FIRST DETECTION OF *NOSEMA CERANAE* IN JORDAN

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Abstract

Nosema diseases is known to have two infecting *Apis mellifera*, *Nosema apis* and *Nosema ceranae*. Historically *N. apis was* known to be well established pathogen of *A. mellifera. Whoever N. ceranae* was frequently reported to infect the honeybees during the last decade. Both *N. apis* and *N. ceranae* are now thought to be widespread across the world. Honey bees are also found to be co-infected with both species. This is the first report of *N. ceranea*,in Jordan with a low infestation level (23.9%) of the inspected apiaries comparing with most references, while no *N. apis* was found infestation was found.

Keywords: Nosema apis, Nosema ceranae, Apis mellifera, honeybees Jordan

Introduction

Honey bees are social insects that are playing a vital role in the sustainability of the ecosystems and biodiversity, it was estimated that one-third of the human diet depends directly or indirectly on the role of pollinators, of which honey bees represent a large proportion (Delaplane and Mayer, 2000; Haddad N.J., 2007; Shammout A. et.al. 2014). Worldwide high mortality of honey bee colonies (*Apis mellifera*) is therefore a worrying problem for beekeeping and beyond. Although several factors may play a role in these declines (vanEngelsdorp et al., 2010, 2013; Cornman et al., 2012), most mortalities have been attributed to high loads of parasites and pathogens, such as high infestations by the Ectoparasitic mite *Varroa destructor,* together with associated viruses (Cox-Foster et al., 2007; Berthoud et al., 2010; Dainat et al. 2012).

The microsporidian *Nosema ceranae* has also been incriminated in colony mortality in southern Europe (Higes et al., 2013). The presence of various parasites and pathogens and their relationships with mortality of bees is a cause of concern that is being studied all over the world (Tentcheva et al.

2004, Chen et al. 2008; Dainat et al. 2012), but the information about *Nosema ceranae* in the most of the Middle East countries is very limited.

Materials and methods

Samples: Honeybee samples were collected from 46 apiaries, from the northern and the central parts of the country where most of the beekeeping sector is located, in each location the country, one pool sample per apiary of approximately 100 worker bees and preservative in buffer (RNA Later®) during April 2014, preservative buffer (RNA Later®) and stored in our laboratory at -80°C.

Nosema species identification

DNA extraction: NucleoSpin® DNA extraction kit (MACHEREY-NAGEL) was used to obtain DNA from a homogenate of 50 honeybees sample, representative of each apiary. The DNA was suspended in 100 µl of 5 mM Tris (pH 8.0), and stored at –20 ◦C until PCR analysis.

PCR amplification: Polymerase chain reaction specific of the rRNA genes of *N. ceranae* and *N. apis* was conducted using specific primers (see Table 1)

Table 1: Sequences of the primers used the PCR.

Nosema	Primer	Volume (bp)
N. apis	Forward:5'GGGGGCATGTCTTTGACGTACTATGTA-3' Reverse:5'GGGGGGCGTTTAAAATGTGAAACAACTATG-3'	321
N. ceranae	Forward: 5'-CGGCGACGATGTGATATGAAAATATTAA-3 Reverse : 5'-CCCGGTCATTCTCAAACAAAAAACCG-3'	218

The thermocycler profile consisted of an initial denaturation at 95 °C for 2 min, followed by 35 cycles of 20 s at 95 °C, 20 s at 56 °C and 30 s at 72 °C, and a final extension step at 72 °C for 5 min. PCR products were analyzed on 1% (w/v) agarose gels, and products were visualized under UV light.

Results and Discussions

In 46 inspected samples of *Apis mellifera* apiaries spread in the northern and the central regions of Jordan, only 11 samples were found to be infected with *N. ceranae* positive samples were sequenced (see table 2).

Table 2: sequence of *N. ceranae* detected in Jordan

Direction	Sequencing results
Forward	TNNNNTAATATAGAATTTGAGTTTTTTGGCTCTGGGGATAGTATGATC GCAAGATTGAAAATTAAAGAAATTGACGGAAGAATACCACAAGGAG TGGATTGTGCGGCTTAATTTGACTCAACGCGAGGTAACTTACCAATAT TTTATTATTTTGAGAGAACGGTTTTTTGTTTGGAATGACCGGGAGACT TTCACTCAACCTCAGCACCCCCTATGCCACCCCTCTATGACTGCTGAC CGGTGATACCACTGCCTGGCCCCAAAGCCCTCCAGTGACTTTCTCCGT GCA
Reverse	GNGTCCNNCAANATAAAATATTGGTAAGTTACCTCGCGTTGAGTCAA ATTAAGCCGCACAATCCACTCCTTGTGGTATTCTTCCGTCAATTTCTTT AATTTTCAATCTTGCGATCATACTATCCCCAGAGCCAAAAAACTCAAA TTTCTATTATGTAATACAAATTAATATTTTCATATCACATCGTCGCCA AGGAAGACGCATGTTAACACACTGCAGACTACATCCGCTGCCGCCAC CAGCCCCCGGGCAGCCACCTGCTCTATGCCAAGATGATCCAGAAGCT ACCGAAAA

None of the samples was infected with *N. apis, this level of infestation can be* considered as a very low level in comparison with most known levels infestation in other parts of the world, for example Nosema has been found at high prevalence at colony and apiary level reaching 95-100% (Bollan et al., 2013; Higes et al., 2010; Hong et al. 2011; Medici et al. 2012; Nabian et al., 2011). *N. ceranae* was found at higher prevalence than *N. apis* in the majority of samples (e.g. Strauss et al., 2013; Gisder *et al*., 2010; Higes et al., 2010; Medici et al., 2012; Nabian et al., 2011; Whitaker et al., 2011; Martin Hernandez, 2007; Stevanovic, 2012). Our data correspond to the global trend of *N. ceranae* dominance (Fries, 2010).

But due to the absence of any historical data from Jordan and most of the region, we cannot confirm the hypothesis of the replacement of *N. apis* by *N.* ceranae (Klee et al., 2007; Paxton et al., 2007; Higes et al., 2006, 2008, 2013; Martín-Hernández et al., 2012, Teixeira et al. 2013). However the total absence of *N. apis* might be due to the better adaptation of *N. ceranae* to warm climates (Fries and Forsgren, 2009; Martín-Hernández *et al*., 2012), or due to the a direct impact of honeybee queens and packaged bees importation, as far as *N. ceranae* is widely spread in the countries of origin of the packaged bees and queens. No significant difference in the geographic distribution was found.

Conclusion

Our results lightshade on the status of Nosema disease in Jordan, it increase the responsibility of scientists in the region to develop cross boarder research projects to understand the status of Nosema disease in the Middle Eastern region. Further studies need to be conducted on the effect of importation of packaged bees and queens on the spreading of this disease.

Studies to explore the reason behind the comparatively low infestation with Nosema disease is needed, The relationship between the season, climatic conditions and possible resistance of local honeybee breeds to this disease needs to be investigated.

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