

First Spatial Distribution of Nosemosis (*Nosema* sp.) Infected Local Bee, *Apis mellifera intermissa* L. in Algeria

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ABSTRACT

Nosema ceranae and *N. apis* are microsporidian parasitoids of the European honey bee *Apis mellifera* L. A study to estimate infection levels with *Nosema* sp. in local bees and their spatial distribution in Algeria was carried out in 2013. Bees' samples were collected from *A. mellifera intermissa* in 10 apiaries at 24 prefectures, representing different hives or areas in Algeria. Using spore counts, 65% and 85% of hives were found infected with *Nosema* sp. in Algeria. Setif and Tipaza were characterized by the highest levels of infection. Insignificant differences were found in infection levels among 164 hives and different areas. This study provides the first spatial distribution for the presence of *Nosema* sp. in Algeria.

Key words: *Apis mellifera intermissa*, *Nosema* sp., Infection level, Spatial distribution, Algeria.

INTRODUCTION

Honey bees (*Apis mellifera* Linnaeus, 1758) are major pollinators of plants and crops in agriculture and ecosystems. Their health is challenged on many factors (Muñoz *et al.*, 2014). Pathogens causing bacterial, fungal and viral diseases (Foley *et al.*, 2014), varroa mites (*Varroa destructor*) (Hamiduzzaman *et al.*, 2015), honeybee tracheal mites (*Acarapis woodi*), Nosemosis and pesticides are the dangers facing beekeepers (Soroker *et al.*, 2011). In nature, frequently infected insects are highly widespread by a large group of obligate intracellular parasites belong to the class Microsporidia. The genus *Nosema* has more than 150 species, usually infecting invertebrate hosts including at least 12 orders of insects. *Nosema* spp. are found in Hymenoptera causing some common diseases (Higes *et al.*, 2007). One of the two species of microsporidia in genus *Nosema* causes the *Nosema* disease (Traver and Fell, 2011). *Nosema apis* and *N. ceranae* are parasites of the European honeybee, *A. mellifera* and Asian honeybee, *A. ceranae*, respectively. The disease has spread worldwide and is currently responsible for considerable economic losses in apiculture (Fries, 2010).

However, both *N. ceranae* and *N. apis* appear in four continents (Asia, Europe, North- and South America) (Yoshiyama and Kimura, 2011 and Fernández *et al.*, 2012). *N. ceranae* is highly pathogenic when experimentally inoculated into European honey bees (Higes *et al.*, 2007) and with association of honey reduced production and increased winter mortality.

The present study aimed to record the first spatial distribution of *Nosema* sp. and to estimate infection levels with it in European honey bees (*Apis mellifera intermissa*) in Algeria.

