IMPACT OF NATIONAL BEEKEEPING APPROACHES AND STRUCTURES ON *NOSEMA* SPP. PREVALENCE IN HONEYBEE (*APIS MELLIFERA* L.) COLONIES

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(Received 7th Jan 2024; accepted 19th Apr 2024)

Abstract. In order to achieve the objective of this study, two Mediterranean countries on two different sides of the Mediterranean were chosen as examples: Turkey and Algeria. General information on the current structure of beekeeping was obtained from beekeepers in both countries. The results provided general information on beekeeping structure, beekeeping culture, bee management strategies, current knowledge on *Nosema* spp. and control methods. Two diagnostic methods, haemocytometry and PCR analysis, were used to scientifically monitor the social information obtained from the survey results. *Nosema* spp. spores were found in the majority of samples examined by haemocytometric analysis. In the samples from both countries, the PCR results showed the dominance of *N. ceranae*. In addition, this study also showed that the microscopic method should be essential for the diagnosis of nosemosis and that the combination of microscopic and molecular examination gives more reliable information on the prevalence of the disease, especially when some results showed the absence of *Nosema* spp. by PCR but their spores were observed by microscopy and the opposite situation was also recorded. Furthermore, the study showed similarity in both field and laboratory results of the two countries, as there are great similarities between the countries, especially in the structure of beekeeping and in the culture in general. It was therefore concluded that further studies could be conducted in countries with different geographical and cultural backgrounds.
Keywords: *questionnaire, diagnosis of nosemosis, beekeeping management, Turkiye, Algeria*

Introduction

Honeybees play an important ecological role, not only in the production of honeybee products but they are an important link in the chain that maintains the balance of the ecosystem through pollination. If the bees disappear, many plants will not be able to reproduce and will die over time. The absence of bees not only affects plant life, but also threatens the extinction of many animals in the human food chain (FAO, 2018).

Honey bees are under threat from many types of problems such as pesticides, lack of plants, climate change, poor nutrition, supplements, additives (Hristov et al., 2021) and diseases are where all these factors come together. Bacteria, viruses, fungi, and parasites can easily infect bees as these factors directly affect their immune system, physiology, and biology.

Varroa destructor, one of the parasites that feed on bees, is well known to beekeepers. Beekeepers may see *Varroa* bodies on honeybees or symptoms on larvae or adult bees. Another major risk to honey bees is nosemosis (Botías et al., 2013). The causative agent of nosemosis is an intracellular microsporidian called *Nosema* spp. (*Vairimorpha* spp. (Tokarev et al., 2020)). There are two different species of *Nosema*, *N. apis* and *N. ceranae*, responsible for two different forms of the disease affecting *Apis mellifera* L. with varying prevalence (Botías et al., 2013). While nosemosis caused by *N. apis* and *N. ceranae* is specific to honey bees such as *Apis mellifera*, it has been noted in the literature that other species of *Nosema* spp. can infect species other than bees, such as *N. bombycis*, which causes nosemosis infection in silkworms such as *Bombyx mori* (Su et al., 2023). Another microsporidian *Nosema* species is *N. granulosis*, which is one of the *Nosema* species that infect amphipods and is known to infect two host species. *Nosema* is as diverse in aquatic as in terrestrial hosts (Bacela-Spychalska et al., 2023). *N. bombi* is also one of the *Nosema* species that infect a variety of bumblebee species (Yanagisawa et al., 2023). Infection by other *Nosema* spp. is observed in the adult western bean cutworm, as described in the study (Bunn and Miller, 2023). However, the current study focuses only on nosemosis infection of the honey bee *Apis mellifera* L. (caused by *N. apis* and *N. ceranae*).

The various factors influencing the prevalence of *Nosema* spp. disease began years ago and continue to attract the attention of many studies. Nosemosis threatens honey bee colonies as much as varroasis (Salkova and Gurgulova, 2022). However, nosemosis is more difficult for beekeepers to detect than varroasis because *Nosema* spp. spores can only be identified by microscopic analysis and are invisible to the naked eye (Galajda et al., 2021). In addition, *Nosema* spp. spores grow inside the bees (intracellular parasitism). The symptoms of nosemosis are not clear and are mixed with other beekeeping issues, e.g. pesticides, malnutrition, etc. Thus, the level of knowledge about *Nosema* spp. among beekeepers and the approaches of beekeepers become more important every day. Currently, nosemosis can only be diagnosed by microscopic examination or PCR. It is therefore essential that beekeepers are aware of this information and send samples of suspect bees to laboratories. It can be seen that if the beekeeping structure in a country continues in the traditional way, it can affect the whole application, beekeeping methods and also the diagnosis and treatment of the disease.

