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ORIGINAL ARTICLE

First detection of *Nosema* sp., microsporidian parasites of honeybees (*Apis mellifera*) in Riyadh city, Saudi Arabia

Abdel-Azeem S. Abdel-Baki [a,b,*], Mohammed M. Mares [a], Mohamed A. Dkhil [a,c], Saleh Al-Quraishy [a]

[a] *Zoology Department, College of Science, King Saud University, P.O. Box: 2455, Riyadh 11451, Saudi Arabia*
[b] *Zoology Department, Faculty of Science, Beni-Suef University, Egypt*
[c] *Department of Zoology and Entomology, Faculty of Science, Helwan University, Egypt*

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Abstract *Nosema* sp. is recorded in Saudi Arabia for the first time, in adult *Apis mellifera* collected from apiaries in Riyadh city. Samples of 100 workers were collected and examined for the infection with *Nosema* sp. 5% of the bees were found positively infected with *Nosema* sp. Spores were oval to elliptical shaped and measuring 6.4 (5.0–7.0) μm in length, 3.4 (3.0–4.5) μm in width. The conclusive identification of the present *Nosema* species will preclude until further ultrastructure and molecular studies. The present study concluded that intensive surveys are prerequisite to identify the species of *Nosema* and to estimate their distribution and prevalence in different regions of Saudi Arabia.

1. Introduction

Honey bees play a substantial role in the environment by pollinating wild flowers and agricultural crops as they forage for nectar and pollen, as well as manufacturing honey and beeswax. Beekeeping in Saudi Arabia is a growing business; indeed, Saudi Arabia is the third biggest producer of honey in the Middle East with an estimated 4000 beekeepers and 700,000 bee hives, providing a total of about 3500 tons of honey per year (Alqarni et al., 2011; Alattal et al., 2014). The fundamental and beneficial activities of bees depend on beekeepers safeguarding a healthy population of honey bees, because, like other insects and livestock, honey bees are subject to a range of diseases and pests. Nosemosis is one of the most widespread diseases affecting honey bees worldwide and it is caused by two distinct species of unicellular microsporidian parasites, *Nosema apis* and *Nosema ceranae* (e.g., Farrar (1947), Weiser (1961), Moeller (1978), Liu (1984), Fries (1988), Charbonneau et al. (2016)). *N. apis* was isolated in the European honey bee (*Apis mellifera*) (Zander, 1909) and *N. ceranae*

* Corresponding author at: Zoology Department, College of Science, King Saud University, 11451 Riyadh, Saudi Arabia. Tel.: +966 14675754; fax: +966 14678514.
E-mail address: azema1@yahoo.com (A.-A.S. Abdel-Baki).
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was isolated from the Asian honey bee (*Apis cerana*) in China (Fries et al., 1996). In essence, however, both species have an essentially global distribution and the two species can co-infect honey bees (Chen et al., 2009; Burgher-MacLellan et al., 2010; Charbonneau et al., 2016). It has been proven, however, that the epidemiological pattern and pathology of *N. ceranae* and *N. apis* are different and therefore, now, the disease caused by *N. ceranae* is named nosemosis type C while that caused by *N. apis* is known as "nosemosis type A" (COLOSS workshop, 2009).

The symptoms of infection by *N. apis* are easy to recognize; there are large numbers of dead bees within the colony and diarrhea stains at entrances of hive indicating gastrointestinal disorders (Bourgeois et al., 2010; Araneda et al., 2015). By contrast, the symptoms of nosemosis caused by *N. ceranae* are less apparent; the growth of colonies grow is weak producing significant reductions in colony size and it is possible to detect the disease throughout the entire year (Bourgeois et al., 2010; Higes et al., 2010; Araneda et al., 2015). The prevalence of nosemosis disease has been proven to vary among regions and years (Mulholland et al., 2012). Although, *N. apis* has a world-wide distribution it is not considered an important problem in tropical and sub-tropical regions (Wilson and Nunamaker, 1983). However, in temperate regions *N. apis* infections typically peak in the spring, decrease during the summer and then increase again in the fall before declining during the early winter months (Higes et al., 2010). On the other hand, *N. ceranae* show less seasonality and can be detected in all four seasons (Higes et al., 2010).

Symptoms of nosemosis have been reported before among honey bees in Saudi Arabia (e.g. Al Ghamdi (1990), Alattal and Al Ghamdi (2015)), but the presence of *Nosema* spores themselves has not yet been described.

In the present brief study we report for the first time the presence of *Nosema* spores in honey bees in Saudi Arabia.

2. Materials and methods

One hundred honey bee workers (*A. mellifera*) were collected from five apiaries in Riyadh city (twenty from each apiary) and examined one by one for the presence of any microsporidian spores following the method adapted by Bollan et al. (2013). Briefly, the abdomen of honeybees was isolated from their bodies, squashed, homogenized employing a mortar and pestle and resuspended in distilled water (1 ml water/bee). Then, a few drops of the suspension were placed on the slides and examined under a microscope at a magnification of 400×, to detect any *Nosema* spore. Photographic documentation and spore measurements were performed using an Olympus BX51 microscope equipped with an Olympus DP71 camera (Olympus, Japan). Measurements are presented in micrometers and data are expressed as the mean followed by the range in parentheses.

