Tehran University of Medical Sciences Publication
http:// tums.ac.ir

Iran J Parasitol

Open access Journal at
http:// ijpa.tums.ac.ir

Iranian Society of Parasitology
http:// isp.tums.ac.ir

Short Communication

Does *Nosema ceranae* Wipe Out *Nosema apis* in Turkey?

***Rahşan IVGIN TUNCA [1], Devrim OSKAY [2], Ayhan GOSTERIT [3], Olgay Kaan TEKIN [4]**

1. *Dept. of Plant and Animal Breeding, Ula Ali Kocman Vocational School, Muğla Sitki Kocman University, Muğla, Turkey*
2. *Dept. of Agricultural Biotechnology, Faculty of Agriculture, Namık Kemal University, Tekirdağ, Turkey*
3. *Dept. of Animal Science, Faculty of Agriculture, Suleyman Demirel University, Isparta, Turkey*
4. *Dept. of Agricultural Biotechnology, Faculty of Agriculture, Ahi Evran University, Kırşehir, Turkey*

Received 11 Oct 2015
Accepted 20 Jan 2016

Keywords:
Nosema apis,
Nosema ceranae,
Honey bees,
Molecular diagnosis,
Turkey

***Correspondence**
Email:
rivgin@gmail.com

Abstract

Background: The aim of this study was to determine the prevalence of the *Nosema ceranae* and *Nosema apis* among apiaries using both spore counts and multiplex PCR and the replacement of *N. apis* by *N. ceranae* in some regions of Turkey.

Methods: A hundred honey bee samples were collected from 99 apiaries in 11 different locations in 2011-2012 in Turkey. *Nosema* infection degree from collected samples was determined using light microscope and molecular detection of *Nosema* spp. (*N. ceranae* and *N. apis*) was performed using specific primers by multiplex PCR.

Results: *N. ceranae* was only found spores in sampling areas using molecular diagnosis. *N. apis* was not detected in whole sampling areas using both techniques. There are no *Nosema* spores detected in Konya one location using two techniques. The nucleotide sequences from amplification products of the *Nosema* infested honeybee samples were (98%) identical with the sequence of *N. ceranae* for many countries deposited in the GenBank database in this study.

Conclusion: The present study illustrated that *N. ceranae* is the only spores for sampled areas in 2011-2012. The study could also indicate that *N. ceranae* has been replaced instead of N. *apis* in Turkey. In addition, the prevalence of *N. ceranae* and two microsporodia spores effects on honey bee colonies in Turkey were needed to determine with intensive sampling, periodically.

Introduction

The microsporodia having more than 160 genera and 1300 species has been determined from insects and other species (1- 3). The most majority of *Nosema* species from microsporidia are parasitic for invertabrate (4). In honey bees, *N. apis* and *N.*

ceranae are called microsporidia and found in adult bees (2). Previously, it was thought that *N. apis,* caused nosema disease, was specific for *Apis mellifera* but *N. ceranae* was found only in *Apis ceranae.* For the last decade, *N. ceranae* has been found widespread in Europe, Africa, North America and Australia (5- 15).

The life cycles of two *Nosema* spores are similar but both are distinguished from each other using molecular diagnostic methods. Generally, nosema causes digestive system disorders, shortening the life span, decreasing pollen collection, reducing the colony population size and honey production, increasing the dead bees behind the hive entrance and the losses of colonies (16). The spores spread by using contaminated beekeeping equipments, poor beekeeping practices, temperature fluctuations and the movement of honey bee colonies (17). For the Western honey bee, these two microsporidian species are pathogenic (18, 19). Although, *N. apis* infection is restricted to the adult bees midgut epithelium (20), *N. ceranae* also was infect other tissues (21). The type C *nosemosis* caused by *N. ceranae* is one of the most prevalent bee pathogens (22, 23). Many factors such as weather conditions, host susceptibility, the age of the bees and beekeeping practices may contribute different effects of *N. ceranae* on infected colonies (16). Furthermore, the *N. apis* replacement by *N. ceranae* was reported by many researchers in the world (13, 22, 23-25).