MATERIALS AND METHODS

Samples

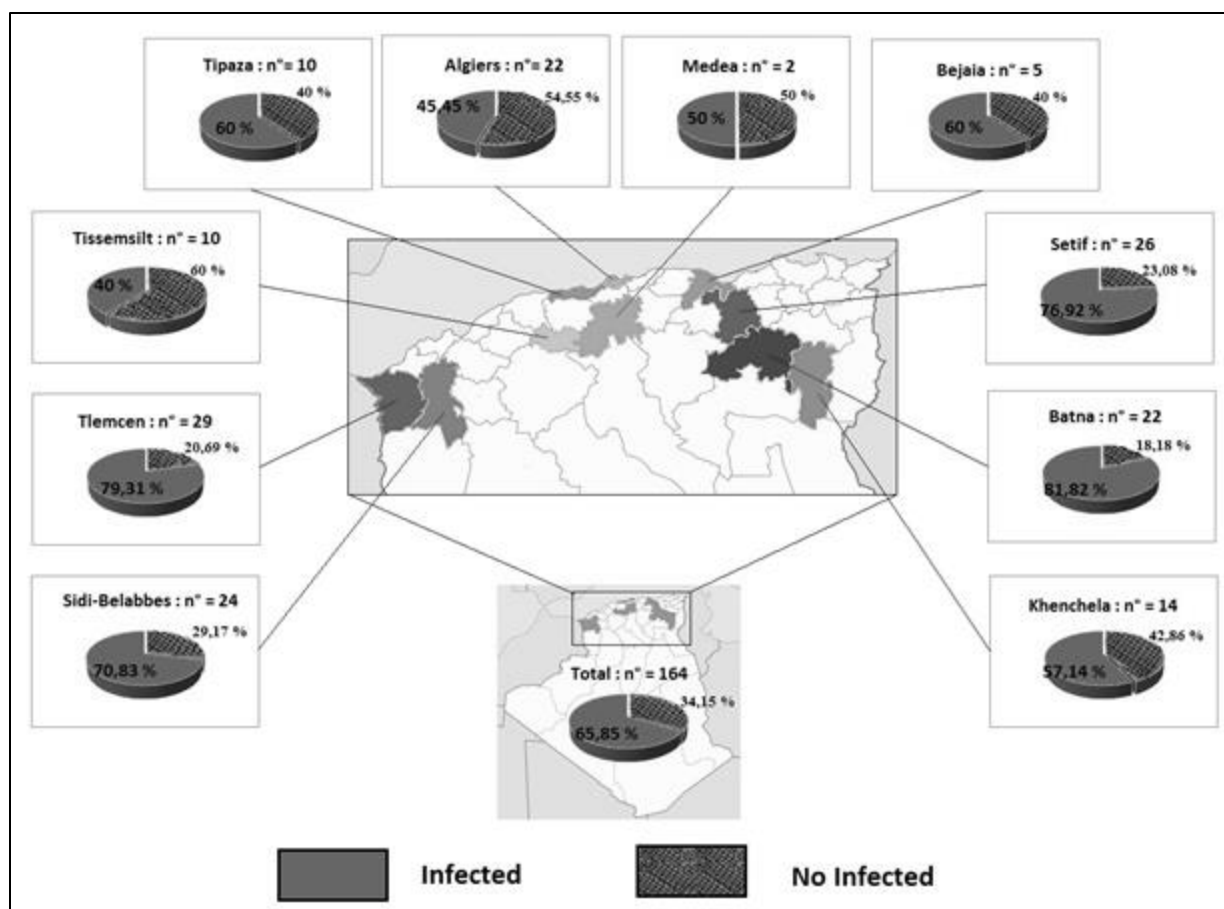
In June 2013, the 24 prefectures located at 10 areas in Algeria; Algiers, Tipaza, Medea, Bejaia, Setif, Batna, Khenchela, Tissemsilt, Sidi-Belabbes and Tlemcen were selected for sampling. In each prefecture, some hives were monitored for nosemosis. Inside the colonies, the live bees were collected and placed directly into pure ethanol (96%). Samples were placed in icebox and then stored at -20°C until processing. Sampling locations are listed in (Table 1) and (Fig. 1).

Detection

In the laboratory, spores of *Nosema* sp. were counted separately according to the protocol suggested by the International Office of Epizootics (OIE, 2013). A total of 164 searches for spores were carried out. Abdomens of 10 individuals were finely crushed in 5 ml of water. The suspension was filtered through two layers of fabric (muslin type) placed on a funnel on the top of a tube graduated to centrifuge. The contents of the tubes were equalized with water; the suspensions were then centrifuged at 800G for 6 min.. The supernatants were elutriated and the tubes were supplemented to 10 ml. A volume of 15 µl was deposited in the cell of Malassez, and observed with a photonic microscope with enlargement × 400. Spores of *Nosema* seemed to be transparent but with a very distinct dark contour, measuring between 5 and

Table (1): Locations and sample numbers of Nosemosis collected from apiaries in Algeria, 2013

