Saudi Journal of Biological Sciences xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Geographical distribution and molecular detection of *Nosema ceranae* from indigenous honey bees of Saudi Arabia

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ARTICLE INFO

Article history:
Received 12 January 2017
Revised 23 January 2017
Accepted 23 January 2017
Available online xxxx

Keywords:
Nosema ceranae
Prevalence
Molecular detection
Honeybee: Saudi Arabia

ABSTRACT

The aim of the study was to detect the infection level of honey bees with *Nosema apis* and/or *Nosema ceranae* using microscopic and molecular analysis from indigenous honeybee race of eight Saudi Arabian geographical regions. A detailed survey was conducted and fifty apiaries were chosen at random from these locations. Infection level was determined both by microscope and Multiplex-PCR and data were analyzed using bioinformatics tools and phylogenetic analysis. Result showed that *N. ceranae* was the only species infecting indigenous honeybee colonies in Saudi Arabia. As determined by microscope, *Nosema* spores were found to be in 20.59% of total samples colonies, while 58% of the samples evaluated by PCR were found to be positive for *N. ceranae*, with the highest prevalence in Al-Bahah, a tropical wet and dry climatic region, whereas low prevalence was found in the regions with hot arid climate. Honeybees from all eight locations surveyed were positive for *N. ceranae*. This is the first report about the *N. ceranae* detection, contamination level and distribution pattern in Saudi Arabia.

1. Introduction

Western honey bees (*Apis mellifera*) are a highly valued resource worldwide and are of great relevance for humans and the entire ecosystem, not only as a honey and wax producer but also as a pollinator of agricultural and horticultural crops and wild flora (vanEngelsdorp and Meixner, 2010). The total annual global economic worth of pollination amounts to 212 billion USD, representing 9.5% of the value of the global agricultural production (Gallai et al., 2009). However, very unfortunately, honeybee is facing enormous threat worldwide (USA, Europe, Middle East) (Crailsheim et al., 2009; vanEngelsdorp et al., 2009, 2010; Haddad et al., 2009; Soroker et al., 2009) including Saudi Arabia (Alattal and AlGhamdi, 2015).

Beekeeping is one of the long-standing practices in rural Saudi Arabia and is one of the most important economic activities for the communities (Al-Ghamdi and Nuru, 2013). Approximately 5000 beekeepers maintain more than one million honeybee colonies and produce approximately 9000 metric tons of honey annually (Al-Ghamdi, 2007). *Apis mellifera jemenitica* Ruttner (= yemenitica auctorum: vide Engel, 1999), the smallest race of *A. mellifera*, is the only race of *A. mellifera* naturally found in the country and has been used in apiculture at least 2000 BC. Traditional beekeeping is mostly practiced using this race, because it is well adapted to the semi-arid to semi-desert conditions of Saudi Arabia (Alqarni et al., 2011; Al-Ghamdi and Nuru, 2013). Also, honey produced by this native bee (*A. m. jemenitica*) is sold at 10–20 times high rates than imported honeys (Al-Ghamdi personal Comm.)

Despite the great potential and multiple opportunities for beekeeping in Saudi Arabia, the bee-keeping industry is steadily growing in the country with different opportunities and, of course, many challenge. The major challenge is occurrence and distribution of honeybee disease in the country (Al-Ghamdi, 1990, 2010; Alattal and AlGhamdi, 2015; Ansari et al., 2016a,b).

A mysterious decline in honeybee colonies has gained worldwide attention, including in Saudi Arabia. In the last decades, significant losses have been observed in indigenous honeybee colonies in Saudi Arabia (Alattal and AlGhamdi, 2015). Much attention has been given to Colony Collapse Disorder (CCD), which is a syndrome specifically defined as a dead colony with no adult bees and with no dead bee bodies but with a live queen, and usually honey and immature bees, still presents (vanEngelsdorp et al., 2009). Several causes of these large-scale losses have been reported, including honey bee parasites (*Varroa destructor*, *Acarapis*

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

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http://dx.doi.org/10.1016/j.sjbs.2017.01.054
1319-562X/

woodi); pathogens (*Nosema* spp. and bee viruses); pesticides, harsh environment, use of antibiotics, poor nutrition, and migratory beekeeping practices (Kevan et al., 2007; Higes et al., 2008; Naug, 2009; vanEngelsdorp et al., 2009; Bacandritsos et al., 2010; vanEngelsdorp and Meixner, 2010; Alattal and AlGhamdi, 2015).

