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ORIGINAL ARTICLE

Survey and molecular detection of *Melissococcus plutonius*, the causative agent of European Foulbrood in honeybees in Saudi Arabia

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Abstract A large-scale field survey was conducted to screen major Saudi Arabian beekeeping locations for infection by *Melissococcus plutonius*. *M. plutonius* is one of the major bacterial pathogens of honeybee broods and is the causative agent of European Foulbrood disease (EFB). Larvae from samples suspected of infection were collected from different apiaries and homogenized in phosphate buffered saline (PBS). Bacteria were isolated on MYPGP agar medium. Two bacterial isolates, ksuMP7 and ksuMP9 (16S rRNA GenBank accession numbers, KX417565 and KX417566, respectively), were subjected to molecular identification using *M. plutonius* -specific primers, a BLAST sequence analysis revealed that the two isolates were *M. plutonius* with more than 98% sequence identity. The molecular detection of *M. plutonius* from honeybee is the first recorded incidence of this pathogen in Saudi Arabia. This study emphasizes the need for official authorities to take immediate steps toward treating and limiting the spread of this disease throughout the country.

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1. Introduction

Beekeeping is one of the long-standing practice in rural Saudi Arabia and is one of the most important economic activities for the communities (Al-Ghamdi and Nuru, 2013a). Approximately 5000 beekeepers maintain more than one million honeybee colonies and produce approximately 9000 metric

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tons of honey annually (Al-Ghamdi, 2007). *Apis mellifera jemenitica* is the only race of *A. mellifera* naturally found in the country and traditional beekeeping is mostly practiced using this race, because it is well adapted to the semi-arid to semi-desert conditions of Saudi Arabia (Al-Ghamdi and Nuru, 2013a). Indigenous honeybee colonies are too scarce with low productivity per hive, and did not fulfill the increasing demand for honey in Saudi Arabia. Consequently, significant annual losses occur during the summer season, because of short flowering season and long hot summer (Al-Ghamdi et al., 2013; Alqarni et al., 2011). To compensate these annual losses, the country annually imports around 100,000 *A. m. carnica* and *A. m. lingustica* Bee colonies from Egypt and Australia (Al-Ghamdi and Nuru, 2013b). Most of the bee packages imported from Egypt lack quality control parameters and may include disease agents and parasites (Alattal et al., 2014). 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 challenges. The major challenge is occurrence and distribution of honeybee disease in the country (Al-Ghamdi, 1990, 2010; Alattal and Al-Ghamdi, 2015). These conditions greatly affect the health, performance and productivity of imported honeybee colonies. A decline in bee populations leads to a decline in pollination, crop yield, and food supply (Potts et al., 2010). Hence, researching these factors and diseases, including potential treatments and preventative measures, is beneficial to the agricultural industry and conservation strategies in general.

In the last decades, significant losses have been observed in imported bee colonies in Saudi Arabia (Alattal and Al-Ghamdi, 2015). A mysterious decline in honeybee colonies has gained worldwide attention, including in Saudi Arabia. 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 (Evans et al., 2009). Five major abiotic and biotic factors (parasites and pests, pathogens, poor nutrition, sublethal exposure to pesticides and harsh environmental conditions) threaten honey bee health on a global scale. In reality though, these factors tend to overlap and interact with one another, which complicates issues and synergistically result in the abrupt disappearance of worker bees from the colony. Abiotic factors include environmental stresses, such as high summer and low winter temperatures, a lack of foraging capacities and the use of insecticides in agriculture (Naug, 2009; Watanabe, 2008), whereas biotic factors include a range of disease causing organisms such as bacteria, viruses, protozoa, fungi and parasitic mites. Two of the most economically important diseases of honey bees are bacterial diseases affecting the brood. American foulbrood (AFB) and European foulbrood (EFB) are both widely distributed and potentially lethal to infected colonies (Forsgren, 2010).

European foulbrood (EFB) is an economically important disease of honey bee (*Apis mellifera* L.) larvae caused by the anaerobic Gram-positive lanceolate bacterium *Melissococcus plutonius* (ex White 1912) (Aleksandrova, 1949; Bailey and Collins, 1982). EFB is well distributed across every continent that honey bees inhabit (Matheson, 1993). EFB affects mainly unsealed larvae and kills them at the age of 4–5 days and in severe cases entire colonies can be lost. The dead larvae turn yellowish, then brown, decompose, and become watery. The larval remains often give off a foul or sour smell due to secondary invaders, such as *Enterococcus faecalis* and *Paenibacillus* sp. (Arai et al., 2012).

These findings have led to a demand for research that explores the disease-causing agents of *A. m. jemenitica* and imported Honeybees in relation to honeybee health under the local environmental conditions in the Kingdom of Saudi Arabia. However, even though some studies have reported on disease causing organisms of honeybee in Saudi Arabia (Nixon, 1982; El-Naga, 1987; Al-Ghamdi, 1990; Matheson, 1993; Ellis and Munn, 2005; Alattal and Al-Ghamdi, 2015; Abdel-Baki et al., 2016). El-Naga (1987) reported European Foulbrood infection in two out of 40 colonies of imported honeybees from Egypt. This was the first report of EFB infection in Saudi Arabia, Later on, Al-Ghamdi (1990), Alattal and Al-Ghamdi (2015) also confirmed, EFB infection in Saudi Arabia. There has been very little research reported on the molecular characterization honeybee pathogens in Saudi Arabia. Therefore, detailed studies of various honeybee pathogens, including their identification and characterization using various molecular and microbiological methods, are needed. The fundamental goal of the research described herein was to characterize pathogenic agents from different geographical locations in Saudi Arabia that infect honeybees, particularly bacterial pathogens, such as *M. plutonius*.

2. Materials and methodology

The presence of European Foulbrood (EFB) in honeybee colonies was investigated in different beekeeping locations during the spring season (March–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). At least 10 apiaries were visited in each area, and 10 colonies in each apiary were inspected.

2.1. Sampling

A total of 800 hives in eight targeted localities (100 hives each) of *A. m. jemenitica* and imported bees (*A. m. carnica* and *A. m. ligustica*) were investigated in this study. Samples were collected from local (*A. m. jemenitica*) and imported bee races. Honeybee broods were visually inspected for any signs of abnormality and the clinical disease status. The clinical signs of AFB are very diverse and depend on the genotype involved, the stage of the disease and the strength of the bee colony (OIE, 2008). To preliminarily confirm EFB, suspect larvae were removed from the combs and tested using an EFB diagnostic field test kit (Vita, Europe) Limited, Basingstoke, UK according to the manufacturer's instructions. EFB-suspected honeybee broods were collected for further lab examination. A piece of brood comb (10 × 10 cm) containing suspect larvae was excised, wrapped in paper towels, packaged in a plastic bag, labeled and transported to the laboratory of the Bee Research Unit (BRU) at the Department of Plant Protection

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