ORIGINAL ARTICLE

Veterinary Research Forum. 2018; 9 (3) 259 - 263
doi: 10.30466/vrf.2018.32082
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Distribution of *Nosema Spp.* in climatic regions of Iran

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Article Info

Article history:

Received: 25 June 2017
Accepted: 09 January 2018
Available online: 15 September 2018

Key words:

Climate
Honeybee
Iran
Nosemosis
Prevalence

Abstract

Nosemosis is one of the most prevalent bee diseases in the world causing significant economic losses in the global bee-keeping industry. This cross-sectional study was conducted during April-September, 2016 to investigate the prevalence of nosemosis in different climatic regions of Iran. A total of 183 apiaries were selected based on cluster sampling and the climate of apiaries under study was classified using Domarten method. In each apiary, five percent of the colonies were randomly sampled. A total of 183 adult bee samples were taken and examined by microscopic and polymerase chain reaction (PCR) methods for the presence of *Nosema* infections. According to the results, infection caused by *Nosema ceranae* was observed in all regions under study. The prevalence of *N. ceranae* was 46.40% (42.70–50.10). However, infection with *Nosema apis* was not observed in the samples in either pure form or as associated infection. Based on the results of PCR, the prevalence of *N. ceranae* was 53.80% (46.60– 61.00) in humid, 71.00% (53.70–77.50) in semi-humid, 68.10% (61.40–74.80) in very humid, 29.40% (22.70–36.10) in arid, 34.30% (27.40–41.20) in semi-arid and 24.00% (17.90–30.00) in Mediterranean climates. The prevalence of infection in different climatic zones of the country was found to have significant differences ($p < 0.001$). According to the findings, *N. ceranae* was the only *Nosema* species in honeybees with a broad geographical dispersion in Iran. It seems that climate can influence the prevalence of mentioned parasite.

پراکنش گونه‌های *نوزما* در مناطق اقلیمی ایران

چکیده

نوزموزیس یکی از شایعترین بیماریهای زنبورعسل در جهان می‌باشد که موجب خسارات اقتصادی در صنعت زنبورداری می‌شود. این مطالعه‌ی مقطعی در فاصله‌ی زمانی فروردین تا مهر ۱۳۹۵ به منظور بررسی شیوع نوزموزیس در مناطق اقلیمی متفاوت ایران انجام شد. تعداد ۱۸۳ زنبورستان بر اساس نمونه گیری خوشه‌ای انتخاب و اقلیم زنبورستانهای مورد مطالعه با استفاده از روش دومارتن تعیین شد. در هر زنبورستان از ۵/۰۰ درصد از کلونی‌ها به صورت تصادفی نمونه گیری شد. تعداد ۱۸۳ نمونه‌ی زنبور بالغ اخذ و به وسیله روش های ریزبینی و واکنش زنجیره ای پلی مراز (PCR) جهت حضور آلودگی با گونه‌های *نوزما* ارزیابی شدند. بر اساس نتایج، آلودگی با *نوزما سرانه* در همه‌ی نواحی اقلیمی تحت مطالعه مشاهده گردید. شیوع *نوزما سرانه* (۴۲/۵۰–۵۰/۱۰) ۴۶/۴۰ درصد بود. در حالی که آلودگی با *نوزما آپیس* به صورت آلودگی خالص یا به صورت آلودگی همراه در نمونه‌ها مشاهده نشد. بر اساس نتایج PCR، شیوع *نوزما سرانه* در اقلیم مرطوب (۴۶/۶۰–۶۱/۰۰) ۵۳/۸۰ درصد، نیمه مرطوب (۵۳/۷۰–۷۷/۵۰) ۷۱/۰۰ درصد ، خیلی مرطوب (۶۱/۴۰–۷۴/۸۰) ۶۸/۱۰ درصد ، خشک (۲۲/۷۰–۳۶/۱۰) ۲۹/۴۰ درصد، نیمه خشک (۲۷/۴۰–۴۱/۲۰) ۳۴/۳۰ درصد و مدیترانه‌ای (۱۷/۹۰–۳۰/۰۰) ۲۴/۰۰ درصد بود. شیوع آلودگی در مناطق اقلیمی مختلف کشور واجد اختلاف معنی‌دار بود ($p < ۰/۰۰۱$). بر اساس یافته‌های این مطالعه، *نوزما سرانه* تنها گونه‌ی نوزما با پراکنش جغرافیایی وسیع در زنبورستان‌های ایران بود. به نظر می‌رسد که اقلیم می‌تواند بر شیوع انگل مذکور تأثیر بگذارد.

