Journal of Entomological Society of Iran https://jesi.areeo.ac.ir

Entomological Society of Iran
2023, 43 (4), 383-392

Research Article
https://doi.org/10.61186/jesi.43.4.6

pISSN: 0259-9996
eISSN: 2783-3968

OPEN ACCESS

Parasitological and molecular study of nosemosis in migratory apiaries in Hormozgan Province, southern Iran

Bahareh Meftahi[1], Saeed Yaghfouri[2], Sadegh Mosazadeh[2], Reza Sheibani Tezerji[2], Mostafa Fakhrabadipour[2], Esmaeil Javdan[2] & Gholam Reza Razmi[1]

1- Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
2- Hormozgan Veterinary Head Office, Bandar Abbas, Iran

✉b.meftahi.m@gmail.com https://orcid.org/0000-0003-2799-8387
✉saeed.yaghfoori@gmail.com https://orcid.org/0000-0002-5844-2464
✉Sadeghmosaadeh16@gmail.com https://orcid.org/0009-0006-7628-7353
✉Shahriarsheibani1313@gmail.com https://orcid.org/0009-0004-0769-3628
✉m.fakhrabadi1983@gmail.com https://orcid.org/0009-0008-7584-8459
✉ horvet@yahoo.com https://orcid.org/0009-0003-3047-2148
✉razmi@um.ac.ir https://orcid.org/0000-0002-0754-1278

Abstract. Nosemosis is a microsporidian disease caused by *Nosema ceranae* and *N. apis* and transmitted via oral-fecal and oral-oral routes. It is globally distributed among adult bees in honeybee colonies. Considering the health importance of nosemosis in honeybees, the study aims to determine the frequency of *Nosema* spp. infection in migratory apiaries in Hormozgan province by microscopic and molecular methods. In the present study, 20 bees from ten randomly selected hives in 84 migratory apiaries were collected. In the laboratory, the abdomen of the bees was separated from the rest of the body with entomological tweezers and scissors and then ground up in a mortar containing saline serum. The prepared suspension was filtered by passing through a sieve, then the prepared suspension was transferred to test tubes and centrifuged. The pellets were repeatedly washed by saline solution and centrifuged. Finally, the pellets were examined for spores of *Nosema* spp. by light microscopy and conventional PCR. In microscopy, 38.2 % of apiaries were positive for *Nosema* spp. spores. By PCR however, DNA of *N. ceranae* was detected in 39.2. % of apiaries with no samples positive for *N. apis*. Due to the considerable frequency of infection in migratory apiaries in Hormozgan province, it is necessary to carry out appropriate health measures such as screening of apiaries with appropriate diagnostic methods and training of beekeepers to disinfect hives in order to control *Nosema* infection in Iranian apiaries by the veterinary health officials.

Keywords:Insect pathology, characterization, honeybee, molecular detection

Article History
Received:
31 July 2023
Accepted:
30 October 2023
Subject Editor:
Alireza Sazmand

Citation: Meftahi, B., Yaghfouri, S., Mosazadeh, S., Sheibani Tezerji, R., Fakhrabadipour, M., Javdan, E. & Razmi, G. R. (2023) Parasitological and molecular study of nosemosis in migratory apiaries in Hormozgan Province, southern. *J. Entomol. Soc. Iran* 43 (4), 383-392.

Introduction

Nosemosis is one of the most destructive diseases of adult honeybees around the world (Bailey & Ball, 1981). The causative agents are unicellular microsporidian parasites *Nosema cernea* and *Nosema apis* (WOAH, 2019). Adult honeybees become infected during consumption of food and water contaminated with spores, during cleaning contaminated combs, robbing contaminated hives, or by infected bees drifting to new hives (Galajda *et al.*, 2021). The wall of ingested spores is broken by the enzymes of honeybees' gut and then sporoplasm is injected into the cell mid-gut by polar tubes. *Nosema* spp. grows and produces several million spores and damages the intestinal cells. Honeybees infected with *N. apis* may be shown dysentery, but bees infected with *N. ceranea* show no symptoms. They often die away from the hive and only a few sick or dead bees may be found

Corresponding author: (Gholam Reza Razmi, E-mail: razmi@um.ac.ir)

near the hive entrance (Galajda *et al.*, 2021). *Nosema apis* infection is more distributed among apiaries located in cold and temperate climates during spring and winter seasons, while *N. ceranae* infection is in tropical and subtropical climates (Fries *et al.*, 1996). The prevalence of nosemosis has been reported in the range of 23% to 53% in honeybee colonies from different climatic regions of Iran (Mohammadian *et al.*, 2018). Hormozgan province is the southernmost region of Iran with a tropical climate and mild weather which is suitable for the growth of flowering plants in the winter season. Every year, many honeybee colonies from northernmost regions migrate to Hormozgan province in winter. Considering the effect of nosemosis on the health of honeybee colonies, the study aimed to determine the rate of *Nosema* spp. in the migratory honeybee colonies in the Hormozgan province using microscopical and molecular tools.