Prefectures	N° samples	Detected	Not detected	Maximum $\times 10^5$ spores/bee	Minimum $\times 10^5$ spores/bee	% Infected colonies	% Healthy colonies
Algiers 1	5	5	0	104	28	100	0
Algiers 2	7	5	2	70	6	71.43	28.57
Algiers 3	10	0	10	0	0	0	100
Tipaza 1	5	5	0	78	18	100	0
Tipaza 2	5	1	4	2	2	20	80
Medea	2	1	1	36	36	50	50
Bejaia	5	3	2	20	14	60	40
Khenchela 1	6	3	3	6	2	50	50
Khenchela 2	3	1	2	2	2	33.33	66.67
Khenchela 3	5	2	3	44	2	40	60
Batna 1	10	9	1	40	2	90	10
Batna 2	8	7	1	10	2	87.5	12.5
Batna 3	4	2	2	4	4	50	50
Setif 1	9	6	3	532	2	66.67	33.33
Setif 2	8	8	0	124	2	100	0
Setif 3	9	6	3	168	2	66.67	33.33
Tissemsilt 1	5	2	3	6	4	40	60
Tissemsilt 2	5	2	3	6	6	40	60
Tlemcen 1	10	9	1	12	2	90	10
Tlemcen 2	9	7	2	6	2	77.78	22.22
Tlemcen 3	10	7	3	4	2	70	30
Sidi-Belabbes 1	10	6	4	6	2	60	40
Sidi-Belabbes 2	7	5	2	14	2	71.43	28.57
Sidi-Belabbes 3	7	6	1	56	2	85.71	14.29
Total prefectures=24	164	108	56	532	2	65.85	34.15

Fig. (1): Locations and distribution of honeybee samples that infected with *Nosema* sp. in apiaries in Algeria, 2013.

7 μm in length and 3 to 4 μm in width.

Quantification

Quantification of spores' number was calculated using the cell of Malassez. The counting was done on five frameworks that in the cell of Malassez where represented a volume of 0,05 mm³ which was divided for the volume of dilution of the sample to obtain the final number of spores (OIE, 2013).

Classification

A scale of classification of the infection was used to determine the degree of the infection of bees' colonies by the nosemosis according to the number of spores (Soerensen, 2009) as follows:

- 1= Very strong infection (more than 5 million spores per bee),
- 2= Strong infection (2 to 5 million spores per bee),
- 3= Weak infection (0, 5 to 2 million spores per bee),
- 4= Very weak infection (Less to 0,5millions spores per bee), and
- 5= No infection (0 spores per bee).

Statistical analyses

All statistical analyses were performed using XLSTAT 7.5.2. Infection levels among the hives and areas, multiples comparisons and Newman-Keuls Analyze (SNK) for differences among groups and significance as $\chi = 0.05$ in ANOVA were analyzed. As well, the correlation between rates of infections and average numbers of spores per bee and region was determined. To analyze *Nosema*-negative samples, infection level data were analyzed by a principal component analysis (PCA in Statistica6) which was used to model the proportion of the population that was infected based on hive, area and level of infection as factors.

RESULTS AND DISCUSSION

This study provides the first spatial description for the presence and distribution of *Nosema* sp. in Algeria.

Distribution of *Nosema*

Locations of the samples were plotted on the Algerian map to visualize the distribution of *Nosema* sp. (Fig. 1). Out of the 164 colonies surveyed, *Nosema* was detected in 108 colonies (65.85%) from 10 apiaries at the prefectures of Algiers, Medea, Tipaza, Bejaia, Setif, Batna, Khenchela, Tissemsilt, Sidi-Belabbes and Tlemcen (Table 1). All the 24 selected prefectures showed bees infection with *Nosema*, except one case in Algiers, where the nosemosis was not detected. Adjlane *et al.* (2012) reported that in the last years, nosemosis infections occurred in the Algerian honey bee populations. *Nosema* sp. can be accidentally introduced into an

area by imports of the queens (Muñoz *et al.*, 2014). But in Algeria, no signs for introducing honey bee stock have been detected (Chahbar *et al.*, 2013).

Spore counts and infection levels

The easiest way to count *Nosema* sp. spores was to use a traditional haemocytometer as described by (Cantwell, 1970 and Human *et al.*, 2013). In the present study, average numbers of spores per bee in a pooled sample for the evaluation of *Nosema* sp. infection in a colony was counted as described by (Soerensen, 2009 and OIE, 2013). A pooled sampling is probably remains an important tool for quantifying infections in colonies (Fries *et al.*, 2013). Levels of *Nosema* sp. in commercial colonies were 200.000 and 53.200.000 spores equivalents per bee, as minimum and maximum, respectively (Fig. 3). Among infected colonies (108 colonies), more than 74% of them had weak or very weak infection's level with the nosemosis, of which it could justify the absence of the acute symptoms of nosemosis in the majority of the monitored colonies. Cantwell (1970) reported that the intestine of the strongly infested bees can contain up to 180 million spores.