The aim of this study is to determine if there is a bridge between social and scientific approaches in beekeeping by measuring knowledge of *Nosema* spp. diseases and beekeeping structure based on social approaches and *Nosema* spp. infection levels based on scientific approaches.

Materials and methods

For this study, two Mediterranean countries from two different sides of the Mediterranean were chosen as examples: Turkey and Algeria. Socially, the two countries have similarities and differences in their populations, but what about beekeeping?

In order to understand the beekeeping approach and the social structure of beekeeping, a questionnaire was administered to beekeepers from different provinces of the two countries, the questions of this questionnaire being part of an international working group survey of the COLOSS network ("Prevention of honey bee COLony LOSSes" , network). The survey was distributed during the period April 2020 to April 2021, in an electronic form (Google forms), a paper form or verbally after direct interviews with beekeepers in both countries, with the help of the beekeepers associations of each country and through social media (Facebook).

For the scientific basis, to present the current situation of *Nosema* spp. prevalence, 157 and 48 samples, each containing 30-50 adult honey bees, were collected from 54 and 15 apiaries in Turkey and Algeria, respectively in the period March to October 2021 (location of the different provinces on the map is indicated in *Figure 1* and *Figure 2*).

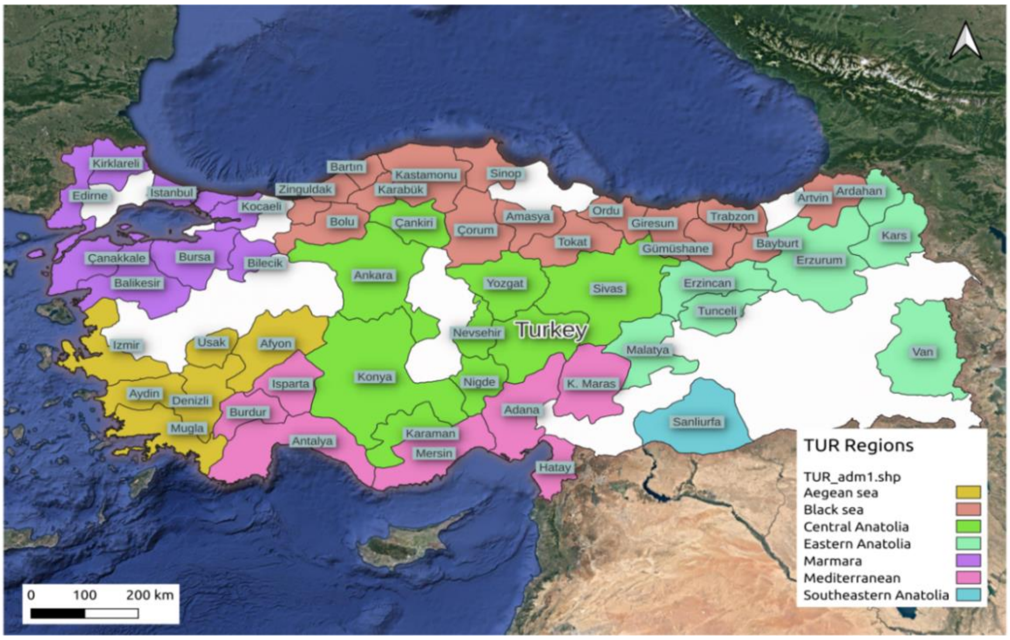

Figure 1. *Provinces from each region of Turkey affected by sampling for Nosema diagnosis*