Materials and methods

Study area

Hormozgan province is located in the South of Iran, the North of Hormoz Strait, with geographical coordinates between 25° 23′ to 28° 57′ N and 52° 41′ and 59° and 15′ E. The boundaries of the province lie on the Oman Sea from the East to the Persian Gulf on the West. More than 70% of the province is covered by mountains and hills. The province is bounded by Kerman province in the north and northeast, Fars and Bushehr provinces in the west and northwest, and Sistan and Baluchistan province in the east. Three types of climates exist in this province, i.e., forest, rangeland, and desert. The average temperature affected by humidity is moderate and rarely gets higher than 45 °C in summer. In the deserts, the temperature is about 0 °C but there is no frigid weather in winters. The annual rainfall is less than 250 mm and the relative humidity is more than 80% (Safa *et al.*, 2012)

Sample collection

The sample size was calculated 840 hives. The sample size was calculated using a 98% confidence level with 4% desired absolute precision (Thursfield, 1986), based on the prevalence of *Nosema* spp. infection (54%) that was previously reported in humid climate (Mohhamadian *et al.*, 2018). After visiting, we obtained the data such as the apiary address, the name of the owner, and bee population from the beekeeper. Then, the samples were collected from 10 seemingly healthy hives in each apiary, consisting of 20 old worker bees from peripheral frames of each hive (200 honeybees in each apiary) (WOAH, 2019). The collected bees were put in storage containers and transported immediately to the laboratory under cold conditions. From December 2022 to March 2023, 84 migratory apiaries were sampled one to two weeks after entering migratory apiaries in autumn and winter to Hormozgan province (Fig. 1) No clinical symptoms related to nosmosis were observed in hives of each apiary during sampling.

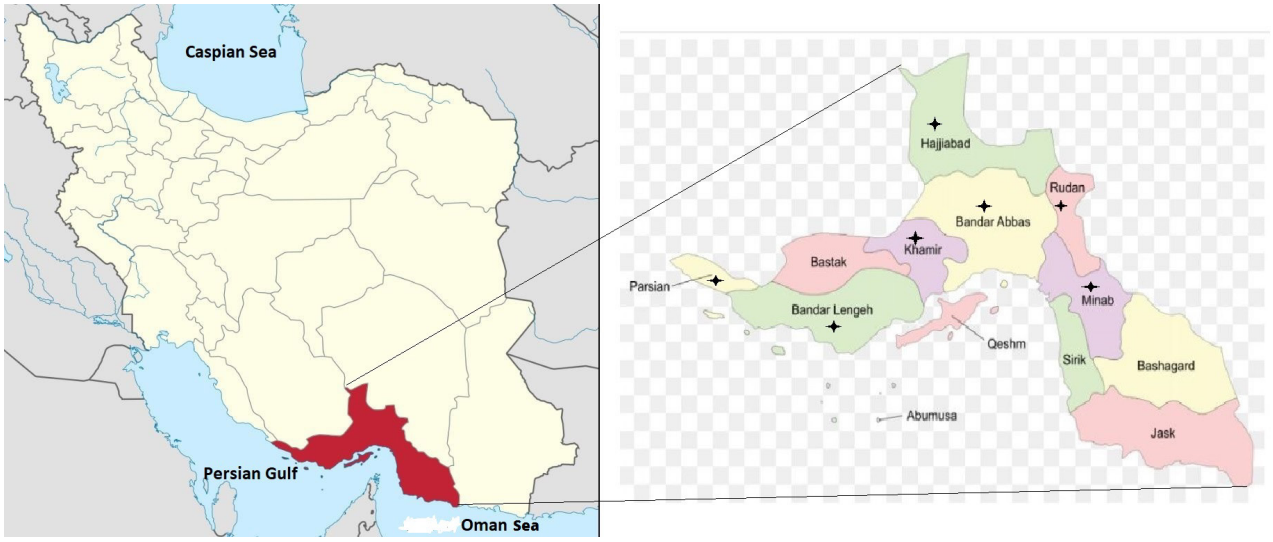

Fig. 1. Sampling areas in Hormozgan province.

Microscopy Examination

The collected bees were put in storage containers and transported immediately to the laboratory under cold conditions. Abdomens of 20 honeybees from each hive were washed with normal saline solution and ground in 5 ml of this solution. The suspensions were then filtered through two layers of muslin to remove coarse bee parts and centrifuged at 2500 g for 5 min. The supernatants were removed and pellets were mixed with saturated saline solution and again centrifuged at 2500 g for 5 min. One milliliter of supernatants was taken, and the rest of the solution was discarded. The supernatants were washed three times with distilled water and each time they were centrifuged at 2500 g for 3 min and the upper parts were discarded. The final pellets were resuspended in 1.5 ml of distilled water. Three drops of the suspension were put on a microscopic slide covered with an 18×18 mm coverslip and examined by a light microscope at ×400 magnification (Shirzadi & Razmi, 2021). The rest of the homogenate was transferred to an Eppendorf tube and at kept at -20°C until molecular examination.

DNA extraction and PCR assay

Genomic DNA of samples was extracted with a commercial DNA extraction kit (MBST, Tehran, Iran) according to manufacturer's instructions. A multiplex PCR targeting 16S rRNA region was employed for simultaneous detection of two *Nosema* species in a single reaction (Martín-Hernández *et al.*, 2007) (Table 1). In positive samples, 321 bp product of *N. apis* and 218 bp product of *N. ceranae* could be detected.

Amplification was conducted in 25 µl reaction volumes (Accupower PCR premix kit, Bioneer®, Soeul, South Korea) with a final concentration of each dNTP of 250 µM in 10 mM Tris-HCl pH 9.0, 30 mM KCl and 1.5 mM $MgCl_2$, 1U Taq DNA polymerase (Takapouzist, Tehran, Iran) and 10 pmol of each PCR primer, then 1 µl of DNA template was added to each reaction. The remaining 25 µl reaction volume was filled with nuclease-free distilled water. The thermocycler program consisted of 94°C for 2 min, followed by 10 cycles of 15 s at 94°C, 30 s at 61.8°C, 45 s at 72°C, 20 cycles of 15 s at 94°C, 30 s at 61.8°C, and 50 s at 72°C plus an additional 5 s of elongation for each successive cycle and a final extension step at 72°C for 7 min. The PCR products were electrophoresed in a 2% agarose gel with TBE buffer and visualized using ethidium bromide. The positive DNA controls were obtained from a previous study (Shirzadi & Razmi, 2021) and nuclease-free distilled water was used as a negative control for each PCR amplification.