The recorded weak infection levels in the bees may be due to the timing of collection (June 2013), as June is characterized by a reduction in bees' activity in Algeria (Haderbache *et al.*, 2013). Highest levels of *N. ceranae* were noticed during spring, just before and during nectar flows peak (Traver *et al.*, 2012). Contrary to the observation of Martín-Hernández *et al.* (2007) *N. ceranae* was detected along the year and based on this finding it was suggested that there is no seasonality to the infection and there is no annual infection cycle.

Nosemosis and colony collapse disorder

The nosemosis infections can be present in honey bee colonies without causing depopulation or colony collapse. Some authors (Martín-Hernández *et al.*, 2007 and Higes *et al.*, 2007) suggested that *N. ceranae* causes colony collapse within 18 months of a colony becoming infected and infections left uncontrolled. These authors did not take into account the rate of infection. The results of the Algerian hives monitored showed that the same hive infected by *Nosema* sp. remained alive with normal production and without collapsing according to the beekeeper's answers made 19 months after sampling.

Data obtained in the present study are complementary and consistent with the results reported by Invernizza *et al.* (2009), whose hives were monitored for decades and demonstrated no correlation between arrival of *N. ceranae* and its presence in the hives or hive losses due to CCD. Available data strongly suggest that this parasite can

Table (2): Total percentage of variance using the axis 1, 2 (PCA in Statistica 6)

Values	Eigen value	% total variance	Cumul Eigen value	Cumul %
1	2,037688	50,94219	2,037688	50,94219
2	1,473866	36,84665	3,511554	87,7888
3	0,459102	11,47755	3,970656	99,2664
4	0,029344	0,73361	4,000000	100,0000

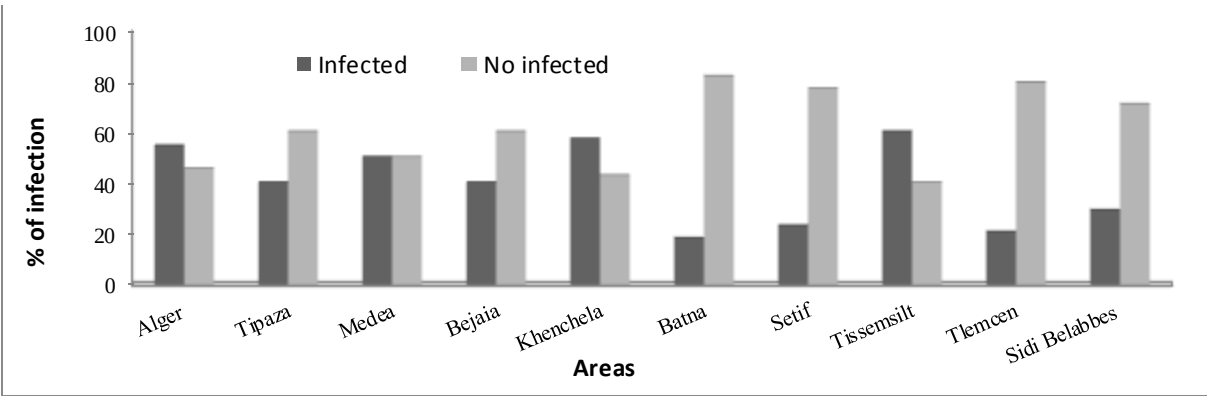


Fig. (2): Percent of colonies infected with *Nosema* sp. in apiaries in the study areas (Algeria) in June 2013 (n=164).