Nosemosis is a fungal infection of honey bees caused by either *Nosema apis* or *N. ceranae*. *N. apis* was the historic species infecting European honey bees (Matheson, 1996). *N. ceranae* was previously isolated in naturally infected *Apis cerana* worker bees in China (Fries et al., 1996) and later has been described infecting *Apis mellifera* in Europe (Higes et al., 2006). Currently, this parasite is widespread all over the world and has shown the capacity of infection in other Hymenoptera different from the honeybees (Plischuk et al., 2009), and it is now a common infection of European honeybees and is highly virulent to its new host (Chen et al., 2009a). This is problematic for beekeepers because *N. ceranae* has a different seasonal phenology than *N. apis*, causing more significant problems for beekeepers in summer months and in warm climates (Bourgeois et al., 2010). Both *Nosema* spp. can co-infect honey bees (Chen et al., 2009b; Paxton et al., 2007; Forsgren and Fries, 2010). Although co-infections occur, *N. ceranae* has become the predominant species in many regions (Chen et al., 2009a; Klee et al., 2007; Williams et al., 2008; Razmaraii et al., 2013; Haddad, 2014).

Both *Nosema* spp. invade the midgut epithelial cells of adult honeybees (*A. mellifera*), i.e. worker bees, drones, and queens, and caused honey bee disease known as nosemiasis or nosemosis (Fries, 1988, 2010; Higes et al., 2007). This disease negatively affects productivity and survival of honeybee colonies, adult bee longevity, queen bees, brood rearing, bee biochemistry, pollen collection, and other bee behaviors (Botías et al., 2013; Huang, 2012). In contrast to the clinical symptoms of *N. apis*, such as crawling bees and dysentery (Liu, 1988), infection with *N. ceranae* is symptomless apart from reports of a massive colony depopulation and collapse (Huang et al., 2007; Paxton, 2010), reduced honey production (Higes et al., 2008). The impact of *N. ceranae* infection on colony survival is unclear and has been found in both healthy colonies (Vanengelsdorp et al., 2009; Cox-Foster et al., 2007; Gisder et al., 2010) and those undergoing sudden collapses (Higes et al., 2008, 2009; Bacandritsos et al., 2010; Martín-Hernández et al., 2007). Thus, investigation of *Nosema* species seems important.

Routine optical microscopy assessment can confirm infection with *Nosema* species, but it is impossible to distinguish between the species because the spores of the two *Nosema* species are very similar and can hardly be distinguished by light microscopy, so that in the absence of clear morphological characteristics for species recognition, other techniques using molecular markers may greatly assist in the diagnosis and identification of honeybee microsporidians Thus, it is necessary to use molecular diagnostic tools and identification methods (Gajger et al., 2010). The PCR technique provides a very sensitive test for detecting microsporidian infection because it enables detection of the parasite even at very low levels of infection.

In some bordered countries of Saudi Arabia (Egypt, Israel, Jordan, Iraq and Iran), *Nosema* infection in honeybee colonies has been reported previously (Alzubaidy and Ali, 1994; El-Shemy et al., 2012; Nabian et al., 2011; Razmaraii et al., 2013; Aroee et al., 2016; Soroker et al., 2009). However, even though some preliminary studies have been conducted on Nosemosis in honeybee in Saudi Arabia (Al-Ghamdi, 1990; Alattal and AlGhamdi, 2015; Abdel-Baki et al., 2016). Recently, in Riyadh region of Saudi Arabia, Nosemosis has been recognized by the presence of *Nosema* spores through light microscopy, assuming *N. apis* to be the causal agent (Abdel-Baki et al., 2016). These findings have led to a demand for PCR based research that determine, which species of *Nosema* have been present in *A. m. jemenitica,* the indigenous honeybee race of Saudi Arabia in a recent past using detailed survey and molecular characterization.

