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ORIGINAL ARTICLE

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



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KEYWORDS

Parasite; Microsporidia; Nosemosis; Bee 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.

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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 bees-

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wax. 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*

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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 coinfect 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 $400\times$, 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) μ m in length and 3.4 (3.0–4.5) μ m 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

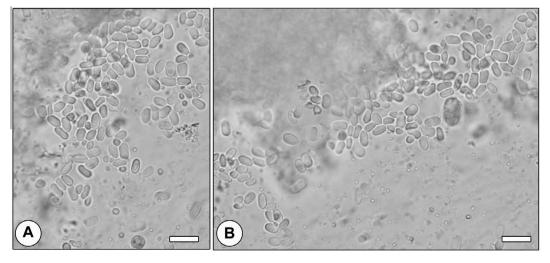


Figure 1 Fresh spores of *Nosema* sp. infected the gut of bee (*Apis mellifera*) collected from different apiaries in Riyadh city, Saudi Arabia. Scale-bar = 10 µm.

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