Statistics analysis

The relationship between the *Nosema* infection rate by molecular results and the region of each migratory apiary was analyzed by the Chi-square test. *P*-value below 0.05 was considered statistically significant. The agreement between the molecular and microscopic tests was shown as a Kappa-coefficient. The agreement is poor if Kappa-the coefficient is between 0.2 and 0.4, moderate if between 0.4 and 0.6, substantial if 0.6 and 0.8, and good if it exceeds 0.8 and 1 (Petrie & Watson, 2006). Analyses were performed in SPSS ver. 18.0.

Results

During the study, honeybees of sampled hives looked to be healthy. Microscopy positivity rate of *Nosema* spp. infection was detected in 38.2% (32/84) of migratory apiaries (Table 2). Molecular examination showed that 39.2% (33/84) of apiaries were infected with *N. ceranea* (Table 2). The highest prevalence of *Nosema* spp. infection was detected in the west and northwest regions while the lowest frequency was in the east and northeast regions in Iran ($P<0.05$) (Fig. 2) (Table 3). A good agreement was observed in the results between the microscopy and PCR methods (Fig. 3) (Table 4) (Kappa= 0.975).

Table 1- Primers selected for detection of *N. ceranae* and *N. apis* in Duplex PCR (Martín-Hernández et al., 2007).

Primer	Sequences
N. ceranae forward	5'-GGCGACGATGTGATATGAAAATATTAA-3'
N. ceranae reverse	5'-CCCGGTCATTCTCAAACAAAAAACCG-3'
N. Apis forward	5'-GGGGGCATGTCTTTGACGTACTATGTA-3'
N. Apis reverse	5'-GGGGGGCGTTTAAAATGTGAAACAACTATG -3'