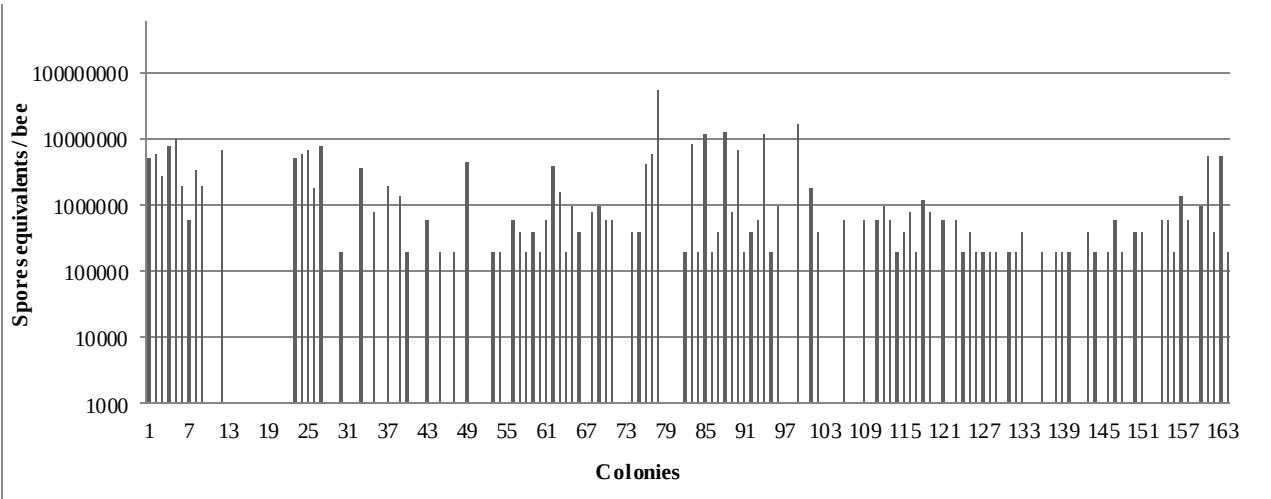


Fig. (3): *Nosema* sp. spore numbers per bee for 164 honey bee colonies collected in June 2013. Data have been calibrated with spore counts from microscopy data.

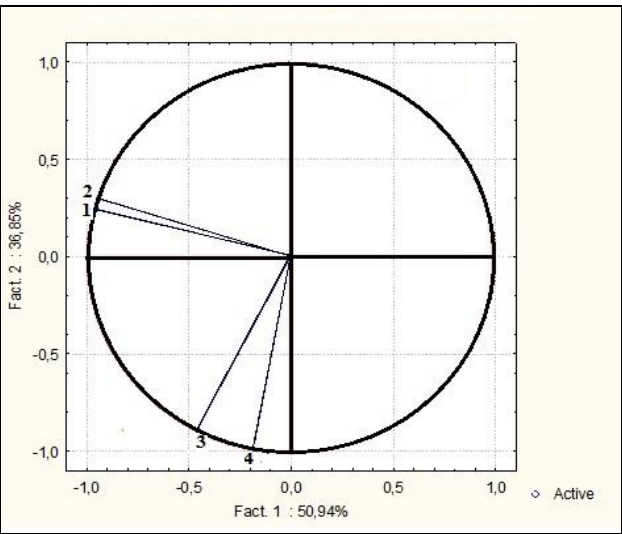


Fig. (4): Projection of variables on the factorial plan (1x2) using PCA in Statistica6.

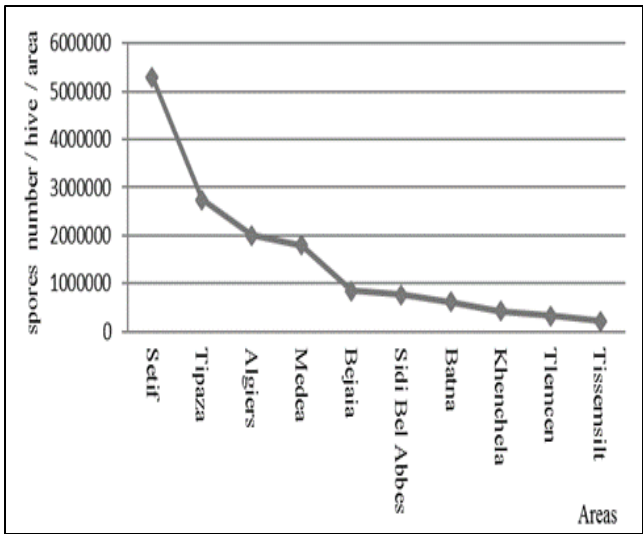


Fig. (5): Graph of the average numbers of spores per hive and area.

be present in honey bee colonies for extended periods without causing symptoms or colony losses (Paxton, 2010). The same case was found in Germany (Siede *et al.*, 2008) and in the Spanish honey bee population (Fernández *et al.*, 2012) as the monitored hives showed no devastating effects as described by (Higes *et al.*, 2009).