واژه های کلیدی: اقلیم، ایران، زنبورعسل، شیوع، نوزموزیس

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Introduction

Nosemosis is known as an important bee disease with worldwide distribution. It is an infectious disease of honeybees that leads to the weakening and eventually death of colony.[1] The disease was first described by Zander (1909), although the spores had been shown to cause the disease in much earlier.[2] Nosema spores are not relatively sensitive to heat and drought and survive in unfavourable environmental conditions for a long time.[3] The disease is transferred horizontally by worker bees during ingestion of the spores when cleaning the fecal stains on the frames.[4]

Two species known as *N. ceranae* and *N. apis* are pathogenic for bees. All the bees in a colony (queen, workers and male bees) are vulnerable to be infected with *Nosema* species.[5] The spores of these species share similar morphological characteristic making the distinction quite difficult by traditional microscopic methods.[6] Recently, the polymerase chain reaction (PCR) technique has been used for successful detection of the entire life cycle of the microsporidia and determines the parasite even at a very low level of microsporidia infection.[7]

Since *Nosema apis* has been the pathogen of European honey bees for several years, numerous studies have been conducted on nosemosis with *N. apis* agent, but studies on *N. ceranae* are limited.[8] The studies carried out before 2003 suggest the worldwide expansion of *N. apis* in *A. mellifera*, though *N. ceranae* has been shown to spread among *A. mellifera* in Europe, America and Asia after 2003.[9]

Higes *et al.* for the first time have reported the natural infection of *A. melifera* with *N. ceranae* among the apiaries in Spain.[10] However, it was found that *N. ceranae* is not a new pathogen for European honeybee and its previously unknown nature may have been due to the difficulty of differential diagnosis of the *Nosema* species.[11]

N. ceranae was first reported by Nabian *et al.* among *A. mellifera* colonies in Mazandaran, Iran.[12] later, the infection with the parasite has been reported by further studies. The study by Razmaraii *et al*. showed that *N. ceranae* was the only *Nosema* species in East Azerbaijan province.[13] According to the findings of a study by Modirrousta *et al.*, the samples collected from five provinces in Iran from 2004 to 2013 were positive for *N. ceranae*. The identification of *N. ceranae* in the samples of recent years in Iran indicates the transition of *N. ceranae* to *A. mellifera* before its identification.[14] Besides, the study by Aroee *et al.* reported the infection of honeybee samples with *N. ceranae* microsporidia in three provinces of Esfahan, Chaharmahal and Bakhtiari, and Fars.[15]

Since *N. ceranae* infection has been reported in different regions of Iran in the recent years, this study was conducted to estimate the dispersion of *Nosema Spp.* in different climatic regions of the Iran.

Materials and Methods

This cross-sectional study was performed during April-September, 2016. A total of 183 apiaries located in different provinces of Iran were selected by cluster sampling (Table 1). The climate of selected apiaries was determined based on Domarten approach. In this approach, the climate of region is assessed based on the following drought indicator: [16]

$$Aridity\ Index = \frac{P}{T} + 10$$

where, P is the average annual rainfall (mm) and T is normal rate of annual temperature (˚C). In this study, the type of climates was determined by calculating the drought indicator of each region.

Sample collection. In each apiary, five percent of colonies were sampled randomly. All samples were stored at –20 ˚C before analyzed. Adult bee samples were then examined for the presence of *Nosema* infections using molecular and microscopic diagnostic approaches.

Preparation of samples for microscopic examination. The abdomen of 20 adult dead honeybees from each apiary were macerated in 10 mL distilled water and crushed in a mortar. Then, the suspension was passed through a 100 µm mesh sieve to remove the debris and centrifuged for 6 min at 800 *g*. Finally, the supernatant was discarded and the pellet was examined under a common light microscope at 400× magnification.[17]

Preparation of samples for PCR. The abdomen of 20 adult dead honeybees from each apiary were macerated in 10 mL distilled water (PCR grade) and the suspension was then filtered and centrifuged at 800 *g* for 6 min. Spore germination was induced with 200 µL freshly prepared germination buffer and the mixture was incubated at 37 ˚C for 15 min.[17]

DNA extraction. The DNA was isolated using DNA extraction kit (Takapozist, Tehran, Iran) according to the manufacturer's instructions.