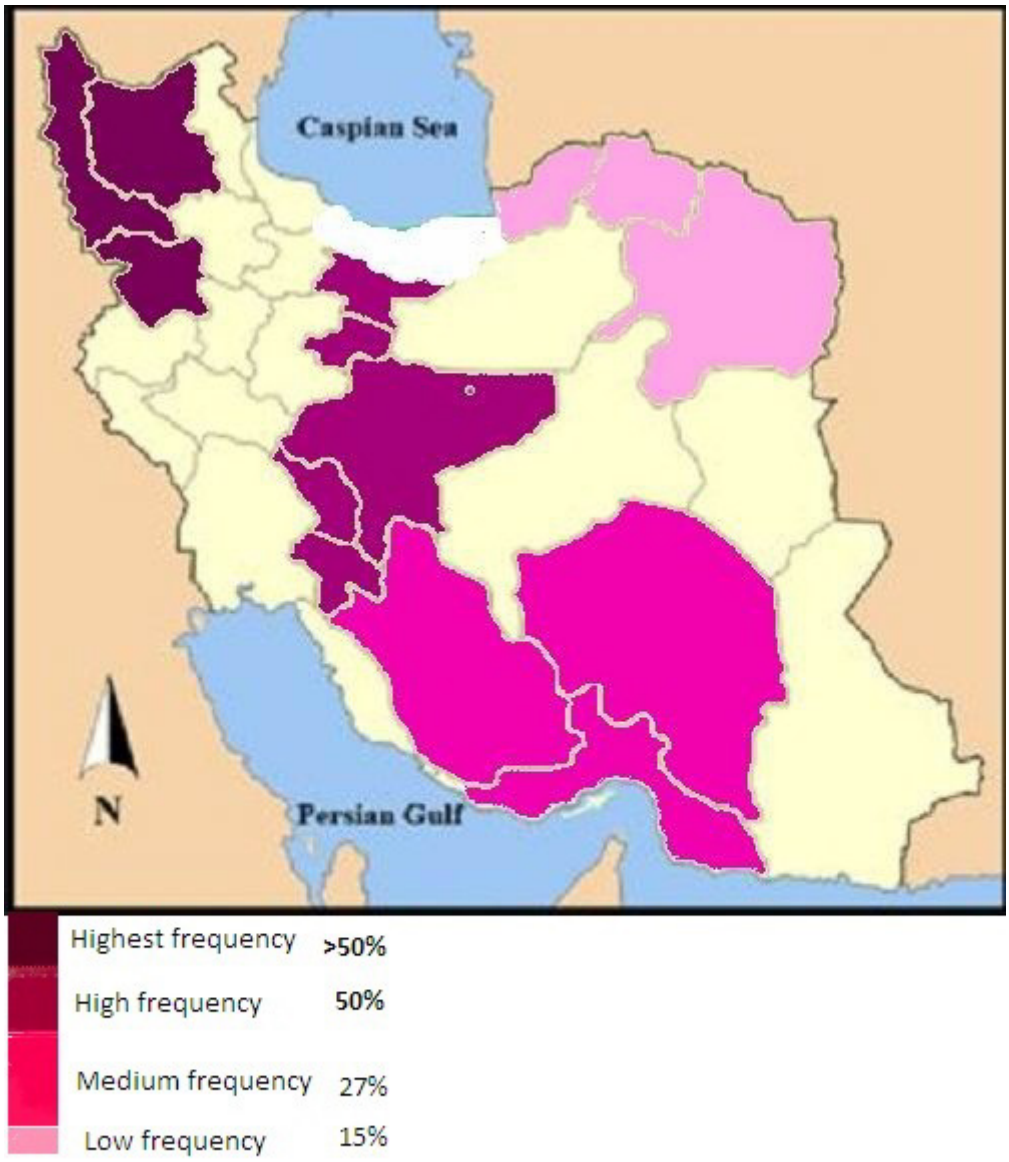

Fig. 2- The frequency of *Nosema* infection in different regions of Iran

Discussion

The range of frequency of *Nosema* infection was determined from 15.7% to 57.14% in migratory apiaries in Hormozgan province. The highest frequency of *Nosema* spp. infection was reported in the north and northwest regions of Iran and the lowest frequency of *Nosema* spp. in the east and northeast regions. The frequency of *Nosema* spp. infection was determined in the range of 9 59% to 67% in the apiaries of different areas in East and West Azerbaijan (Lotfi *et al.*, 2009; Razmaraii, *et al.*, 2013; Moeini *et al.*, 2022). The prevalence of *Nosema* spp. infection was also reported in the range of 10-60% in different areas of Kurdistan province (Khezrei *et al.*, 2018). The reported frequency of *Nosema* spp. infection in the above studies is consistent with the reported frequency in the west and northwestern areas in this study. Mohammadian *et al.* (2018) showed a high frequency of *Nosema* spp. infection in humid and semi-humid climates and the lowest frequency in semi-arid and arid areas of Iran. A study showed that increasing the humidity and temperature caused high *N. ceranae* density and low *N. apis* density in honey bee colonies in Turkey (Özgör *et al.*, 2015).

Table 2. The frequency of microscopy and PCR positivity rate in migratory apiaries in Hormozgan province, Iran.

Pronince	PCR		Microscopy method	
	n. examined	*n. positive*	*n. positive*	*n. doubtful*
East Azerbaijan	3	1	1	
West Azerbaijan	27	17	16	2
Isfahan	5	1	1	1
Alborz	2	1	1	
Chaharmahal and Bakhtiari	3	1	1	
South Khorasan	3	-	-	1
Khorasan Razavi	15	3	3	4
North Khorasan	1	-	-	
Fars	5	3	3	
Qom	2	2	2	
Kerman	2	-	-	1
Kurdistan	5	2	2	
Golestan	1	-	-	
Lorestan	2	2	2	
Hormozgan	4	-	-	
Mazandaran	2	-	-	
No data	2	-	-	1
Total	84	33	32	10

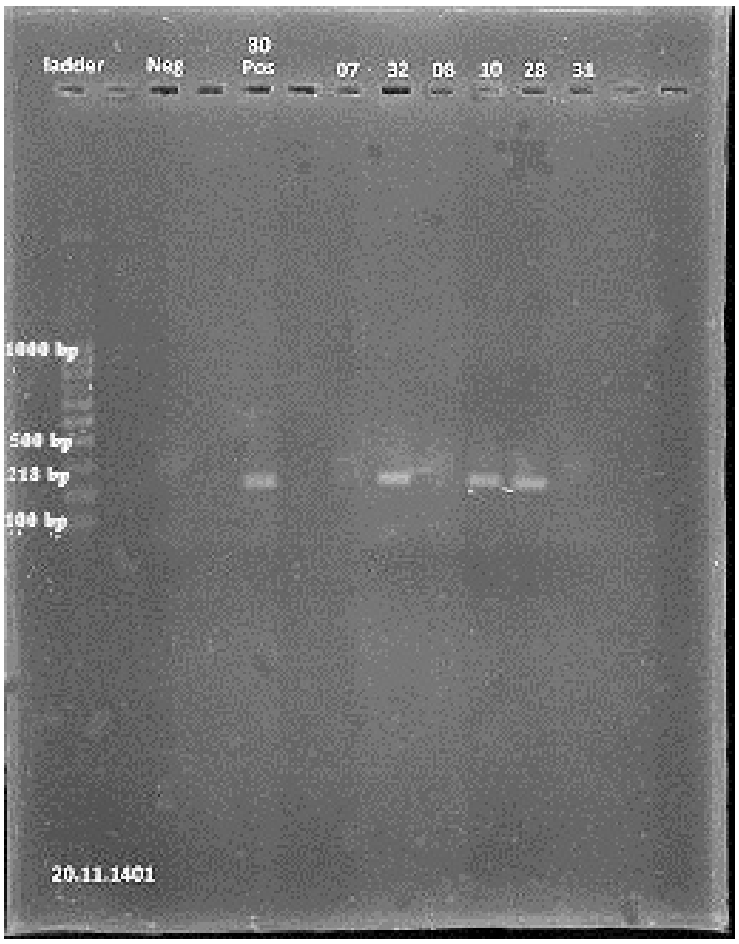

Fig. 3. Electrophoresis results of 16 *SSUrRNA* gene with special primers, M: Marker, P: Positive control, N: negative control, *Nosema ceranea* positive samples (218bp).

Table 3. The frequency of PCR positivity of *Nosema* spp. based on the region of migratory apiaries in Hormozgan province (2021-2022).

