Plant Archives Vol. 20 Supplement 1, 2020 pp. 3027-3030 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EVALUATION OF THE FIRST REPORT OF (*NOSEMA CERANAE*) DISEASE ON HONEY BEES IN IRAQ

Afrah sabah Abd Al-Hameed and Hayder Abd Al-Abbas Hadi*

Honeybee department, Plant protection directorate, Ministry of Agriculture, Iraq.

Abstract

This study was conducted to investigate the presence of Nosema ceranae disease on honeybees (*Apis mellifera*) in Iraq. The study was carried out in AL-rashidia beekeeping station in the northern of Baghdad.

350 samples of honey bees were collected from five Provinces of Iraq (Baghdad, Diala, Alnajaf, Karbala and Babil). The results showed depending on the characteristics of the pathogen that the disease found in three Provinces (Baghdad, Diala and Babil) with incidence ranged between (50-100)%. Genetic marker for the pathogen, simple sequence repeat (SSR) was used to differentiate between *Nosema ceranae* and *Nosema apis*. primer pair product a band with (218bp).

Key words: *Nosema ceranae*, honey bees, *Nosema apis*.

Introduction

Honey bees consider one of the most important beneficial insects and its importance comes through the production of honey, which is considered an important health food, in addition to that it is a good income for many beekeepers in the world. In addition to its honey production, it produces royal gel as well as bee wax which is a good resource of income too and play an important role in pollinating plants (A. shamout *et al*., 2014).

In the last years the beekeeping are expanded in Iraq due to the many facilitations which are given by plant protection directorate, ministry of agriculture to them. This expansion was accompanied by many emerge diseases that must be considered. One of these diseases is *Nosema ceranae* which is consider one of the most important modern disease. The disease is wide world and exists on all seasons because it can live in wide range of temperature. (Higes *et al*., 2010).

The disease was isolate for the first time from Asia bees (*Apis ceranae*) in china 1996 (Fries *et al*., 1996). After that the disease record on Europe bee (*Apis mellifera*) in 2006 (Higes *et al*., 2012). Higes *et al.,* (2013) said the disease is one of the important of the colony collapse disorder phenomenon and also said that disease contributes in destruction of bees colony in south Europe. The disease did not lead any emerge significant sign and did not cause death to the colony directly but it is destroying the queen activity and the larvae cannot reach to the adult stage because they cannot obtain the royal gel due to the hypo pharyngeal glands of bees become infected with the disease so they cannot supply the royal gel (Kauko *et al*., 2003; Webster *et al*., 2011). Because of the presence of signs and cases that the disease may be exist in Iraq, this study has been suggested.

Nosema ceranae has elliptical shape with dimensions average (2.2 × 4.4) micrometer (Chen *et al*., 2009). The information of this disease in most of middle east countries is too limited (N.J. Haddad, 2014)

Materials and Methods

Collecting samples

350 Samples of old bee were collecting from 7 provinces, 50 bee from each province.

The sample then soaked in alcohol. after that alcohol was drain off and the samples were sealed in a plastic container till examination.

Pathogen Isolation

Laboratory tests were performed in order to discover spores of disease according what has been mentioned by (Bollan *et al*., 2013). Where as the abdominal area of the samples have separated and buffer added in ratio 1:1

(1 ml to each bee) and the mix was crushed to obtain required extract.

Morphological examination

Bees samples were place in freezer to immobilize, after abdominal area was separated added 1 ml water / bee in a ceramic mortar and mix with a pestle until mixture in uniform. One drop from suspension was added on microscope slide and covered with cover slip.

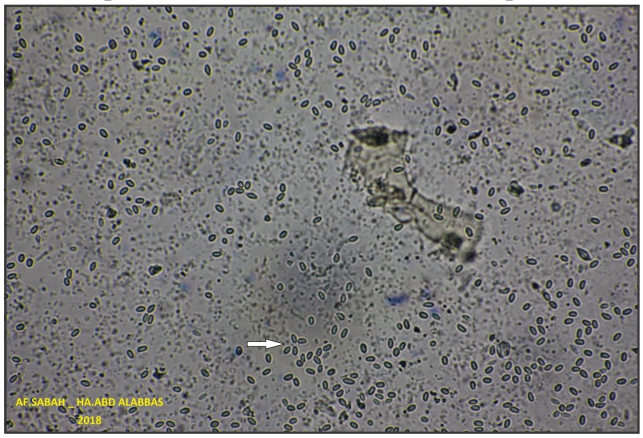

Fig. 1: *Nosema ceranae* spores (400X) magnification.