The molecular diagnosis was performed in order to distinguish *N. apis* from *N. ceranae* in honey bees from Turkey in 2010. That was the first time which indicated the presence of *N. ceranae* in these studies and samples were collected in 2005-06, 07- 08 and 09 periods in these studies (8, 26, 27).

In the present study, our goals were to gain a better understanding of the prevalence of the *N. ceranae* and *N. apis* among apiaries using both spore counts and multiplex PCR and also the replacement of *N. apis* by *N. ceranae* in some regions of Turkey compared with previous studies.

Materials and Methods

Samples were collected from 99 apiaries in eleven different locations in 2011-2012 in Turkey (Fig. 1). A hundred honey bee samples were collected from each apiary. *Nosema* infection degree from collected samples was determined using light microscope and molecular detection of *Nosema* spp. (*N. ceranae* and *N. apis*) was performed using specific primers.

Fig. 1: Map of sample collection sites in Turkey. The total numbers of apiaries / infected apiaries for *Nosema* spores using microscopic diagnose were given in parentheses

Determination of Nosema infection level

Abdomens of twenty adult bees from a single colony were crushed in 10 ml. of distilled water. The suspended samples were filtered through two layers of muslin and centrifuged at 1000 g for 5 min. and the supernatants were removed. Pellets were resuspended in 10 ml of distilled water. Each homogenate was microscopically examined for the presence of *Nosema* spp. spores at 400X magnification and the spores were counted on the haemocytometer (28).

Molecular detection of Nosema spp.

Each homagenate was kept at 4 °C. Total DNA was extracted from samples using DNA isolation kit (Fermentas K512) and isolated DNA was analyzed in order to confirm the *Nosema* species of the spores by PCR as previously described using 218MITOC FOR/218MITOC REV and 321APIS FOR/321APIS REV primers specific for *N. ceranae* or *N. apis,* respectively (29). Multiplex PCR reaction mix contains 10 ng DNA, 0.5 U Taq DNA polymerase (Fermentas), 10XPCR buffer (Fermentas), 0.3 mM of each dNTP (Fermentas) 2.5 mM $MgCl_2$ (Fermentas), 0.4 µM primers and high pure H_2O as required in order to make up to 20 µl total volume. The PCR protocol was 2 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 55 °C, and 1 min at 72 °C and a final extension step at 72 °C for 5 min. PCR amplification was detected agarose gel (1.5%) electrophoresis and was visualized by ultraviolet (UV) after ethidium bromide staining (29). The positive PCR products were compared with positive for *N. ceranae* and *N. apis* provided from Etlik Veterinary Control Central Research Institute. Three positive PCR products for *N. ceranae* were sequenced and the sequence similarity analyses were performed using BLAST database search.

Results

The microscopic examination results of *Nosema* spores in samples were illustrated in Table 1 and Fig. 1. The highest percentages (100%) of *Nosema* positive samples were found in Muğla located in the southwestern part of Anatolia having typical Mediterranean climatic condition. There were no *Nosema* spores observed in three locations (Isparta, Konya and Zonguldak) in microscopic diagnosis. The lowest percentage (8.3%) of *Nosema* spores was detected in Kırşehir located in the central part of Anatolia. After microscopic diagnosis, the homogenates from all different 99 apiaries were used for DNA isolation and *N. apis* and *N. ceranae* were investigated by multiplex PCR.

Molecular diagnosis of the samples illustrated that *N. ceranae* spore was the only *Nosema* species found to honeybees from in Turkey (Fig. 2).

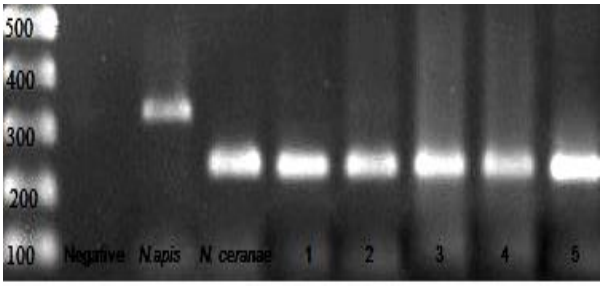

Fig. 2: PCR products of negative, positive controls and infected colonies: Line 1: 100bp DNA ladder, line 2: negative control, line 3: *N. apis*, line 4: *N. ceranae*, line 5- 9: nosema positive samples