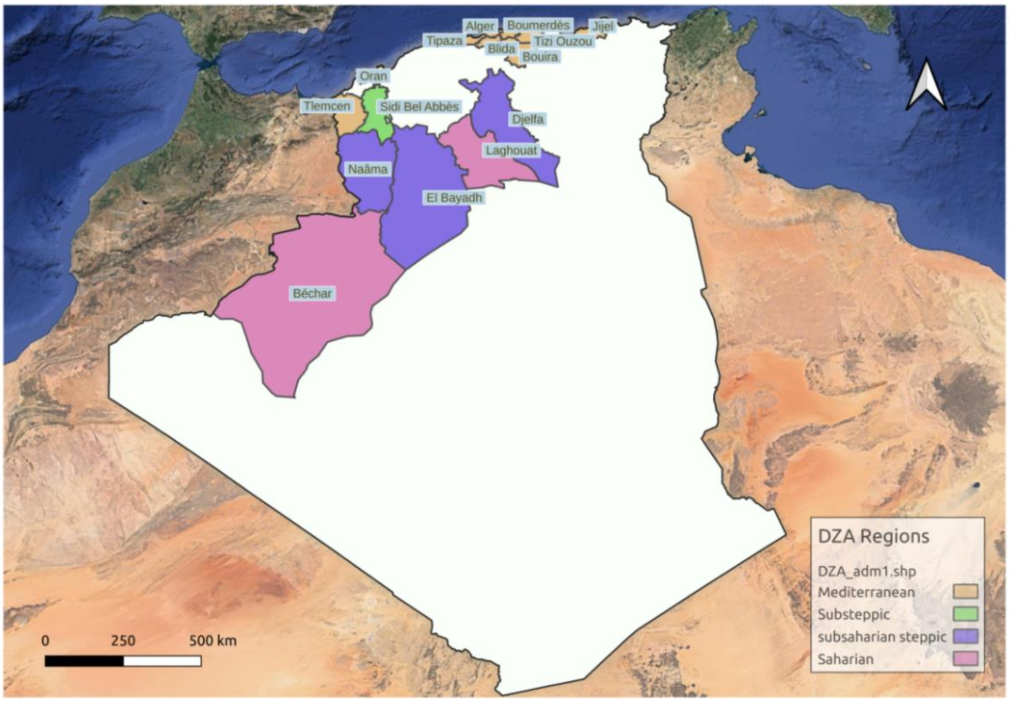

Figure 2. *Provinces from each region of Algeria affected by sampling for Nosema diagnosis*

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DOI: http://dx.doi.org/10.15666/aeer/2203_27852795

The diagnosis of *Nosema* spp. infection was carried out using microscopic (spore counting by haemocytometry method (Human et al., 2013), (Cantwell, 1970), (OIE, 2018)) and molecular (PCR method (Evans et al., 2013)) methods, in Turkiye: at Hacettepe University Microbeeotic Laboratory and Ankara University Insect Morphology and Molecular Systematics Laboratories, and in Algeria: at Algiers Kouba Higher Normal School (ENS-Kouba) Biology and Animal Physiology Laboratory.

For the haemocytometry method used in this study, a Neubauer Improved Slide (*Figure 3*), a type of haemocytometer, was used for spore counting. The abdomen of each of 15 bees is taken and crushed by softening it with distilled water, and after filtering this suspension, 15 ml of distilled water is added. 0,1 ml of the prepared mixture is taken and the number of *Nosema* spp. spores is counted on the haemocytometer slide using a light microscope with a ×400 magnification (objective of 40).

Figure 3. *Neubauer Improved Slide used for the microscopic analysis (Yalçınkaya, 2008)*

For the molecular diagnosis, as described in (Evans et al., 2013), DNA extraction was performed on 30 bee homogenates according to the CTAB protocol (CTAB: cetyltrimethylammonium bromide ionic detergent). For the multiplex polymerase chain reaction (PCR), the appropriate primer combination is used to amplify *N. apis* and *N. ceranae* DNA sequences (see *Table 1*). The PCR products are passed through a 1-2% agarose gel, then stained with ethidium bromide and visualised by photography using a UV transilluminator.

Table 1. *Nosema apis and N. ceranae primers used for the PCR analysis*

Primers	Sequence	Product size (bp)	Source
Nosema ceranae 218MITOC-FOR 218MITOC-REV	5’-CGGCGACGACGATGTGATATGAAAATATTAA-3’ 5’-CCCGGTCATTCTCAAACAAAAAACCG-3’	218-219	(Martin-Hernandez et al., 2007)
Nosema apis 321APIS-FOR 321APIS-REV	5’GGGGCATGTCTTTGACGTACTATGTA-3’ 5’-GGGGGGCGTTTAAAATGTGAAACAACTATG-3’	321	(Martin-Hernandez et al., 2007)

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DOI: http://dx.doi.org/10.15666/aeer/2203_27852795

Statistical analysis

Data analysis in this study was carried out using Microsoft Excel, Google Analytics and QGIS software (Quantum Geographic Information System: software was used to plot the locations of sampling sites on maps of the two countries).