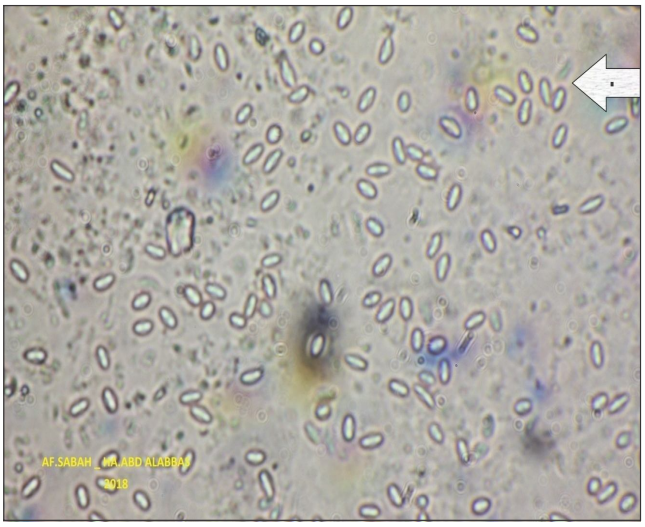

Fig. 2: *Nosema ceranae* spores (1000X) magnification.

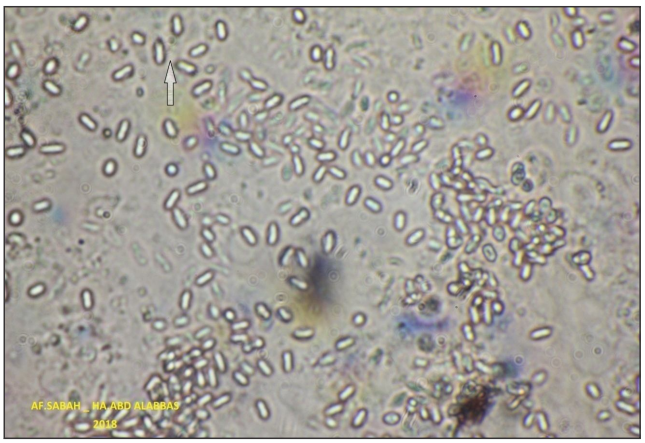

Fig. 3: Size of *Nosema ceranae* spore.

Table 1: Thermo cycle program.

PCR Program			
Steps	**°C**	**m:s**	**Cycle**
Initial Denaturation	95	05:30	1
Denaturation	95	00:30	35
Annealing	56	00:30	
Extension	72	00:30	
Final Extension	72	07:30	1
Hold	10	10:00	

The *Nosema ceranae* examined with compound microscope (type Meiji) under objective (400x), show the spores with elliptical shapes and the dimensions range between (2-2.5) micrometer width and (3.9-5.3) micrometer length. (Fig. 1).

Pathogenecity test

An infection of the disease was done by feeding bees with sugar solution contains spores of *Nosema ceranae*, after assurance that the bees are not infected before, after that samples were taken and inspected which show existing of too much spores of disease this means infection by *Nosema ceranae*.

Molecular test

• DNA Isolation: In case of positivity samples 1 ml of suspension was filtered and centrifuged for 5 min. at 8000 rpm and supernatants were removed. Spores were stored -20°C until they were used for DNA extraction (Utuk *et al.,* 2010).

Genomic DNA extraction was done from the pellets using DNaseTM tissue kit (promega, USA) by following the manufacturer's instructions. Before to DNA extraction, pellets were washed for 5 times with phosphate buffer solution (PBS).

• PCR technique: The SSR method depend on PCR.

Total DNA was extracted from samples which amplified by using one pair primer. PCR amplification were performed in 20ml volume *via* thermo cycler

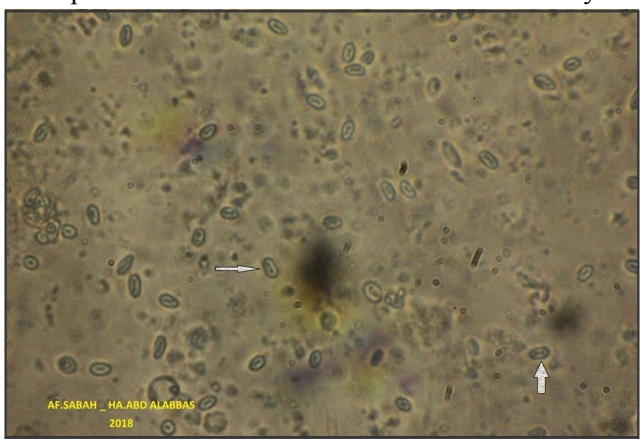

Fig. 4: Nuclei of *Nosema ceranae* spores.

Table 2: Sequence the primer used the PCR.

N. ceranae	Forward: 5'CGGCGACGATGT GATATGAAAATATTAA-3'	Volume (bp) 218
	Reverse:5'-CCCGGTCATTCT CAAACAAAAAACCG-3'	

machine (BIO Rad, USA).