PCR. Amplification was performed by the PCR kit (Sinacolon, Tehran, Iran) in a Mastercycler gradient (Eppendorf, Hamburg, Germany) under conditions as follows: 50 µL reaction cocktail containing 25 µL of high-fidelity PCR master mixture, 0.40 µM of each primer, 0.40 mM of each deoxynucleoside triphosphate, 3 mM Cl_2Mg, 0.20 mg mL^{-1} bovine serum albumin, 0.10 Triton X-100 and 5 µL *N. apis* or *N. ceranae* DNA template, initial denaturation at 94 ˚C for 2 min, 10 cycles of 94 ˚C for 15 sec, 61.80 ˚C for 30 sec and 72 ˚C for 45 sec and 20 cycles of 94 ˚C for 15 sec, 61.80 ˚C for 30 sec and 72 ˚C for 50 sec along with the final extension at 72 ˚C for 50 sec and at 72 ˚C for 7 min. Amplicons were run on a 2.00% agarose gel electrophoresis, stained with a safe stain and visualized by ultraviolet transillumination.[7] In this study, a small subunit 16S rRNA gene of *N. apis* and *N. ceranae* was used (Table 2). Positive controls for the mentioned species were

prepared from Honeybee-Silkworm and Wildlife Diseases' Department of Razi Vaccine and Serum Research Institute, Karaj, Iran. The results of PCR are shown in Fig. 1.

Data Analysis. Descriptive results were prepared using SPSS (version 21.0; SPSS Inc., Chicago, USA). The chi-square test was used to compare the level of infection with *N. ceranae* in all regions under study. Cohen's kappa value was used to evaluate the agreement between molecular and microscopic tests by SPSS.

Table 1. Frequency of studied apiaries in different provinces of Iran in 2016.

Provinces	No. of apiaries*	Frequency	Valid (%)
Kerman	194	2	1
Qom	187	2	1
Yazd	668	9	5
South Khorasan	337	2	1
Khorasan Razavi	1078	8	4
Lorestan	1808	13	7
North Khorasan	1400	4	2
Chaharmahal and Bakhtiari	1268	7	4
Fars	2688	14	8
West Azerbaijan	3882	26	14
Kurdistan	3606	22	12
Gilan	2579	17	9
Ardabil	705	4	3
Qazvin	1344	10	5
Golestan	1824	13	7
Mazandaran	3160	21	12
Semnan	283	9	5
Total	27011	183	100

* Iran Veterinary Organization data (2015).

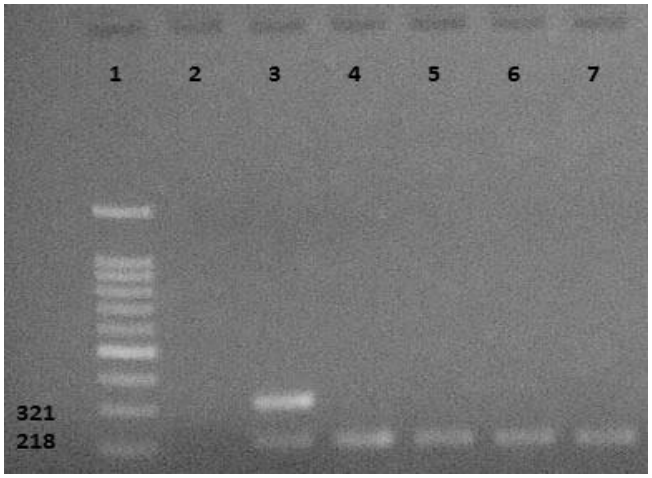

Fig. 1. The PCR results of *N. ceranae* detection. 1: Lane Marker, 2: Negative control, 3: Positive control (*N. apis & N. ceranae*), 4, 5, 6, 7 (*N. ceranae*).

Results

Based on the findings, *N. apis* was not found in any of the samples. In contrast, *N. ceranae* parasite was found in all studied regions.

The prevalence of *N. ceranae* was 46.40%. Table 3 shows the prevalence of *N. ceranae* based on microscopic and molecular diagnostic approaches. The confidence interval of total prevalence was calculated based on the 183 samples in the climatic regions of the country. The results indicated that contamination with *N. ceranae* was significantly different in various climatic regions of the country ($p < 0.001$).