Results

Questionnaire

The number of responses to the questionnaire used in this study is directly proportional to the beekeeping activity in a given province. From the provinces where beekeeping activity is high, we received more responses than from the provinces where beekeeping activity is low.

According to the beekeepers' answers (about 66 answers from 38 different provinces of both Turkey and Algeria) to the conducted questionnaire, the knowledge about *Nosema* spp. disease ranged from zero to sufficient in both Turkey and Algeria, where most of the beekeepers have no or partial knowledge about the disease (*Table 2*).

Table 2. *Knowledge of Nosema and sending of suspect samples to laboratories - information provided by beekeepers in some provinces of Turkey and Algeria*

Turkiye			Algeria		
Province	***Nosema* knowledge**	**Sending samples to laboratory**	**Province**	***Nosema* knowledge**	**Sending samples to laboratory**
Bartın	-	No	Béchar	-	No
Kayseri	±	No	Blida	-	No
Sinop	±	No	Boumerdes	±	No
Malatya	-	No	Naama	-	No
Çanakkale	-	No	Alger	-	No
Uşak	±	Yes	Oran	-	No
Ankara	±	Yes	Tizi Ouzou	-	No

±: partial information; -: lack of the information

Regarding the methods of treatment/control of *Nosema* spp. used by the beekeepers interviewed, it was observed that the greater number of beekeepers in both Turkey and Algeria did not use any direct method against *Nosema* spp. infection because, as mentioned above, the majority of them had no or only partial knowledge about the disease. In general, the use of chemicals (such as fumagilin-B by beekeepers of both countries, Nosembee plus by beekeepers of some provinces in Turkiye) and the extensive use of various aromatic plants were the most commonly used treatment methods by beekeepers (Thyme extracts in both countries, *Artemisia* spp. herb extracts in some provinces in Algeria). Besides, some beekeeping practices that can indirectly protect against *Nosema* spp. infection, such as: vitamin supplements, keeping the hives in sunny positions from autumn to spring, feeding the colonies to enter winter, some natural control methods for *Varroa*, disinfection of equipment, replacement of old brood combs, etc., were used by a significant number of beekeepers from both countries who responded to the questionnaire.

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DOI: http://dx.doi.org/10.15666/aeer/2203_27852795

When beekeepers were asked whether they send samples from colonies they consider suspicious to specialised laboratories, it was found that the majority of them don't prefer to do so (*Table 2*).

Nosema diagnosis

The diagnosis of *Nosema* spp. infection using the haemocytometric method showed the presence of *Nosema* spores in the majority of samples tested (*Figure 4*). Molecular analysis revealed the presence of *N. ceranae* species in the samples examined (*Figure 5*).

The main results of microscopic and PCR analysis of *Nosema* spp. in the samples taken from both countries are shown in *Table 3*.

Figure 4. *Nosema spp. spores detected in a sample from Muğla province of Turkiye*

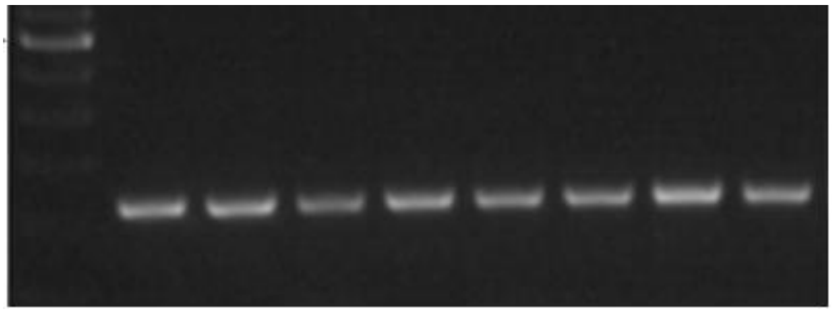

Figure 5. *Agarose gel showing Nosema ceranae PCR products: M: 100 bp. marker, colomn 1-8: samples showing N. ceranae amplification*

An unintended but very interesting result also emerged from this study (*Table 3*). The PCR results for the Algerian samples on the one hand confirmed the results of the microscopic analyses and on the other hand showed the presence of *Nosema* spp. in some negative samples after microscopic analysis and the absence of *Nosema* in some other positive samples also after microscopic analysis.