The thermal profile for the sequencing PCR was:

95°C for 5 min (initial denaturation), 95°C for 30 sec. (denaturation), 56°C for 30 sec. (annealing), 72°C for 30 sec. (extension) after 30 cycles. 72°C for 7 min (final extension) then the hold in 10°C for 10 min. (Table 1).

• Electrophoresis: After PCR amplification, agarose gel electrophoresis was adapted to confirm the presence of amplification. PCR was completely dependable on the extracted DNA criteria.

Results

From total of (7) honey bees apiaries, (350) samples of bees were tested in order to discover the disease (*Nosema ceranae*), the results showed that (4) honey bees apiaries were infected, also the ratio of infection was between (50% to 100%), where as noted existing of spores of the disease by microscope, on other side no infection was found in other honeybees apiaries, also has been noted that the spores of disease have elliptical shapes and have different sizes ranging between (2-2.5) micrometer width and (5.3-3.9) micrometer length. (Fig. 2 & 3).

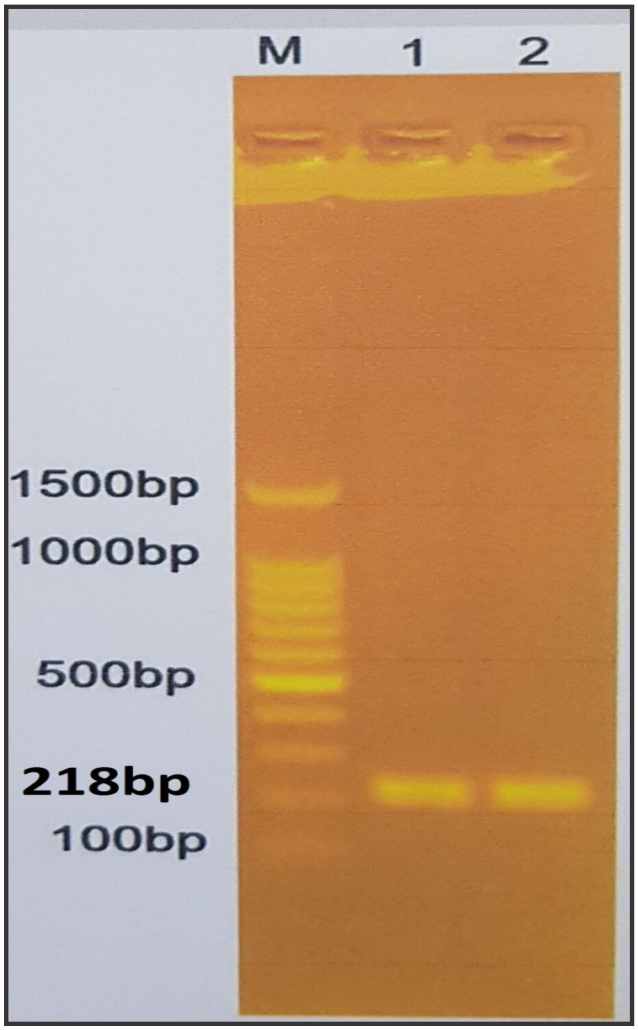

Fig. 5: Gel electrophoresis result.

Nosema ceranae strain AH17 small subunit ribosomal RNA gene, partial sequence

GenBank: MK072950.1

GenBank Graphics

>MK072950.1 Nosema ceranae strain AH17 small subunit ribosomal RNA gene, partial sequence
TTTGAGTTTTTTGGCTCTGGGGATAGTATGATCGCAAGATTGAAAATTAAAGAAATTG
ACGGAAGAATAC
CACAAGGAGTGGATTGTGCGGCTTAATTTGACTCAACGCGAGGTAACTTACCAATATTT
TATTATTTTGA
GAGAACGGTTTTTTGTTTGA

Nosema ceranae strain AH17 small subunit ribosomal RNA gene, partial sequence

GenBank: MK072950.1

FASTA Graphics

Go to:

LOCUS MK072950 160 bp DNA linear PLN 26-OCT-2018
DEFINITION Nosema ceranae strain AH17 small subunit ribosomal RNA gene, partial sequence.
ACCESSION MK072950
VERSION MK072950.1
KEYWORDS .
SOURCE Nosema ceranae
ORGANISM Nosema ceranae
Eukaryota; Fungi; Fungi incertae sedis; Microsporidia; Nosematidae; Nosema.
REFERENCE 1 (bases 1 to 160)
AUTHORS Abdulhameed,A.S. and Hadi,H.A.
TITLE Direct Submission
JOURNAL Submitted (21-OCT-2018) Department of Bees, Iraqi Ministry of Agriculture/Directorate of Plant Protection, Baghdad, Baghdad 10001, Iraq
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..160
/organism="Nosema ceranae"
/mol_type="genomic DNA"
/strain="AH17"
/isolation_source="Midgut of Honey bees"
/host="Bees"
/db_xref="taxon:40302"
/country="Iraq"
/collection_date="01-May-2017"
rRNA <1..>160
/product="small subunit ribosomal RNA"
ORIGIN
1 tttgagtttt ttggctctgg ggatagtatg atcgcaagat tgaaaattaa agaaattgac
61 ggaagaatac cacaaggagt ggattgtgcg gcttaatttg actcaacgcg aggtaactta
121 ccaatatttt attattttga gagaacggtt ttttgtttga