The main point is that all samples were negative for *N. apis* despite of the presence of *N. apis* illustrating in previous studies. The positive samples were from beekeepers located in Aegean, Mediterranean, Thrace, Black Sea and Central Anatolia regions in Turkey. There is no *Nosema* spore detected in Konya (in Central Anatolia) using both microscopic and molecular diagnosis. Other important point is that *Nosema* spores were not observed in Isparta and Zonguldak honey bee samples in microscopic diagnoses; however, *N. ceranae* was detected in samples using multiplex PCR. The nucleotide sequences of amplification prod-

ucts from the *Nosema* infested samples were (98%) identical with the sequence of *N. ceranae* from many countries deposited in the GenBank database in this study. A nucleotide blast illustrated that *N.* ceranae samples from Turkey was highly identical (98%) from *N. ceranae* sequences from many European countries (Lithuania, Poland, France, Italy, Germany and Austria), Morrocco, Lebonan, Iran, Mexico, Argentina, Australia and Thailand.

Table 1: Results of light microscopy examination and PCR results for *Nosema* spp. spores in common samples originating from each location in Turkey

Locations	# of Apiaries	Negative		Positive			
	(N)	(n-)	(%)	(n+)	(%)	***N. ceranae***	***N. Apis***
İzmir	13	11	84.6	2	15.4	Yes	No
Aydın	14	9	64.3	5	35.7	Yes	No
Muğla	12	0	0.0	12	100.0	Yes	No
Tekirdag	10	2	20.0	8	80.0	Yes	No
Kırklareli	13	11	84.6	2	15.4	Yes	No
Zonguldak	5	5	100.0	0	0.0	Yes	No
Artvin	5	4	80.0	1	20.0	Yes	No
Isparta	5	5	100.0	0	0.0	Yes	No
Adana	5	4	80.0	3	60.0	Yes	No
Konya	5	5	100.0	0	0.0	No	No
Kırşehir	12	11	91.7	1	8.3	Yes	No

(N=Total number of Apiaries; (n-)= Negative number of infected colonies; (n+)= Positive number of infected colonies)

Discussion

The presence of *N. ceranae* and *N. apis* in Turkey were determined using molecular techniques in previous studies (8, 26, 27). The sampling period of these studies included 2005 to 2009. The detection of the two *Nosema* spores was important suspicious for colony losses in Turkey in those periods. Because *N. ceranae* were detected in Artvin, Muğla and Hatay provinces (8) and high rates of colony losses were detected by Giray et al. (30). At that point, Whitaker et al. (8) mentioned that "Though the cause of the colony losses cannot be conclusively attributed to *N. ceranae* infection, there were a potentially significant relationship between its presence and the occurrence of the losses".

Besides, *N. ceranae* is more pathogen than *N. apis* and it has been spread all over the world (8, 13, 22, 24). In 2005 and 2006, *N. ceranae* was detected from three samples from the Hatay, Muğla and Artvin provinces (8). *N. apis* was detected in samples from the Sivas, Izmir, Gaziantep and Bitlis provinces (8). In the samples from Black Sea (Samsun and Giresun), *N. ceranae* were detected in 2007 and 2008 (26). The other study informed the presence of *N. cerana* and *N. apis* in Hatay and southeastern Marmara region between 2007 and 2009 (27).

The present study results illustrated that *N. ceranae* is the only *Nosema* species detected into infect honey bee in Turkey and *N. apis* were not detected using both techniques for collected samples between 2011-2012. But, the presence of *N. apis* was informed in previous studies (8, 26, 27). The other study illustrated the presence of *N. ceranae* in the samples of migratory beekeepers but not within the samples of local beekeepers (31). They also concluded that the infectivity of *N. ceranae* expands with migratory beekeeping activities (31). However, the samples of the present study were collected mostly in local beekeepers in Zonguldak, Isparta, Izmir, Kırşehir and others except Muğla and *N. ceranae* spores found nearly all locations except Konya samples, which were also collected from local

beekeepers. The present study does not support that *N. ceranae* expands with migratory beekeeping activities or found in migratory beekepers. The recent studies indicated the replacement of *N. apis* by *N. ceranae* in many regions in Turkey (31, 32).