Regions	Provinces	*n* positive (%)	Total
West and Northwest	West Azerbaijan, East Azerbaijan, Kurdistan	20 (57.14)	35
East and Southeast	Khorasan Razavi, South Khorasan, North Khorasan	3 (15.7)	19
Central	Alborz, Isfahan, Qom, Chaharmahal and Bakhtiari, Lorestan	7 (50)	14
South	Kerman, Fars, Hormozgan	3 (27.27)	11
Total		33	79

Other studies in China and Saudi Arabia reported a high prevalence of *N. ceranea* in tropical wet and dry regions and a low prevalence in the regions with hot arid climates (Ansari *et al.*, 2017; Wang *et al.*, 2019). However, some studies showed that temperature and humidity do not have a positive or even negative effect on increasing the *N. ceranae* incidence in four Mediterranean countries and Serbia (Jabal-Uriel *et al.*, 2022; Vejnovic *et al.*, 2017). A good agreement was obtained between detecting *Nosema* infection in the examined apiaries by microscopic and molecular methods. The result was in line with the results of the two studies that have reported substantial to good agreement between microscopy and PCR methods (Khezri *et al.* 2018; Papini *et al.*, 2017).

The low cost and ease of work of the microscopic method could be used to prove the *Nosema* infection in the apiaries, although detection of the *Nosema* species needs the molecular methods application. In this study, the samples were tested by Multiplex PCR method using two primer pairs of *N. apis* and *N. ceranea* at the same time. All samples were positive for *N. ceranea* infection. Our results are consistent with other molecular studies that determined *N. ceranae* as the only causative agent of nosemosis in Iranian apiaries. (Nabian *et al.*, 2007; Razmaraii *et al.*, 2013; Modirrousta *et al.*, 2014; Aroee *et al.*, 2014; Shirzadi & Ramzi, 2021; Moeini *et al.*, 2022). The results of these studies have been summarized in Table 5. In the neighboring countries, a high prevalence of *N. ceranea* infection has been reported in apiaries in Turkey (Ivgin Tunca *et al.*, 2016), Azerbaijan (Ütük *et al.*, 2019), Iraq (Kareem *et al.*, 2021), and Saudia Arabia (Ansari *et al.*, 2017). To date, *N. ceranea* have been detected in honeybee colonies in different countries of all continents including Asia (Martín-Hernández *et al.*, 2018), Africa (Higes *et al.*, 2009), Europe (Higes *et al.*, 2006; Fries *et al.*, 2010), Australia (Giersch *et al.*, 2009), North America (Williams *et al.*, 2008; Huang *et al.*, 2015), and South America (Medici *et al.*, 2012; Teixeira *et al.*, 2013).

Diarrhea is the only detectable clinical sign in honey bees infected with *N. apis*. This symptom is not observed in hives infected with *N. ceranea* (Huang *et al.*, 2015; Papini *et al.*, 2017). The main clinical symptom is decreasing honeybees' population with the progression of the disease (Huang *et al.*, 2015). It has also been reported that *N. ceranae* is more virulent than *N. apis*; affects learning and homing behavior, causes higher energy costs and immune suppression, and affects queen health (Huang *et al.*, 2015). Despite the similar lifecycle of both *Nosema* species in midgut of honeybees, the reason for the difference in the symptoms is unknown (Huang *et al.*, 2015). However, no hives of *N. ceranae*-positive apiaries in the present study showed any clinical sign at the time of sampling.

Conclusion

Nosema ceranea was the only species of *Nosema* in local and migratory apiaries in Hormozgan province. The major limitation of this study was in sampling of some apiaries that beekeepers were not willing to cooperate with us in taking bees samples. Due to the high frequency of infection in migratory apiaries in Hormozgan province, it is necessary to carry out appropriate health measures such as screening of apiaries with appropriate diagnostic methods and training of beekeepers to disinfect hives in order to control *Nosema* infection in Iranian apiaries by the veterinary health officials. However, more epidemiological studies are needed to determine the actual frequency of nosemosis in apiaries in different parts in Iran.

Table 4. The comparison of the results of *Nosema* spp. detection in apiaries by microscopy and PCR.

		Microscopy Method			
	Results Number	Positive	Negative	Doubtful	Total
PCR Method	Positive	32	0	1	**33**
	Negative	0	42	9	**51**
	Doubtful	0	0	0	**0**
	Total	32	42	10	**84**

Table 5. Studies on frequency of *Nosema* infection in different areas of Iran until October 2023.

Province	Study year	Method	*n* examined	*n* infected	*Nosema* species	References
West Azarbijan	2002–2003	Microscopy	478	138	*N. apis?*	Tavassoli et al., 2009
Ardabil	2008	Microscopy	294	59	*Norsema* spp.	Lotfi et al., 2009
North Khorasan	2011	Microscopy	54	12	*N. apis?*	Moshaverinai et al., 2012
Mazandaran	2011	PCR	6 microscopy positive	6	*N. ceranae*	Nabiean et al., 2011
East Azarbaijan	2011	Microscopy	387	225	*N. ceranae*	Razmaraii et al., 2013
		PCR	387	260		
Alborz, East Azarbaijan, Ghazvin, Gilan, Tehran	2013	PCR	41 microscopy positive	41	*N. ceranae*	Modirrosta et al., 2014
Chaharmahal and Bakhtiari, Isfahan, Fars	2017	Microscopy	180	-	*N. ceranae*	Aroee et al., 2017
		PCR	180	-		
Kurdistan	2018	Microscopy	100	29	*N. ceranae*	Khezeri et al., 2018
		PCR	100	32		
Different provinces	2019	Microscopy	183	85	*N. ceranae*	Mohammadian et al., 2018
		PCR	183	66		
Mazandarn	2020	Microscopy	320	250	*N. ceranae*	Shirzadi and Razmi, 2021
		PCR	320	278		
West Azarbaijan	2022	Microscopy	840	269	*N. ceranae*	Moeini et al., 2022
		PCR	840	488		
East Azarbaijan	2022	Microscopy	165	165	*Nosema* spp.	Imani and Hamidiam, 2022

Acknowledgments

Authors are very grateful to Hamid Eshrati for his technical assistance.