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Table 3. *Results of both microscopic and PCR analyses for the two countries*

Turkiye				Algeria			
Province concerning by sampling	***Nosema* spp. Spore number ($\times 10^6$)**	**PCR result (*Nosema* spp. Presence/absence)**		**Province concerning by sampling**	***Nosema* spp. Spore number ($\times 10^6$)**	**PCR result (*Nosema* spp. Presence/absence)**	
		N. apis	***N. ceranae***			***N. apis***	***N. ceranae***
Bursa	0,94	-	+	Blida	2,4	-	+
Muğla	6,28	-	+	Tipaza	2,5	-	+
Karabük	0,61	-	+	Boumerdes	2,8	-	+
Bartın	0,39	-	+	Tizi Ouzou	0	-	+
Erzincan	2	-	+	Alger	0	-	+
Tunceli	0,94	-	+	Bouira	1,1	-	-
Ankara	5,33	-	+	Sidi Belabbes	0,2	-	-
Antalya	3,56	-	+	Bechar	0	-	-

Discussion

The results obtained in this study helped us, on the one hand, to approach a part of the beekeeping structure that concerns more knowledge about *Nosema* spp. between the beekeepers of each of Turkey and Algeria (questionnaire results). On the other hand, the use of scientific methods for the diagnosis of *Nosema* spp. disease revealed important information about the existing *Nosema* spp. prevalence.

Despite the serious damage it can cause to honeybee colonies, it was found that nosemosis is less considered by beekeepers than varroasis. This may be justified by the fact that the symptoms of nosemosis are not all known to beekeepers, although some symptoms of this disease may be confused with other bee diseases (Galajda et al., 2021). In its acute form, nosemosis is characterised by trembling worker bees or dead bees around the hive. It causes diarrhea and feces can be found on the comb and front of the hive. The posterior part of the bee abdomen becomes dilated, whitish and does not show the usual constriction. Heavily infected colonies show a decrease in brood production and an increase in foraging, resulting in the death of the colony with large supplies of honey and pollen. It has been described that *Nosema ceranae* infection differs from that caused by *N. apis*. *N. ceranae* causes gradual depopulation of adult bees, higher mortality and disruption of foraging behavior (Adjlane, 2012; Fries et al., 2015).

It was observed that beekeepers in both countries do their best to solve their problems without going to any bee research institution or laboratory. This can be explained by the lack of an advisory service, as is the case in some industrialised countries, as well as the lack of trust in science on the part of beekeepers and the lack of effort on the part of bee researchers and scientists specializing in this field to strengthen their relationship with beekeepers. It should be mentioned that in recent years scientists from Turkey and Algeria are doing their best to solve this problem of scientist-beekeeper relationship by being integrated in international projects such as B-Rap (Bridging bee Research And beekeeping Practice) project which is being carried out by an important international network of scientists (a network that includes an important number of countries from all over the world including Turkey and Algeria [COLOSS B-RAP CP workshop, www.coloss.org]). This is even less the case in Algeria than in Turkey, and it is thought that this may be due to a general problem in Algeria, namely the lack of a coherent

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relationship between the different sectors of the country (Regreg et al., 2020). Secondly, the beekeeping sector is an important economic income for the country of Turkey, compared to the importance given to it in Algeria. In another viewpoint, the abundance of beekeepers in Turkey, which prompted the Turkish government to give more interest to this sector, compared to the number of beekeepers in Algeria, since apiculture in Turkey is experienced in almost all regions of the country, while in Algeria is done more in the northern and central regions of the country because of the harsh climatic conditions in the Algerian southern regions.

The results of *Nosema* spp. diagnosis (*Table 3*) showed that the highest level of infection was recorded mainly in the coastal and plateau areas of both countries. This may be explained by the relationship between the climatic conditions of a given geographical region and the prevalence of *Nosema* spp. infection. This relationship has been reported in several research studies, such as Özgör et al. (2015), Mohammadian et al. (2018) and Gisder et al. (2010). This increase in infection levels in these regions may also be due to the degree of disease susceptibility of the bee race in these regions. This latter factor can also be used to justify the increase in infection in Turkey compared to Algeria. In Algeria, the fact of having two races of the European honey bee *Apis mellifera* (*A. m. intermissa* and *A. m. sahariensis*) represents an advantage compared to the different races and ecotypes of honey bees found in Turkey, in addition to the effect of migratory beekeeping on the immunity and resistance of honey bees to diseases (Simone-Finstrom et al., 2016). In addition, the results of this study showed that the Saharan breed of honeybee found in Algeria appears to be more resistant to *Nosema* infection than the other breed found in the country or those from Turkey. Algeria is a vast country with many geographical gaps, especially between the north and the south. In addition, the southern regions of Algeria have a kind of harsh and specific geographical and climatic conditions. This leads to the growth of specific fauna and flora that can adapt to these critical conditions. Consequently, this may be the reason for the resistance of the Saharan honeybee race to nosemosis (CETAM 2012).