The present results illustrated that *N. ceranae* is the only spore in sampled areas which is found instead of *N. apis* in Turkey. The sequence results for present study also showed high-level identity of *N. ceranae* sequences from many European countries Morrocco, Lebanon, Iran, Mexico, Argentina, Australia and Thailand. This result can be explained with the properties of pathogen, which has high level of pathogenicity and transmission rate. The other reason of *N. ceranae,* which is the only spores in sampled areas, could be explained the lack of symptoms of *N. ceranae* led to insufficient attention or neglect nosemosis of beekeepers (12, 17).

Conclusion

N. ceranae is the only spores for sampled areas in 2011-2012. The study could also indicate that *N. ceranae* has been replaced instead of *N. apis* in Turkey according to sampling areas. Also the prevalence of *N. ceranea* and two microsporidia spores effects on honey bee colonies in Turkey were needed to determine with intensive sampling, periodically.

Acknowledgments

The authors declare that there is no conflict of interest.

References

1. Canning EU. Microsporidia. In: Kreier JP, Baker JR, editors. Parasitic protozoa. New York: Academic Press; 1993. p. 299-385.
2. Chen YP, Evans JD, Murphy C, Gutell R, Zuker M, Gundensen-Rindal D, Pettis JS. Morphological, molecular, and phylogenetic characterization of *Nosema ceranae*, a microsporidian parasite isolated from the European honey bee, *Apis mellifera*. J Eukaryot Microbiol. 2009; 56: (2):142–7.
3. Singh T, Bhat MM, Khan MA. Microsporidiosis in the Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). Pertanika J Trop Agric Sci. 2012; 35(3):387–406.
4. Canning EU, Curry A, Cheney SA, Lafranchi-Tristem NJ, Kawakami Y, Hatakeyama Y, Iwano H, Ishihara R. *Nosema tyriae* and *Nosema* sp., microsporidian parasites of cinnabar moth *Tyria jacobaeae.* J Invertebr Pathol. 1999;74 (1):29–38.
5. Higes M, Martin R, Meana A. *Nosema ceranae,* a new microsporidian parasite in honey bees in Europe. J Invertebr Pathol. 2006;92 (2):93–5.
6. Higes M, Martin-Hernandez R, Garrido-Bailon E, Gonzalez-Porto AV, Garcia-Palencia P, Meana A, Del Nozal MJ, Mayo R, Bernal JL. Honeybee colony collapse due to *Nosema ceranae* in professional apiaries. Env Microbiol Rep. 2009;1:110–3.
7. Huang WF, Jiang JH, Chen YW, Wang CH. *A Nosema ceranae* isolate from the honeybees *Apis mellifera*. Apidologie 2007;38:30–7.
8. Whitaker J, Szalanski AL, Kence M. Molecular detection of *Nosema ceranae* and *N. apis* from Turkish honey bees. Apidologie 2011;42:174-80.
9. Chauzat MP, Carpentier P, Martel AC, Bougeard S, Cougoule N, Porta P, Lachaize J, Madec F, Aubert M, Faucon JP. Influence of pesticide residues on honey bee (Hymenoptera: Apidae) colony health in France. Environ Entomol. 2009; 38 (3):514–23.
10. Williams GR, Shafer ABA, Rogers REL, Shutler D, Stewart DT. First detection of *Nosema ceranae*, a microsporidian parasite of European honeybees (*Apis mellifera*) in Canada and central USA. J Invertebr Pathol. 2008;97 (2):189–92.
11. Klee J, Besana A, Genersch E, Gisder S, Nanetti A, Tam DQ, Chinh TX, Puerta F, Ruz JM, Kryger P, Message D, Hatjina F, Korpela S, Fries I, Paxton RJ. Widespread dispersal of the microsporidium *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. J Invertebr Pathol. 2007;96 (1):1–10.
12. Martin-Hernandez R, Meana A, Prieto L, Martinez- Salvador A, Garrido- Bailon E, Higes M. Outcome of colonization of *Apis mellifera* by