Funding

This study was supported by grant no- 102305 from the Hormozgan Veterinary Head Office.

REFERENCES

Ansari, M. J., Al-Ghamdi, A., Nuru, A., Khan, K. A. & Alattal, Y. (2017) Geographical distribution and molecular detection of *Nosema ceranae* from indigenous honeybees of Saudi Arabia. *Saudi Journal of Biological Sciences* 24(5), 983–991. https://doi.org/10.1016/j.sjbs.2017.01.054

Aroee, F., Azizi, H., Shiran, B. & Pirali Kheirabadi, K. (2017) Molecular identification of *Nosema* species in provinces of Fars, Chaharmahal and Bakhtiari and Isfahan (Southwestern Iran). *Asian Pacific Journal of Tropical Biomedicine*, 7, 10–139. https://doi.org/10.1016/j.apjtb.2016.11.004

Bailey, L. & Ball, B. V. (1981) Honeybee Pathology. Academic Press, London.

Fries, I. (2010) *Nosema ceranae* in European honeybees (*Apis mellifera*). *Journal of Invertebrate Pathology* 103(1), S73–S79. https://doi.org/10.1016/j.jip.2009.06.017

Fries, I., Feng, F., Silva, A. D., Slemenda, S. B. & Pieniazek, N. J. (1996) *Nosema ceranae* n. sp. (Microspora, Nosematidae), morphological and molecular characterization of a microsporidian parasite of the Asian honeybee *Apis cerana* (Hymenoptera, Apidae). *European Journal of Protistology* 32(3), 356–365. https://doi.org/10.1016/S0932-4739(96)80059-9

Galajda, R., Valenčáková, A., Sučik, M. & Kandráčová, P. (2021) *Nosema* disease of European honeybees. *Journal of Fungi (Basel).* 7(9), 714. https://doi.org/10.3390/jof7090714

Giersch, T., Berg, T., Galea, F. & Hornitzky, M. (2009) *Nosema ceranae* infects honeybees (*Apis mellifera*) and contaminates honey in Australia. *Apidologie*, 40(2), 117–123 https://doi.org/10.1051/apido/2008065

Higes, M., Martín, R. & Meana, A. (2006) *Nosema ceranae*, a new microsporidian parasite in honey bees in Europe. *Journal of Invertebrate Pathology* 92(2),93–95. https://doi.org/10.1016/j.jip.2006.02.005

Higes, M., Martín-Hernández, R., Garrido-Bailón, E., Botías, C. & Meana, A. (2009) The presence of *Nosema ceranae* (microsporidia) in North African honeybees (*Apis mellifera intermissa*) *Journal of Apicultural Research* 48(3),217 –219. https://doi.org/10.3896/IBRA.1.48.3.12

Huang, W. F., Solter, L., Aronstein, K. & Huang, Z. (2015). Infectivity and virulence of *Nosema ceranae* and *Nosema apis* in commercially available North American honeybees. *Journal of Invertebrate Pathology*, 124, 107–113. https://doi.org/10.1016/j.jip.2014.10.006

Human, H., Brodschneider, R., Dietemann, V., Dively, G., Ellis, J. D., Forsgren, E., et al (2013). Miscellaneous standard methods for *Apis mellifera* research. *Journal of Apicultural Research* 52(4),1–53. https://doi.org/10.3896/IBRA.1.52.4.10

Imani- Baran, A., Hamidiam, G. (2022). A comparative study of the frequency and intensity of nosemosis based on individual and composite samples of live bees in the apiaries of the cities of East Azerbaijan province. *Veterinary Clinical Pathology* 16(63), 269-280. https://doi.org/10.30495/JVCP.2023.1964566.1376

Ivgin -Tunca, R., Oskay, D., Gosterit, A. & Tekin, O. K. (2016) Does *Nosema ceranae* wipe out *Nosema apis* in Turkey? *Iranian Journal of Parasitology* 11(2), 259–264.

Kareem, J. A., Kareem, A. A. & Shaher, K. W (2021) Detection of *Nosema* Cerranain samples of Iraqi bees using traditional and molecular methods. *Annals of the Romanian Society for Cell Biology* 24, 3008–30013.

Jabal-Uriel, C., Barrios, L., Bonjour-Dalmon, A., Caspi-Yona, S., Chejanovsly, N., Erez, T. & Martín-Hernández, R. (2022) Epidemiology of the microsporidium *Nosema ceranae* in four mediterranean countries. *Insects* 13(9), 844. https://doi.org/10.3390/insects13090844

Khezri, M., Moharrami, M., Modirrousta, H., Torkaman, M., Salehi, S., Rokhzad, B. & Khanbabi, H. (2018). Molecular detection of *Nosema ceranae* in the apiaries of Kurdistan province, Iran. *Veterinary Research Forum* 9(3),273–278. https://doi.org/10.30466/vrf.2018.32086

Lotfi, A., Jamshidi, R., Aghdam Shahryar, H. & Yousefkhani, M. (2009) The prevalence of nosemosis in honey bee colonies in the Arasbaran region (Northwestern Iran). *Journal of Agriculture Environmental Science* 5, 255–257.