3. Results

Of 100 honey bees workers (*A. mellifera*) examined for the infection with *Nosema* spp., five were found infected. The five infected workers are from two different apiaries (3 from one and 2 from the other). Light microscopic examination of the midgut content and fecal matter revealed the presence of huge numbers of microsporidian spores. Spores were oval to elliptical shaped and varied in size, measuring 6.4 (5.0–7.0) μm in length and 3.4 (3.0–4.5) μm in width ($N = 50$) (Fig. 1).

4. Discussion

Nosemosis is a bee disease caused by spore-forming parasites of the genus *Nosema*, which attack the epithelial lining of the middle intestine of the worker bees, queens and drones (Botías et al., 2012; Bollan et al., 2013). For a long time, the only known causative agent of Nosemosis in honeybees (*A. mellifera*) was the unicellular microsporidium *N. apis* (Nabian et al., 2011). Later, Higes et al. (2006) reported a new microsporidium, *N. ceranae*, as the main motive agent of nosemosis in Spain. Shortly after, the presence of *N. ceranae* was confirmed in Europe, America, and Asia (Chen et al., 2008; Chen and Huang, 2010; Nabian et al., 2011). Recent prevalence studies indicate that infections with *N. apis* and

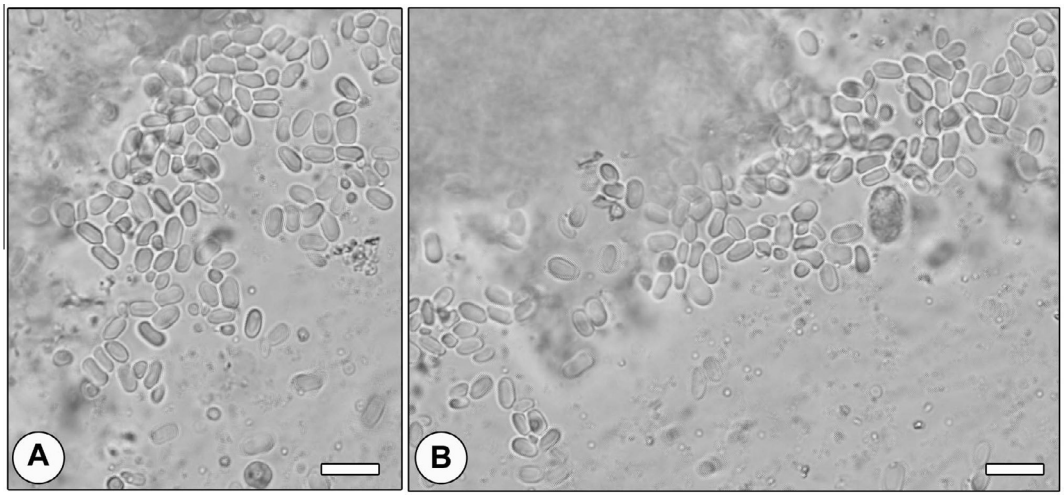

Figure 1 Fresh spores of *Nosema* sp. infected the gut of bee (*Apis mellifera*) collected from different apiaries in Riyadh city, Saudi Arabia. Scale-bar = 10 μm.

N. ceranae can co-occur and be present as mixed infections in both Europe and North America (Copley et al., 2012; Milbrath et al., 2015). Recently, Eiri et al. (2015) proved that *N. ceranae* can infect honey bee larvae and decrease subsequent adult longevity. It has been postulated that *N. ceranae* is more predominant in warmer countries compared to *N. apis* (Nabian et al., 2011; Haddad, 2014; Van der Zee et al., 2014). It seems that *N. ceranae* is better acclimatized to complete its endogenous cycle with a higher biotic index at different temperatures reflecting greater incidence of the disease in warmer regions and the epidemiological differences between both *Nosema* species in field conditions and at the colony level (Martín-Hernández et al., 2007, 2009).

Commonly, spore measurements of *N. apis* are larger than those of *N. ceranae* (Chen and Huang, 2010). In the present study the average spore size of the identified *Nosema* sp. was 6.4 × 3.4 μm, which is very close to the average spore size of *N. apis*, reported by Fries et al. (1996) as 6.0 × 3.0 μm, but quite distinct from the average *N. ceranae* spore size of 4.4 × 2.2 μm (Chen et al., 2009). At the ultrastructural level, on the other hand, the number of polar filament coils inside *N. ceranae* spores was 18–21 compared to more than 30 coils inside *N. apis* (Fries, 1989; Chen et al., 2009). This reiterates that it is only possible to arrive at robust differential diagnosis and classification of *N. apis* and of *N. ceranae* by combining morphological and molecular data (Bollan et al., 2013). The conclusive identification of the species of the present *Nosema* therefore awaits further ultrastructural and molecular studies. Accordingly, the present parasite will be allocated simply as *Nosema* sp.

Although some passing references in the literature can be found to Nosemosis in Saudi Arabia (e.g. Al Ghamdi (1990), Alattal and Al Ghamdi (2015)), this report describes systematically for the first time the spores of *Nosema* sp. in colonies of *A. mellifera* in Saudi Arabia. There is now a clear need for intensive surveys to identify the species of *Nosema*, and to determine their distribution and prevalence in different regions of Saudi Arabia.

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