Nosema ceranae. Appl Environ Microbiol. 2007;73:6331–8.
13. Paxton RJ, Klee J, Korpela S, Fries I. *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. Apidologie 2007;38:558–65.
14. Tapaszti Z, Forgach P, Kovago C, Bekesi L, Bakonyi T, Rusvai M. First detection and dominance of *Nosema ceranae* in Hungarian honeybee colonies. Acta Vet Hung. 2009; 57:383–8.
15. Giersch T, Berg T, Galea F, Hornitzky M. *Nosema ceranae* infects honey bees (*Apis mellifera*) and contaminates honey in Australia. Apidologie 2009; 40:117–23.
16. Higes M, Meana A, Bartolome C, Botias C, Martin-Hernandez R. *Nosema ceranae* (Microsporida), a controversial 21st century honey bee pathogen. Environ Microbiol Rep. 2013;5:17–29.
17. Gajger TV, Vugrek O, Grilec OD, Petrinec Z. Prevalence and distribution of *Nosema ceranae* in Croatian honeybee colonies. Vet Med. 2010;55(9):457–62.
18. Fries I, Feng F, Da Silva A, Slemenda SB, Pieniazek NJ. *Nosema ceranae* n. sp. (Microspora, Nosematidae), morphological and molecular characterization of a microsporidian parasite of the Asian honey bee *Apis ceranae* (Hymenoptera, Apidae). Eur J Protistol. 1996;32:356–65.
19. Fries I, Martin R, Meana A, Garcia-Palencia P, Higes M. Natural infections of *Nosema ceranae* in European honey bees. J Apic Res. 2006;45:230–3.
20. Fries I. The intracellular development of *Nosema apis* Z. Apidologie 1989;20:502–3.
21. Gisder S, Hedtke K, Möckel N, Frielitz MC, Linde A, Genersch E. Five-year cohort study of *Nosema* spp. in Germany: does climate shape virulence and assertiveness of *Nosema ceranae*? Appl Environ Microbiol. 2010;76 (9):3032–8.
22. Higes M, Martin-Hernandez R, Meana A. *Nosema ceranae* in Europe: an emergent type C nosemosis. Apidologie 2010;41:375–92.
23. Fries I. *Nosema ceranae* in European honey bees (Apis mellifera). J Invertebr Pathol. 2010; 103: Suppl 1:S73-9.
24. Paxton RJ. Does infection by *Nosema ceranae* cause "colony collapse disorder" in honey bees (*Apis mellifera*)? J Apic Res. 2010;49:80–4.
25. Martin-Hernandez R, Meana A, Garcia-Palencia P, Marin P, Botias C, Garrido-Bailon E, Barrios L, Higes M. Effect of temperature on the biotic potential of honeybee microsporidia. Appl Environ Microbiol. 2009;75:2554–7.
26. Utuk AE, Piskin FC, Kurt M. First molecular detection of *Nosema ceranae* in Turkey. Vet J Ankara Univ. 2010;57:275–8.
27. Muz MN, Girisgin AO, Muz D, Aydin L. Molecular detection of *Nosema ceranae* and *Nosema apis* infections in Turkish apiaries with collapsed colonies. J Apic Res. 2010; 49(4):342.
28. Office International des Epizooties (OIE) (2008) Manual of Standards for Diagnostic Test and Vaccines. http://www.oie.int/doc/ged/D7710.PDF
29. Fries I, Chauzat MP, Chen YP, Doublet V, Genersch E, Gisder S, Higes M, Mcmahon DP, Martinhernandez R, Natsopoulou M, Paxton RJ, Tanner G, Webster TC, Williams GR. Standard methods for *Nosema* research. J Apic Res. 2013;52(1):1–28.
30. Giray T, Kence M, Oskay D, Doke MA, Kence A. Scientific note: colony losses survey in Turkey and causes of bee deaths. Apidologie. 2010;41:451–3.
31. Tozkar CO, Kence M, Evans J, Kence A. Identification of *Nosema* spp among honey bees from different regions of Turkey. WG2 Workshop: *Nosema*, from knowledge to experimental setup, 2012, Istanbul, Turkey 3rd-4th of March p.25.
32. Yalçinkaya A, Martin-Hernandez R, Higes M, Ozkirim A. Epidemiology of *Nosema* spp. in Turkey. WG2 Workshop: *Nosema*, from knowledge to experimental setup 2012– Istanbul, Turkey 3rd-4th of March p.27.