Martín-Hernández, R., Meana, A., Prieto, L., Salvador, A.M., Garrido-Bailón, E. & Higes, M. (2007). The outcome of colonization of *Apis mellifera* by *Nosema ceranae*. *Applied Environmental Microbiology* 73(20),6331–6338. https://doi.org/10.1128/AEM.00270-07

Martín-Hernández, R., Bartolomé, C., Chejanovsky, N., Le Conte, Y., Dalmon, A., Dussaubat, C., García-Palencia, P., Meana, A., Pinto, M. A., Soroker, V. & Higes, M. (2018) *Nosema ceranae* in *Apis mellifera*: A 12 years postdetection perspective. *Environmental Microbiology* 20(4),1302 –1329. https://doi.org/10.1111/1462-2920.14103

Medici, S. K., Sarlo, E. G., Porrini, M. P., Braunstein. M. & Eguaras, M. J. (2012) Genetic variation and widespread dispersal of *Nosema ceranae* in *Apis mellifera* apiaries from Argentina. *Parasitology Research* 110,859–864 https://doi.org/10.1007/s00436-011-2566-2

Modirrousta, H., Moharrami M. & Mansouri, M. (2014) Retrospective study of the *Nosema ceranae* infection of honeybee colonies in Iran (2004-2013). *Archives Razi Institute* 69(2),197–200.

Mohammadian, B., Bokaie, S., Moharrami, M., Nabian, S. & Forsi, M. (2018) Distribution of *Nosema* spp. in climatic regions of Iran. *Veterinary Research Forum* 9(3),259–263. https://doi.org/10.30466/vrf.2018.32082

Moeini, S., Malekifard F. & Tavassoli, M. (2022) Identification of the *Nosema* spp., a microsporidian parasite isolated from the honeybees (*Apis mellifera*) and its association with honeybee colony losses in apiaries of Iran. *Journal of the Hellenic Veterinary Medical Society* 73(1),3667 –3672.

Moshverinia, A., Abedi, V., Safaie, H. (2012) A survey of *Nosema apis* infection in apiaries of North Khorasan province, Iran. *Iranian Journal of Veterinary Science and Technology* 4, 25–30. https://doi.org/10.22067/veterinary.v4i2.17418

Nabian, S., Ahmadi, K., Shirazi, M. N. & Gerami Sadeghian, A. (2011) First detection of *Nosema ceranae*, a microsporidian protozoon of European honeybees (*Apis mellifera*) in Iran. *Iranian Journal of Parasitology* 6(3), 89–95.

WOAH (World Organisation for Animal Health) (2019) Manual for diagnostic tests and vaccines for terrestrial animals, Nosemosis of honeybees, Paris.

Özgör, E., Güzerin, E. & Keskin, N. (2015). Determination and comparison of *Nosema apis* and *Nosema ceranae* in terms of geographic and climatic factors. *Hacettepe Journal of Biology and Chemistry* 43(1), 9–15.

Papini, R., Mancianti, F., Canovai, R., Cosci, F., Rocchigiani, G., Benelli, G. & Canale, A. (2017) Prevalence of the microsporidian *Nosema ceranae* in honeybee (*Apis mellifera*) apiaries in central Italy. *Saudi Journal of Biological Sciences* 24 (5),979–998. https://doi.org/10.1016/j.sjbs.2017.01.010

Petrie, A. & Watson P. (2006) Statistics for veterinary and animal science. Blackwell, Oxford.

Razmaraii, N., Sadegh-Eteghad, S., Babaei, H., Paykari, H., Esmaeilnia, K. & Froghy, L. (2013) Molecular identification of *Nosem*a species in East Azerbaijan province, Iran. *Archives Razi Institute* 68, 23–27.

Safa, O., Soltanpoor, M., A., Rastegar, S., Kazemi, M., Nourbakhsh, K. & Ghanndi, A. (2012) An ethnobotanical survey on Hormozgan province, Iran. *Avicenna Journal of Phytomedicine* 31, 64-81.

Shirzadi, A. & Razmi, G. (2021). A microscopy and molecular studies of *Nosema ceranae* infection in Mazandaran province of Iran. *Uludağ Arıcılık Dergisi* 21(2), 198–205. https://doi.org/10.31467/uluaricilik.991579

Teixeira, E. W, dos Santos, L. G., Sattler, A., Message D., Alves, M. L. T. M. F., Martins M.F, et al. (2013) *Nosema ceranae* has been present in Brazil for more than three decades infecting Africanized honeybees. *Journal of Invertebrate Pathology* 114, 250–254. https://doi.org/10.1016/j.jip.2013.09.002

Tavassoli, M., Eiganinejad, S. & Alizadeh-Asl, S. (2009) A survey on *Nosema apis* infection in apiaries of Urmia, North-West of Iran. *Iranian Journal of Veterinary Science and Technology* 1(1), 35–40. https://doi.org/10.22067/veterinary.v1i1.2271

Thursfield, M. V. (1986). Veterinary Epidemiology, Butterworth, Guilford, London.

