

Short Communication

Retrospective study of the *Nosema ceranae* infection of honey bee colonies in Iran (2004-2013)

Modirrousta*¹, H., Moharrami¹, M., Mansouri², M.A.

1. Department of Honey Bee, Silk Worm and Wildlife Research Diseases, Razi Vaccine and Serum Research Institute, Karaj, Iran

2. Department of Research, Production & Breeding of Laboratory Animals, Razi Vaccine and Serum Research Institute, Karaj, Iran

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ABSTRACT

Nosemosis is the most common disease in adult bees. *Nosema apis* and *Nosema ceranae* species are agents of important economic losses to beekeepers around the world. The severity of disease at various area is different. Previously, *N. apis* was observed in areas with a long winter, especially in late winter and early spring. But in recent years, disease has been reported in the warm seasons. The studies indicated that a new species as *N. ceranae* is involvement in loss and mortality in adult bees. Therefore, diagnosis and differentiation of *Nosema* species is importance at colony collapse disorders (CCD). The aim of this Research was a retrospective study on *Nosema* samples isolated from apiaries. Forty- one *Nosema* Sp. Positive samples were collected from five provinces during 2004 to 2013. The samples were tested by multiplex PCR method using both primers of *N. ceranea* and *N. apis* were simultaneously. All of samples were positive for *N. ceranea*. The products were sent for sequencing. The results show that *N. ceranea* has spread in Iran, from previous years almost simultaneously with other parts of the world.

Keywords: *Nosema*, *Apis*, *Ceranae*, Honeybee, Iran

INTRODUCTION

To date, two microsporidian parasites have been described from honey bees: *N. apis* (Zander) and *N. ceranae*. *N. apis* is a parasite of the European honey bee (*Apis mellifera*), and *N. ceranae* of the Asian (*Apis cerana*) and the European honey bees (OIE Terrestrial Manual 2013). *N. apis* and *N. ceranae* are parasites exclusive of the epithelial cells of the ventriculus of adult bees and both parasites occur throughout the world (Klee *et al* 2007). Based on molecular evidence,

microsporidia are now included in the cluster Fungi (Adl *et al* 2005). Infection occurs by the ingestion of spores in the feed (Bailey 1981, Webster 1993). In acute forms of infection, especially in early spring, brown faecal marks may be noted on the comb and the front of the hive (Bailey 1967). Lack of seasonal prevalence and symptoms such as faecal deposits have been reported for *N. ceranae* (Higes *et al* 2008). Both parasites have a temperature-dependent multiplication rate. *N. ceranae* may grow better at slightly higher temperatures compared to *N. apis* (Fenoy *et al* 2009). The spores of the two *Nosema* species are very similar and microscopic examination could not differentiate

* Author for correspondence. Email: h.modirrousta@rvsri.ir

between the *N. apis* and *N. ceranae*. Thus molecular techniques can greatly assist in the diagnosis and identification of honey bee microsporidians. Multiplex PCR provides a significant improvement to the conventional technique by incorporating multiple primers that amplify regions of DNA from two honeybee microsporidians simultaneously in a single reaction (Martin-Hernandez *et al* 2007). The aim of this research was a retrospective study on *Nosema* samples isolated from apiaries.

MATERIALS AND METHODS

Sample collection. In this study forty one *Nosema* Sp. samples (previously diagnosed as positive by light microscopy) were used. These samples were collected from 5 Iranian provinces (Alborz - East Azarbaijan - Gazvin - Gilan - Tehran) in 2004-2013.

Preparation of samples. The abdomens of 10–20 adult dead honey bees from each sample were macerated in 10 ml distilled water (PCR grade) and the suspension was then filtered and centrifuged at 800 g for 6 minutes. Spore germination was induced with 200 µl freshly prepared germination buffer (0.5 M sodium chloride, 0.5 M sodium hydrogen carbonate, pH to 6.0 with orthophosphoric acid), and the mixture was incubated at 37°C for 15 minutes. (OIE Terrestrial Manual 2008 & Martin-Hernandez *et al* 2007).

