., 2012; Gisder et al., 2010; Traver & Fell, 2011). On the other hand, rare studies have existed about *Nosema* spp. infection in winter and summer stuation (Martín-Hernández et al., 2007; Pernal et al., 2010). According to the findings of a present study, *Nosema* infections have still appeared and are a problem in the summer months in line with Pernal et al. (2010) and Martín-Hernández et al. (2009).

In Turkey, Basar (1990) reported that the maximum level of infection was seen in spring and winter in the Trakya region, Muğla and İstanbul. Another study conducted in the winter season in Hatay reported a 10% *Nosema* infection between 2010 and 2011 (Muz et al., 2012). In addition, the highest infestation level was observed in June and July in the Eastern Black Sea region of Turkey (Tosun & Yaman, 2016). In our study, 15% (12 out of 80) prevalence was determined during the summer season in Ankara, Turkey. Additionally, infected apiaries were mainly located in the central and north parts of Ankara.

Conclusion

Detection of nosemosis in the hives is very important to prevent colony losses and financial damage. Even though the presence of *Nosema* infection is mostly known in spring and autumn, summer infection prevalence was demonstrated rarely. The results of the present study provide a minor evidence for summer infection. Therefore, more attention should be paid to the presence of infestation and treatment necessity in all-season including summer.

Acknowledgement

The article was presented as an oral presentation at 6th International Congress on Advances in Veterinary Sciences & Technics.

Funding

This work was supported by TÜBİTAK, 2209-A Research Project Support Programme for Undergraduate Students (project no: 1919B011902598).

References

Adl, S. M., Simpson, A. G., Farmer, M. A., Andersen, R. A., Anderson, O. R., Barta, J. R., ... & Taylor, M. F. (2005). The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *Journal of Eukaryotic Microbiology*, 52(5), 399-451.

Aydin, L., Gulegen, E., & Cetinbas H. (2001). Prevalence of *Nosema apis* in Southern Marmara Region in Turkey. Proceeding of 37th Apimondia Congress, Durban, South-Africa. 363-378. ISBN: 0-620-27768-8.

Basar, E. (1990). Ülkemizdeki bal arılarında(*Apis mellifera*) Acarapis woodi ve *Nosema apis* parazitlerinin araştırılması. (Publication No. 8505). [Master Thesis, Hacettepe University]. https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=ayfNPbohfu9KEy40W1Nk4w&no=ayfNPbohfu9KEy40W1Nk4w.

Brenna, E., Traver, B. E., Matthew, R., Williams, M. R., Richard, D., & Fell, R. D. (2012). "Comparison of with inHive Sampling and Seasonal Activity of *Nosema ceranae* in Honey Bee Colonies." *Journal of Invertebrate Pathology,* 109(2), 187-93.

Ceylan, A., Özgenç, Ö., Erhan, F., Sevin, S., & Yarsan, E. (2019). Nosemosis' in (*Nosema*tosis) bal arısı (*Apis mellifera*) midesine etkileri üzerine histokimyasal gözlemler. *Veteriner Hekimler Derneği Dergisi*, 91(2), 98-103.

Cox-Foster, D. L., Conlan, S., Holmes, E. C., Palacios, G., Evans, J. D., Moran, N. A., ... & Lipkin, W. I. (2007). A metagenomic survey of microbes in honey bee colony collapse disorder. *Science*, 318(5848), 283-287.

Fries, I., Feng, F., da Silva, A., Slemenda, S. B., & Pieniazek, N. J. (1996). *Nosema ceranae* n. sp. (Microspora, *Nosema*tidae), morphological and molecular characterization of a microsporidian parasite of the Asian honey bee *Apis cerana* (Hymenoptera, Apidae). *European Journal of Protistology*, 32(3), 356-365.

Fries, I., Martin, R., Meana, A., García-Palencia, P., & Higes, M. (2006). Natural infections of *Nosema ceranae* in European honey bees. *Journal of Apicultural Research*, 45(4), 230-233.

Gisder, S., Hedtke, K., Möckel, N., Frielitz, M. C., Linde, A., & Genersch, E. (2010). Five-year cohort study of *Nosema* spp. in Germany: does climate shape virulence and assertiveness of *Nosema ceranae*?. *Applied and environmental microbiology*, 76(9), 3032-3038.

Güzerin, E. (2013). Investigation of Honey Bees (*Apis mellifera L.*) in Artvin Region in Terms of Microbial and Parasitic Diseases. [Master Thesis, Hacettepe University]. http://www.openaccess.hacettepe.edu.tr:8080/xmlui/handle/11655/2020?show=full.

Higes, M., Martín, R., & Meana, A. (2006). *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. *Journal of invertebrate pathology*, 92(2), 93-95.

