

FIRST DETECTION OF NOSEMA CERANAE INFECTING APIS MELLIFERA IN INDIA

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Abstract

Apis mellifera colonies were sampled for over twelve months to study the *Nosema* infection in different apiaries of Himachal Pradesh, India. We found that the infection incidence was highest in the winter season (48.40 %) followed by autumn and rainy months. The infection was diagnosed conventionally through the microscopic analysis detection of *Nosema* species spores. However, with the recent findings that both *N. ceranae* and *N. apis* affect western honey bees, more sensitive and species specific molecular techniques are required that reliably differentiate between species of microsporidia. Therefore, molecular studies were conducted to precisely differentiate between *Nosema* species infecting honeybee colonies in India. Species specific multiplex PCR amplification using previously reported primers derived from 16S rRNA fragments were performed in a single reaction mixture. A single diagnostic band size of only 143 bp for *N. ceranae* was observed in the agarose gel. Furthermore, a diagnostic band size of 252 bp was observed with primer NoS, thus reconfirming the presence of *N. ceranae*. This study appears to be the first to report the detect of *N. ceranae* species from India in samples of *A. mellifera*.

Keywords: *Apis mellifera*, India, microscopy, Multiplex PCR, *Nosema ceranae*

INTRODUCTION

Nosema apis (Zander, 1909) and *N. ceranae* (Fries et al., 1996; Higes et al., 2006), two microsporidia responsible for *Nosema* infection in both *A. cerana* and *A. mellifera*, are known to cause substantial economic losses to apiarists and beekeepers worldwide. *N. apis* was the only microsporidian identified in *A. mellifera* until Higes et al. (2006) and Huang et al. (2007) found bees infected with *N. ceranae* in Europe and Taiwan, respectively. The morphological features of microsporidia have been studied by Fries et al. (1996) and Shimanuki & Knox (2000) under both light and transmission electron microscopes but morphological distinction of the spores at the species level remains subtle. Species specific microsporidia identification of both *N. ceranae* and *N. apis* can be detailed by advanced electron mi-

croscopic techniques, biochemical analysis and sensitive molecular methods (Fries et al., 2006). *Nosema* infection in India was first scientifically documented by Singh (1974) in the Kumaon region of Uttarakhand. Microscopic examination of adult bee samples revealed the presence of spores of *Nosema* sp., which was at those times considered as *N. apis*. The occasional occurrence of *Nosema* spp. was also reported from other states of India (Kshirsagar, 1982; Rana & Katna, 2011) but the species was not identified with the use of molecular biology. The present study aims to identify the probable *Nosema* species infection of European honey bees in India.

MATERIAL AND METHODS

For these preliminary studies, samples of *A. mellifera* bees were collected during the