DNA was tested for samples which have previously diagnosed microscopically (morphology and size of spores) that give positive results. For molecular test (PCR) specific primer for *Nosema ceranae* (IDT) used and after laboratory steps required show compliance completely. The two positive *Nosema ceranae* samples showed no intraspecific DNA sequence differences among the (218bp) (Table 2) (Fig. 5).

Discussion

Nosema ceranae is one of the most important bee

diseases caused by uni-cellular spore-forming parasite.

Nosema ceranae and honey bee have an obligate parasitic relationship meaning that *Nosema ceranae* benefits while the honey bee is harmed. *Nosema ceranae* forms spores which are then ingested by the honey bees through food water (R. Cornman *et al*., 2009) then infect the intestine of honey bees. (Botias *et al*., 2012; Bollan *et al*., 2013).

The recent studies show that *Nosema ceranae* can infect honey bee larvae and decrease the longevity of adult. (Eiri *et al*., 2015).

Because of the absence of data base for *Nosema ceranae* and enter the bees to Iraq from unknown sources and occurrence of mortality in the bee hives, therefore, this study was done.

Spores measurements of *Nosema ceranae* are (2-2.5) mm width and (3.9-5.3) mm length which is very close to the average spore size reported by (Chen *et al*., 2009) as (4.4 × 2.2) mm.

Nosema ceranae has double nuclei in the center of spore. (Chen *et al*., 2009) (Fig. 4).

We detected *Nosema ceranae* in all four seasons and that agree with (Higes *et al*., 2010).

References

Bollan, K.A., J.D. Hothersall, C. Moffat, J. Durkaczm, N. Saranzewa, G.A. Wright, N.E. Raine, F. Highet and C.N. Conolly (2013).

Botias, C., R. Martin-Hernandez, A. Meana and M. Higes (2012). Critical aspects of the *Nosema* spp. diagnostic sampling in honey bees (*Apis mellifera*) colonies. *parasitol. Res*., **110:** 2557-2561.

Chen, Y., J.D. Evans, L. Zhou, H. Boncristiani, K. Kimura, T. Xiao, A.M. LItkowski and J.S. Pettis (2009). Asymmetrical coexistence of *Nosema ceranae* and *Nosema apis* in honey bees. *J. Invertebra. pathol.*, **101:** 204-209.

Cornman, R., Y. Chen, M. Schatz, C. Street, Y. Zhao, B. Desany, M. Egholm, S. Hutchison, J. Pettis, W. Lipkin and J. Eva (2009). Genomic analysis of the microsporidian *Nosema ceranae*, an Emergent pathogen of honey bees. *PLos pathogens.,* **5(6):** e1000466.

Eiri, D.M., G. Suwannapong, M. Endler and J.C. Nich (2015). *Nosema ceranae* can infect honey bee larvae and reduces subsequent adult longevity. *PLOS one.,* **10:** e01266330.

Fries, I., F. Fen, A. daSilva, S.B. Slemend and N.J. Pieniazek (1996). *Nosema ceranaen* sp. Microspora Nosematida morphological and molecular characterization of a microsporidian parasite of the Asian honey bee Apisceranae (Hymenoptera, Apidae). *Eur. J. Protistol*., **32:** 356-365.

Hadad, N.J. (2014). First detection of *Nosema ceranea* in Jordan.

Higes, M., R. Martin-Hernandez and A. Meana (2010). *Nosema ceranae* in Eourpe. an emergent type c nosemosis. *Apidology.*, **41:** 375-392.

Higes, M., A. Meanna, C. Bartolome, C. Botias and R. Martine-Hernandez (2013). *Nosema ceranae* (Microsporidia), a controversial 21st century honey bee pathogen. *Environ. Microbilol.* **5:** 17-29. doi;10.1111/1758-2229.

Kauko, L., S. Honko and H. Vartiainen (2003). Winter mortality and *Nosema apis* Z. the diagnostic value of Nosema spore countsa clinical approach. Annales universities Marie Curie Sklodowska. *Secto-DD medicina veterinaria.*, **58:** 199-203.

Shammout, A., N. Haddad and O. Auobeid (2014). The Monetary Value of Ecosystem Services Provided by Insects. A case study for selected crops in Jordan. *Jordan Journal of Agriculture Science*., **10(1):** 16-33.