The Kappa coefficient was also calculated in order to determine agreement between microscopic and molecular tests. The kappa value was 0.79 (0.65–0.93), thus the concordance between the two methods was substantial.

Discussion

Nowadays, infection with *N. ceranae* has been proved in all continents. Recent reports have confirmed changes in the clinical and epidemiological patterns of nosemosis suggesting that *N. ceranae* has been one of the most prevalent pathogens in honeybees around the world,[18] which is extremely virulent for its new host, *A. mellifera*.[5] Earlier reports in Iran have indicated that *N. apis* has been the only pathogen of nosemosis.[12] Studies conducted in Iran reported *N. apis* infection in honeybee colonies in Arasbaran,[19] East Azerbaijan province,[20] Tabriz and Meshkinshahr,[21] Urmia[22] and North Khorasan province.[23]

In contrast to previous studies, the findings of the present study indicated a widespread prevalence of *N. ceranae* in Iran. The experimental results indicated pure infection with *N. ceranae* in 85 (46.40%) apiaries. The absence of *N. apis* in its main host, *A. mellifera*, could mean the substitution of *N. ceranae* in Iranian apiaries. Although it is not recognized when and how the host shift happened, several reasons have been proposed including the transfer of honeybees and queen products between apiaries, migration and climate change in the recent years.

According to a study in Turkey, Van province, the prevalence of nosemosis was identified to be 32.50%. However, *N. ceranae* was the only *Nosema* species found to infect honeybees and no *N. apis* or mixed infections were detected in the samples.[24]

Table 2. The sequence of specific primers used for identification of *Nosema* species in PCR according to Martín-Hernández *et al*.[7]

Specificity	Primer sequence (5`-3`)	PCR product size (bp)
N. ceranae	FWD: 5'- CGGCGCGACGATGTGATATGAAAATATTAA-3' REV: 5'-CCCGGTCATTCTCAAAAAACCG-3'	218-219
N. apis	FWD: 5'-GGGGGCATGTCTTTGACGTACTATGTA-3' REV: 5'-GGGGGCGTTTAAAATGTGAAACAACTATG-3'	321

Table 3. The prevalence of *Nosema ceranea* in climatic regions of Iran in 2016.

Climate	No. of Sample	Molecular results		Microscopic results	
		No. of positive	Prevalence (CI: 95%)	No. of positive	Prevalence (CI: 95%)
Humid	39	21	53.80% (46.60–61.00)	15	38.50% (31.50–45.50)
Semi-humid	31	22	71.00% (53.70–77.50)	21	67.70% (60.90–74.50)
Very Humid	22	15	68.10% (61.40–74.80)	14	63.60% (56.60–70.50)
Mediterranean	25	6	24.00% (17.90–30.00)	2	8.00% (4.00–11.90)
Arid	34	10	29.40% (22.70–36.10)	6	17.60% (12.10–23.10)
Semi-arid	32	11	34.30% (27.40–41.20)	8	25.00% (18.70–31.30)
Total	183	85	46.40% (42.70–50.10)	66	36.10% (29.20–43.00)

Similarly, different studies conducted in Croatia,[25] Taiwan,[26] Uruguay[27] and the United States[7,28] confirmed the transition of *N. ceranae* to *A. mellifera*. In contrast, there have been no reports of substitution of parasite with another one in countries such as Germany and Sweden.[18]

In this study, the prevalence of *N. ceranae* in the climatic zones was significantly different and the highest prevalence was found for the semi-humid climate (71.00%) followed by very humid (68.10%) and humid (53.80%) climates. The high prevalence of infection in the humid regions is in accordance with the findings of the study conducted in Turkey indicating that the impact of moisture on *N. ceranae* is more intense in areas with higher humidity and the humidity level was described as a vital factor for *Nosema* species.[29] This finding may allow us to predict the dynamics of *N. ceranae* using average humidity in different regions.

According to De la Rocque *et al.*, climatic changes may influence distribution, intensity and seasonality of infectious diseases such as nosemosis.[30] The multiplication rates of both parasites (*N. ceranae* and *N. apis)* are dependent on temperature but *N. ceranae* exhibits a faster growth in slightly higher temperatures compared to *N. apis.*[31] Higher prevalence of *N. ceranae* in warmer weather was proposed by Fries and Forsgren, while the study by Higes *et al.* reported the low seasonal dependence of *N. ceranae* and its presence in all four seasons.[8]

The study by Martín-Hernández *et al.* also emphasized the presence of the parasite throughout the year,[7] while a study by Stevanovic *et al.* showed a seasonal pattern of the aforesaid parasite.[32]

Nosema ceranae infection has been found to have a wide range of clinical symptoms. Also, since several cases of colony population decline have been attributed to the infection of bees with *N. ceranae,*[33-36] it is necessary to study the causal relation of *N. ceranae* with the sudden decline in the bee population in apiaries across the country.