2. Materials and methodology

The presence of nosemosis in honeybee colonies was investigated in different beekeeping locations and eco-regions, during the spring season (March to April 2015), the active season for honeybees in Saudi Arabia. Eight different geographical localities, where beekeeping is common were included in this survey (Fig. 1): Al-Ahsa (25° 25′ 46″ N, 49° 37′ 19″ E), Abha (18° 13′ 24″ N, 42° 30′ 26″ E), Jazan (16° 53′ 21″ N, 42° 33′ 40″ E), Taif (21° 16′ 0″ N, 40° 25′ 0″ E), Al-Madinah (24° 28′ 0″ N, 39° 36′ 0″ E), Al-Bahah (20° 0′ 0″ N, 41° 30′ 0″ E), Al-Qassim (25° 49′ 19.72″ N, 42° 50′ 6.85″ E) and Riyadh (24°43′19.2″N 46°37′37.2″E). Total 50 random apiaries were visited in all locations, and 10 colonies in each apiary were inspected.

2.1. Sampling

A total of fifty seemingly healthy apiaries of *A. m. jemenitica*, indigenous race of Saudi Arabia, owned by different beekeepers were randomly selected following stratified randomization procedures (Moher et al., 2010). Samples were collected from local (*A. m. jemenitica*) bee races only (10 hives from each apiary) from March to April (major nectar flow period in Saudi Arabia) during the year 2015. The samples were collected from eight major beekeeping regions of Saudi Arabia, based on, the beekeeping management schedule and the categorization of geographical regions, For instance, Riyadh, Al-Qassim, Al-Ahsa, Taif and Jazan (Hot arid climate region), Al-Madinah and Al-Bahah (Tropical wet and dry climate), and Abha (Cold semi-arid climate). The sampling hives had not been treated against *Nosema* disease for at least 6 months. In each hive, approximately 100 worker bees were collected from outer honey frames of the brood chamber, placed in falcon tube containing preservative buffer (RNA Later®), transported to the laboratory of the Bee Research Unit (BRU) at the Department of Plant Protection of the Faculty of Food and Agriculture Sciences at King Saud University and stored at −20 °C until analyzed.

2.2. Data collection

The data collected from the survey included the following information for each inspected apiary: the date of inspection, the apiary location (to facilitate repeat visits), the name of the owner, the hive type (local or modern), the honeybee race (indigenous or imported), the number of honeybee colonies and the colonies having some unusual symptoms.

2.3. Microscopic examination of spores

Samples were initially analyzed by phase contrast microscopy for presence or apparent absence of *Nosema* spp. spores. For each sample, the abdomens of 30 adult bees were macerated in 30 mLof ddH2O, the suspension was filtered and centrifuged for 5000 rpm for 10 min and the homogenate examined under a Phase Contrast Microscope (Olympus BX51, model BX51TF, Japan, equipped with an Olympus DP71 camera (Olympus, Japan) at 400 × magnification (Fig. 2B), and photographed (OIE, 2008). Measurements are presented in micrometers and data are expressed as the mean followed by the range in parentheses. As morphological characteristics of *N. ceranae* and *N. apis* spores are similar and can hardly be distinguished by optical microscopy, all samples were also screened by multiplex 96 polymerase chain reaction

Please cite this article in press as: Ansari, M.J., et al. Geographical distribution and molecular detection of *Nosema ceranae* from indigenous honey bees of Saudi Arabia. Saudi Journal of Biological Sciences (2017), http://dx.doi.org/10.1016/j.sjbs.2017.01.054