Ütük, A. E., Aliyeva, R., Girisgin, A. O., Gökmen, T. G., Özüiçli, M. & Aydın, L. (2019). First molecular detection of *Nosema ceranae* in Azerbaijan. *Journal of Apicultural Research* 58(4),559–561. https://doi.org/10.1080/00218839.2019.1614737

Vejnovic, B., Stevanovic. J., Schwarz. R. S., Aleksic. N., Mirilovic. M., Jovanovic, N. M. & Stanimirovic, Z. (2017) Quantitative PCR assessment of *Lotmaria passim* in *Apis Mellifera* colonies co-infected naturally with *Nosema ceranae*. *Journal of Invertebrate Pathology* 151, 76–81. https://doi.org/10.1016/j.jip.2017.11.003

Wang, Q., Dai, P., Guzman-Novoa, E., Wu, Y., Hou, C. & Diao, Q. (2019) *Nosema ceranae*, the most common microsporidium infecting *Apis mellifera* in the main beekeeping regions of China since at least 2005. *Journal of Apicultural Research* 58, 562–566. https://doi.org/10.3390/vetsci8060107

Williams, G. R., Shafer, A. B., Rogers, R. E., Shutler, D. & Stewart, D. T. (2008) First detection of *Nosema ceranae*, a microsporidian parasite of European honeybees (*Apis mellifera*), in Canada and central USA. *Journal of Invertebrate Pathology* 97(2), 189 –192. https://doi.org/10.1016/j.jip.2007.08.005

مطالعه انگل‌شناسی و ملکولی نوزموزیس در زنبورستان‌های مهاجر در استان هرمزگان، جنوب ایران

بهاره مفتاحی[۱]، سعید یغفوری[۲] ، صادق موسی زاده [۲] ، رضا شیبانی تزرجی[۲] ، مصطفی فخرابادی پور[۲] ، اسماعیل جوان[۲] و غلامرضا رزمی [۱]

۱- گروه پاتوبیولوژی، دانشکده دامپزشکی، دانشگاه فردوسی مشهد، مشهد، ایران
۲- اداره کل دامپزشکی هرمزگان، بندرعباس، ایران

✉b.meftahi.m@gmail.com	https://orcid.org/0000-0003-2799-8387
✉saeed.yaghfoori@gmail.com	https://orcid.org/0000-0002-5844-2464
✉Sadeghmosaadeh16@gmail.com	https://orcid.org/0009-0006-7628-7353
✉Shahriarsheibani1313@gmail.com	https://orcid.org/0009-0004-0769-3628
✉m.fakhrabadi1983@gmail.com	https://orcid.org/0009-0008-7584-8459
✉ horvet@yahoo.com	https://orcid.org/0009-0003-3047-2148
✉ razmi@um.ac.ir	https://orcid.org/0000-0002-0754-1278

تاریخچه مقاله

دریافت: ۱۴۰۲/۰۵/۰۹ | **پذیرش:** ۱۴۰۲/۰۸/۰۵ | **دبیر تخصصی:** علیرضا سازمند

چکیده

نوزموزیس یک بیماری میکروسپوریدیایی ناشی از *Nosema ceranae* و *Nosema apis* است که باعث آلودگی زنبوران بالغ در کلنی‌های زنبورعسل در سراسر جهان می‌گردد. انتقال اسپورهای *نوزما* از طریق مسیرهای مدفوعی–دهانی و دهانی– دهانی اتفاق می‌افتد. با توجه به اهمیت سلامت زنبورهای عسل، هدف از این مطالعه بررسی فراوانی آلودگی با *نوزما* در زنبوداری های مهاجر به استان هرمزگان با استفاده از روش‌های میکروسکوپی و مولکولی بود. بدین منظور از ۸۴ زنبورستان مهاجر در استان هرمزگان نمونه‌برداری شد، بدین صورت که از هر زنبورستان ۱۰ کندو انتخاب شده و از هر کندو ۲۰ تا ۳۰ زنبورعسل مسن به صورت تصادفی انتخاب شده و به آزمایشگاه منتقل شدند. در آزمایشگاه، شکم زنبورها با پنس و قیچی حشره‌شناسی از بقیه بدن جدا شده و در هاون حاوی سرم نمکی آسیاب شد. سوسپانسیون تهیه شده با عبور از الک صاف شده و به لوله‌های آزمایش منتقل و سانتریفیوژ گردید. رسوب ته لوله‌های ازمایش به طور مکرر با افزودن سرم فیزیولوژی و سانتریفیوژ شسته شده و برای تشخیص عفونت *نوزما* با روش‌های میکروسکوپی و مولکولی مورد بررسی قرار گرفتند. در بررسی میکروسکوپی اسپورهای گونه‌های *Nosema* در ۳۸/۲ درصد زنبورستان ها مشاهده شدند. با روش PCR اما ۳۹/۲ درصد زنبورستان ها آلوده به دنای *N. ceranae* تشخیص داده شدند. هیچ موردی از آلودگی *N. apis* مشاهده نشد. با توجه به فراوانی قابل ملاحظه‌ی آلودگی *نوزما* در زنبورستان های مهاجر به استان هرمزگان، انجام اقدامات بهداشتی مناسب همچون غربالگری زنبوستان های کشوز با روش‌های تشخیصی مناسب و آموزش زنبورداران جهت ضدعفونی صحیح کندوها برای کنترل بیماری در زنبورستان های ایران توسط مسئولان بهداشتی دامپزشکی ضروری است.

کلمات کلیدی: بیماری شناسی حشرات، زنبور عسل، شناسایی مولکولی

نویسنده مسئول: غلامرضا رزمی (پست الکترونیک: razmi@um.ac.ir)

Citation: Meftahi, B., Yaghfouri, S., Mosazadeh, S., Sheibani Tezerji, R., Fakhrabadipour, M., Javdan, E. & Razmi, G. R. (2023) Parasitological and molecular study of nosemosis in migratory apiaries in Hormozgan Province, southern. *J. Entomol. Soc. Iran* 43 (4), 383-392.https://doi.org/10.61186/jesi.43.4.6