According to the findings of present study, *N. ceranae* was the main causal agent of nosemosis in the studied apiaries of Iran. It seems that *N. ceranae* has been transferred to its new host, *A. mellifera*. Since the prevalence of the parasite was significantly different among climatic regions, this probably enables to predict the dynamics of *N. ceranae* using climatic factors in different regions.

Acknowledgments

The authors express their deep gratitude to Veterinary Organization for providing the bee samples. We also appreciate the efforts made by veterinarians in different provinces for sampling from the apiaries and offer our thanks to Mrs. Torkaman, Mrs. Salahi and Mr. Garousi for laboratory analysis in Department of Honeybee-Silkworm and Wildlife Diseases at Razi Vaccine and Serum Research Institute, Karaj, Iran.

Conflict of interest

The authors declare no conflict of interest.

References

1. Webster T, Pomper K, Hunt G, et al. *Nosema apis* infection in worker and queen *Apis mellifera*. Apidologie 2004; 35(1): 49-54.
2. Fries I. *Nosema apis* - a parasite in the honeybee colony. Bee World 1993; 74: 5-19.
3. Oranlar E, Lotfi A, Aghdam Shahryar H. Seasonal incidence of some economic bee diseases (varroosis, nosemosis and American foulbrood) in honeybee colonies of northwestern Iran. Uludag Bee J 2011; 11: 25.
4. Paxton R., Klee J, Korpela S, et al. *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. Apidologie 2007; 38: 558-565.
5. Chen YP, Evans JD, Murphy C, et al. Morphological, molecular and phylogenetic characterization of *Nosema ceranae*, a microsporidian parasite isolated from the European honey bee *Apis mellifera*. J Eukar Microbiol 2009; 56: 142-147.
6. Weiss LM, Vossbrinck CR. Molecular biology, molecular phylogeny, and molecular diagnostic approaches to the microsporidia. In: Wittner M, Weiss LM (Eds). The microsporidia and microsporidiosis. Washington DC, USA: American society for microbiology 1999; 129-171.
7. Martín-Hernández R, Meana A, Prieto L, et al. Outcome of colonization of *Apis mellifera* by *Nosema ceranae*. Appl Environ Microbiol 2007; 73(20): 6331-6338.
8. Higes M, Martín-Hernández R, Meana A. *Nosema ceranae* in Europe: An emergent type C Nosemosis.