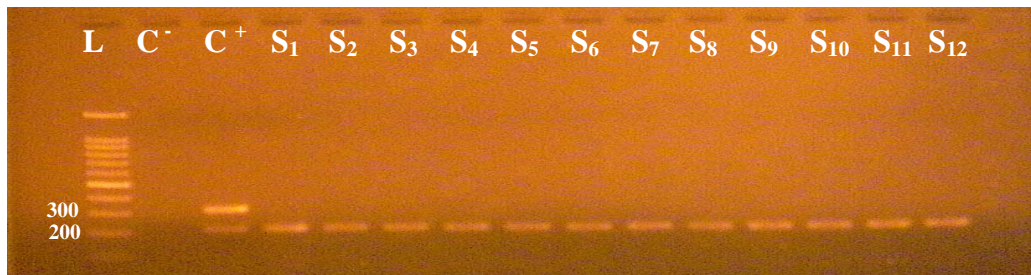
DNA extraction. DNA was extracted from samples of *Nosema* spore suspensions using the High Pure PCR Template Preparation Kit (Qiagen) according to the manufacturer's instructions.

Multiplex PCR. Specific primers for both *N. apis* and *N. ceranae* were designed by Martin-Hernandez *et al*. 2007. Multiplex PCR were simultaneously performed using the High Fidelity PCR Master Kit (Qiagen). Amplification is carried out in a eppendorf mastercycler gradient thermocycler under the following conditions: Initial denaturation step of 94 °C for 2 minutes, 10 cycles of 94 °C for 15 seconds, 61.8 °C for 30 seconds and 72 °C for 45 seconds, and 20 cycles of 94 °C for 15 seconds, 61.8 °C for 30 seconds and 72 °C for 50 seconds with a final extension step of 72 °C for 7

minutes (OIE Terrestrial Manual 2008 & Martin-Hernandez *et al* 2007). Amplicons were run on a 2% agarose gel electrophoresis, and products were visualized under UV. Positive controls consisted of *N. ceranae* from honey bees collected from Iranian apiaries, and *N. apis*, target gene synthesized, cloned into plasmid, then transformed into *E. coli*. and used for positive control (Unpublished). (Size of the *N. ceranae* amplicon was 218-219 bp and for *N. apis* was 321 bp). PCR products were sent for forward and reverse sequencing using senger method (Bioneer Company) and revealed sequences were verified by bioedit software (Ver 5.2).

RESULTS AND DISCUSSION

Nosemosis is the most common disease in adult bees. *Nosema apis* and *Nosema ceranae* species are agents of important economic losses to beekeepers around the world. In the absence of molecular diagnosis, most studies performed in the past decades considered *N. apis* the etiological agent of nosemosis. Recent reports (Faucon 2005) indicate that the clinical and epidemiological pattern of nosemosis is changing. The aim of this study was a retrospective study on *Nosema* samples isolated from apiaries. The results showed that all collected samples (41) since 2004 originated from five provinces were positive for *N. ceranae* by multiplex PCR (12 out of 41 samples were showed). The sequencing and analysis of PCR products reconfirmed *nosema ceranae* too. Our results demonstrated that co-infection of *N. ceranae* and *N. apis* is not present in our samples and that *N. ceranae* prevails over *N. apis*. It is now clear that *N. ceranae* has successfully invaded and become established in honey bee colonies from different provinces of Iran. The results show that *N. ceranae* has spread in Iran from previous years almost simultaneously with other parts of the world. But, due to impossibility of differential diagnosis with *N. apis*, it was not very well known. Findings from this survey correspond with studies by other researchers. Nabian *et al*. 2011 and Razmarai *et al*. 2013 reported *N. ceranae* in



L: Ladder 100 bp , C⁻: Negative control , C⁺: Positive control (*Nosema apis* & *Nosema ceranae*) , S₁- S₁₂(*Nosema ceranae*)

Mazandaran and East Azarbaijan apiaries (Cox-Foster *et al* 2007), detected *N. ceranae* in 30 (100%) of samples from colonies with CCD and 17 (80.9%) of non-CCD samples, suggesting a potential role for *N. ceranae* in CCD. *N. ceranae* was detected in bee samples collected in the USA in 1996 and France in 2002 (Chen *et al* 2008, Chauzat *et al* 2007). Of bees (N = 18) collected in China, 28% were positive for *N. apis* and 61% were positive for *N. ceranae*. Of bees (N = 48) collected in Taiwan, 33% were positive for *N. apis* and 73% were positive for *N. ceranae*. Of bees (N = 12) collected in Japan, 25% were positive for *N. apis* and 75% were positive for *N. ceranae* (Chen *et al* 2009). The results confirm the colonization of *N. ceranae* in Iran and demonstrates the need for additional molecular research at a more extensive monitoring level in order to elucidate possible links between of the infection by *N. ceranae* with honey bee colony losses.

Ethics

Hereby, I declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors have no conflict of interest.

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