Higes, M., Martín-Hernández, R., Botías, C., Bailón, E. G., González-Porto, A. V., Barrios, L., ... & Meana, A. (2008). How natural infection by *Nosema ceranae* causes honeybee colony collapse. *Environmental microbiology*, 10(10), 2659-2669.

Higes, M., Juarranz, Á., Dias-Almeida, J., Lucena, S., Botías, C., Meana, A., ... & Martín-Hernández, R. (2013a). Apoptosis in the pathogenesis of *Nosema ceranae* (M icrosporidia:

*Nosema*tidae) in honey bees (Apis mellifera). *Environmental microbiology reports*, 5(4), 530-536.

Higes, M., Meana, A., Bartolomé, C., Botías, C., & Martín-Hernández, R. (2013b). *Nosema ceranae* (Microsporidia), a controversial 21st century honey bee pathogen. *Environmental microbiology reports*, 5(1), 17-29.

Kurze, C., Le Conte, Y., Kryger, P., Lewkowski, O., Müller, T., & Moritz, R. F. (2018). Infection dynamics of *Nosema ceranae* in honey bee midgut and host cell apoptosis. *Journal of invertebrate pathology*, 154, 1-4.

Kutlu, M. A., & Kaftanoğlu, O. (1990). A study on the distribution and infection rate of adult honey bee (*Apis mellifera L*.) disease *Nosema apis*. *Çukurova University Journal of Science Institute*, 4(2), 41-53.

Martín-Hernández, R., Meana, A., García-Palencıa, P.,Marín, P., Botías, C., Garrıdo-Baılón, E., ... & Higes, M. (2009). Effect of temperature on the biotic potential of honeybee microsporidia. *Applied and Environmental Microbiology*, 75(8), 2554-2557.

Martín-Hernández, R., Higes, M., Sagastume, S., Juarranz, Á., Dias-Almeida, J., Budge, G. E., ... & Boonham, N. (2017). Microsporidia infection impacts the host cell's cycle and reduces host cell apoptosis. *PLoS One*, 12(2), e0170183.

Martín-Hernández, R., Meana, A., Prieto, L., Salvador, A. M., Garrido-Bailón, E., & Higes, M. (2007). Outcome of colonization of *Apis mellifera* by *Nosema ceranae*. *Applied and environmental microbiology*, 73(20), 6331-6338.

Muz, M. N., Solmaz, H., Yaman, M., & Karakavuk, M. (2012). Kış salkımı erken bozulan arı kolonilerinde paraziter ve bakteriyel patojenler. *The Journal of the Faculty of Veterinary Medicine University of Yuzuncu Yıl*, 23(3), 147-150.

Özkırım, A., Schiesser, A., & Keskin, N. (2019). Dynamics of *Nosema apis* and *Nosema ceranae* co-infection seasonally in honey bee (*Apis mellifera L*.) colonies. *Journal of Apicultural Science*, 63(1), 41-48.

Terrestrial Manual of the OIE. (2018). Nosemosis of honey bees, Chapter 3.2.4, pp. 744-49. Paris, France.

Pernal, S., Ibrahim, A., & Melathopoulos, A. P. (2010). Integrated management of *Nosema* and detection of antibiotic residues. *Hivelights*, 23, 12-18.

Smith, M. L. (2012). The honey bee parasite *Nosema* ceranae: transmissible via food exchange?. *PLoS One*, 7(8), e43319.

Tosun, O., Bekircan, Ç., & Yıldırım, H. B. (2019). The presence and distribution of Nosemosis Disease in Turkey. *Journal of Apitherapy and Nature*, 2(2), 71-84.

Tosun, O., & Yaman, M. (2016). The Effects of Temperature and humidity around the beehives on the distribution of *Nosema ceranae,* and also geographical and seasonal activity of the infection in the Eastern Black Sea Region of Turkey. *Mellifera*, 6, 7.

Traver, B. E., & Fell, R. D. (2011). Prevalence and infection intensity of *Nosema* in honey bee (*Apis mellifera L*.) colonies in Virginia. *Journal of Invertebrate Pathology*, 107(1), 43-49.

Tutkun E., & İnci A. (1992). *Honeybee pests, diseases and treatment methods (From Diagnosis to Treatment)*. Demircioglu Printing, Ankara.

Tutun, H., Sevin, S., & Cetintav, B. (2020). Effects of different chilling procedures on honey bees (*Apis mellifera*) for anesthesia. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 67(3), 289-294.

Utuk, A. E., Piskin, F. C., Girisgin, A. O., Selcuk, O., & Aydin, L. (2016). Microscopic and molecular detection of *Nosema* spp. in honeybees of Turkey. *Apidologie*, 47(2), 267-271.

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