The variation in climatic conditions, especially temperature, is certainly not the only explanation for the differences in the peaks of *Nosema* spp. (Chen et al., 2012). Physiological and behavioural changes during the seasons, especially before winter (Winston, 1991), as well as the population dynamics of adult bees and brood, are likely to influence the development of the infection and the ability of the colony to counteract infection (Khoury et al., 2018).

After microscopic examination, the PCR result showed that the presence of *Nosema ceranae* as the only *Nosema* spp. species identified in the samples analysed from both countries seems to be similar to the results of many other studies. They suggested the replacement of *N. apis* by *N. ceranae* (Hauzat et al., 2007; van Engelsdorp et al., 2009; Traver and Fell, 2011). However, there are studies that don't support this idea of replacement between *Nosema* species, since *N. ceranae* occurs in all seasons, but its frequency is always higher than that of *N. apis*. The epidemiology of this species is independent of climatic conditions and geographical regions (Gisder et al., 2010; Özkırım et al., 2019).

The complementary value of the use of both methods in the diagnosis of *Nosema* spp. infection was an additional result of this study. The results of the molecular analysis showing the presence of *Nosema* spp. while the microscopic analysis showed their absence may be explained by the sensitivity of the PCR method (Fries et al., 2013). Nevertheless, the results of the PCR test, which showed the absence of *Nosema* spp. while

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2203_27852795

the latter's spores were detected by the mean of the microscopic analysis, were similarly observed in different previous studies, such as Webster et al. (2004). This study investigated the ability of late autumn worker bees to transfer parasite spores to queen bees. Although *Nosema apis* spores were observed under the light microscope in nosemosis-infected specimens and in the guts of workers and queens, they were not found in DNA analyses. The cited authors suggested that this was due to the presence of ungerminated spores and their deposition in the gut for long periods of time. Undigested spores have flexible walls and are resistant to DNA extraction.

Conclusion

With regard to the influence of the structure and practices of beekeeping on the prevalence of *Nosema* spp., the results of the questionnaire carried out in this study, indicators such as the level of knowledge of beekeepers about nosemosis, the abundant use of chemicals and even the unconscious use of biological products, which are suspected to be responsible for the decrease in the resistance of honeybees to diseases, etc., may justify the prevalence registered after the diagnosis of *Nosema* spp.

The present study showed somewhat similar results between the two countries, Algeria, and Turkey, which is not surprising since the beekeeping structures of these two countries were close to each other. Approaches and answers to questions; the rate of application to science, the effort to find self-solutions such as the use of herbal treatments against *Nosema* spp., the large gap between researchers and beekeepers and the absence of an advisory service have shown that the prevalence is not controlled and there is no strategy for the management of *Nosema* spp.

This study can be carried out in further studies in countries that are completely different in terms of beekeeping structure. Our preliminary results showed that our hypothesis was correct. It has been shown that beekeeping culture, approaches and structure on a social basis can directly influence the prevalence of diseases. If this work were to be carried out in 2-3 other countries, where the level of differentiation is higher, it might be realised that the impact factor is clearer.

Acknowledgements. Authors would like to express their deep gratitude to Professor İrfan KANDEMİR and his team for his help in PCR analysis at Ankara University Insect Morphology and Molecular Systematics Laboratories (Turkey), and for his patient guidance and constructive criticism of this study effort. They are also immensely grateful to the technicians at Algiers Kouba Higher Normal School (ENS-Kouba) Biology and Animal Physiology Laboratory (Algeria) and my supervisor Associate Professor Aslı ÖZKIRIM's assistant at Hacettepe University Bee Health Laboratory (Turkey) for their valuable help in maintaining the good pursuit of the different stages of this work. Finally, they would like to thank all the beekeepers from Turkey and Algeria who participated in answering the questionnaire conducted in this study for their time and contribution in sharing their valuable answers and their beekeeping practices they use. For funding, no funds, grants, or other support were received during the preparation of this manuscript.

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http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2203_27852795