Apidologie 2010; 41: 375-392.
9. Klee J, Besana AM, Genersch E, et al. Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. J Invertebr Pathol 2007; 96(1): 1-10.
10. Higes M, Martín-Hernández R, Meana A. *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. J Invertebr Pathol 2006; 92: 81-83.
11. Higes M, Martin-Hernandez R, Garrido-Bailon E, et al. Honeybee colony collapse due to *Nosema ceranae* in professional apiaries. Environ Microbiol Rep 2009; 1: 110-113.
12. Nabian S, Ahmadi K, Shirazi MN, et al. First detection of *Nosema ceranae*, a microsporidian protozoa of European honeybees (*Apis mellifera*) in Iran. Iran J Parasitol 2011; 6(3): 89-95.
13. Razmaraii N, Sadegh-Eteghad S, Babaei H, et al. Molecular identification of *Nosema* species in East Azerbaijan province, Iran. Arch Razi Inst 2013; 68(1): 23-27.
14. Modirrousta H, Moharrami M, Mansouri MA. Retrospective study of the *Nosema ceranae* infection of honey bee colonies in Iran (2004-2013). Arch Razi Inst 2014; 69(2): 197-200.
15. Aroee F, Azizi H, Shiran B, et al. Molecular identification of *Nosema* species in provinces of Fars, Chaharmahal and Bakhtiari and Isfahan (Southwestern Iran). Asian Pac J Trop Biomed 2017; 7(1): 10-13.
16. Kamyabi S, Farahani S. Effective climatic factors in mountainous regions of Iran for urban planning: A case study in Rudbar Qasran in the central Alborz. e-Bangi: J Soc Sci Humanit 2011; 6(1): 17-28.
17. Office international des epizooties, manual of diagnostic tests and vaccines for terrestrial animals. 2008; Chap. 2.2.4., Nosemosis of honey bees. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.02.04_nosemosis.pdf. Accessed June 25, 2017.
18. Fries I. *Nosema ceranae* in European honey bees (*Apis mellifera*). J Invertebr Pathol 2010; 103(S1): S73-S79.
19. Lotfi A, Jamshidi R, Aghdam Shahryar H, et al. The prevalence of nosemosis in honey bee colonies in Arasbaran region (northwestern Iran). American-Eurasian J Agric Environ Sci 2009; 5(2): 255-257.
20. Razmaraii N, Karimi H. A survey of nosema disease of honey bee (*Apis mellifera*) in East Azarbaijan province of Iran. J Anim Vet Adv 2010; 9(5): 879-882.
21. Davoudi J, Naderi A, Mohammadpour F, et al. Study of infection rate of suburb bee hives to parasites *Nosema apis*, Varroa spp. and *Acarapis woodi* in Miyaneh, Iran. J New Agric Sc 2009; 4(13): 39-43.
22. Tavassoli M, Eiganinejad S, Alizadeh Asl S. A survey on *Nosema apis* infection in apiaries of Urmia, north-west of Iran. Iran J Vet Sci Technol 2009; 1(1): 35
23. Moshaverinia A, Abedi V, Safaei H. A survey of *Nosema apis* infection in apiaries of North Khorasan province, Iran. Iran J Vet Sci Technol 2012; 4(2): 25-30.
24. Oğuz B, Karapinar Z, Dinerç E, et al. Molecular detection of *Nosema spp.* and black queen-cell virus in honeybees in Van province, Turkey. Turk J Vet Anim Sci 2017; 41: 221-227.
25. Gajger IT, Vugrek O, Grilec D, et al. Prevalence and distribution of *Nosema ceranae* in Croatian honeybee colonies. Vet Med 2010; 55: 457-462.
26. Chen YW, Chung WP, Wang CH, et al. *Nosema ceranae* infection intensity highly correlates with temperature. J Invertebr Pathol 2012; 111; 264-267.
27. Invernizzia C, Abuda C, Tomascoa I, et al. Presence of *Nosema ceranae* in honeybees (*Apis mellifera*) in Uruguay. J Invertebr Pathol 2009; 101: 150-153.
28. Chen Y, Evans JD, Smith IB, et al. *Nosema ceranae* is a long-present and wide-spread microsporidean infection of the European honey bee (*Apis mellifera*) in the United States. J Invertebr Pathol 2008; 97: 186-188.
29. Özgör E, Güzerin E, Keskin N. Determination and comparison of *Nosema apis* and *Nosema ceranae* in terms of geographic and climatic factors. Hacettepe J Biol Chem 2015; 43(1): 9-15.
30. de La Rocque S, Rioux JA, Slingenbergh J. Climate change: Effects on animal disease systems and implications for surveillance and control. Rev Sci Tech Off Int Epizoot 2008; 27(2): 339-354.
31. Fenoy S, Rueda C, Higes M, et al. High-level resistance of *Nosema ceranae*, a parasite of the honeybee, to temperature and desiccation. Appl Environ Microbiol 2009; 75: 6886-6889.
32. Stevanovic J, Simeunovic P, Gajic B, et al. Characteristics of *Nosema ceranae* infection in Serbian honey bee colonies. Apidologie 2013; 44: 522-536.
33. Cepero A, Martín-Hernández R, Bartolomé C, et al. Passive laboratory surveillance in Spain: Pathogens as risk factors for honey bee colony collapse. J Apic Res 2015; 54: 525-531.
34. Cornman RS, Chen YP, Schatz MC, et al. Genomic analyses of the microsporidian *Nosema ceranae*, an emergent pathogen of honey bees. PLoS Pathogens 2009; 5(6): 1-14.
35. Cox-Foster DL, Conlan S, Holmes EC, et al. A metagenomic survey of microbes in honey bee colony collapse disorder. Science 2007; 318: 283-287.
36. Higes M, Meana A, Bartolomé C, et al. *Nosema ceranae* (Microsporidia), a controversial 21st century honey bee pathogen. Environ Microbiol Rep 2013; 5: 17-29.