It is necessary to hold accounts on the rates of infection in the colonies with the nosemosis. In this study, 65.85% of the hives were found carrying the disease but without causing CCD that can be explained by the levels of infection, while 74.07% of the hives had a weak or a very weak infection level. The destruction of the colonies by the nosemosis occurs when the rate of infection is marked strong.

Comparison of infection levels among areas and hives

Insignificant relationship was found between colony strength and *N. ceranae* infection level (Traver *et al.*, 2012) which indicating that weak colonies can be heavily infected with *N. ceranae* as might strong colonies do (Traver and Fell, 2011). This is in agreement with obtained results as insignificant differences in the levels of infections among the colonies ($F = 1,534$; $ddl = 162$; $p = 0,579$). Contrary to Bourgeois *et al.* (2010), random nature of the sample may also contribute to the variation seen among colonies, as older bees are more exposed to *Nosema* sp. and the bees sampled might have different stages of infection.

In the present study, insignificant differences ($F = 2,495$; $ddl = 162$; $p = 0,011$) in *Nosema* sp. infection levels among bees sampled from different areas were recorded. The same results were indicated in USA (Traver *et al.*, 2012), where also insignificant differences in *N. ceranae* infection levels among bees sampled from different areas were found. Study of local honeybee populations showed that Algerian *A. mellifera* populations are characterized by high variability in terms of number of alleles (Chahbar *et al.*, 2013 and Loucif-Ayad *et al.*, 2014). *Nosema*-negative samples were analyzed by (PCA in Statistica6). The axis 1, 2 was used which 1 had total percentage of variance of 50.94% and axis 2 with 36.85% (Table 2). Based on this analysis, hives (factor 1) and areas (factor 2) did not have presence or absence of infection (factor 3) and did not have direct effect on the levels of the infection (factor 4) by the nosemosis (Fig. 4). Significant differences between; Setif and Khenchela, Setif and Batna, Setif and Tlemcen, Setif and Sidi-Belabbes, Setif and Algiers were found.

The grouping showed that Setif and Tipaza were set up a group (A) that characterized by a strong

nosemosis infection (Fig. 5). For the remainder of the areas (group B), no differences were found among infection levels, which can be explained by the detection of two different species of *Nosema*. At the area of Setif, number of spores per bee reached a maximum of 53200000 spores. Bourgeois *et al.* (2010) noted that on 104 analyzed colonies, the number of *N. apis* spores did not exceed 1 million per bee and it can reach more than 100 million spores for *N. ceranae*. Accordingly, the most frequent species of *Nosema* in Algeria was *N. apis*, but this result can be confirmed by genetic assay; contrary to Higes *et al.*, (2009a), who reported that only *N. ceranae* was detected in North Africa.

Climate may be an important factor explaining differences in species distribution and impact as some environmental conditions may prevent the development of the disease or the expression of their genetic potential (Fries, 2010). Also, the bees may adapted themselves and the parasite does not produce enough spores, there is a resistance of the bee (may be in Algeria) against the nosemosis, as 100% of the beekeepers do not practice treatment against the nosemoses (according to the beekeeper's answers). Results of Chaimanee *et al.* (2012) demonstrated that *Nosema* sp. infections can be quite different between experimental cages. These differences may be due to genetic variation in the ability of honey bees to fend off *Nosema* infection. Intestinal honey bee parasite, *N. ceranae* (Microsporidia), is at the root of colony losses in some regions, while in others its presence causes indirect mortality. This is the case for Spain and France, respectively (Dussaubat *et al.*, 2013), and there is no specific genetic background of the two *N. ceranae* isolates, from Spain or France.

Rates of infection and average number of spores

No correlation between rates of the infections and the average number of the spores was found. The 10 studied areas did not have same climatic conditions; therefore they did not have a direct influence on the produced number of spores. The number of spores in the case of infection by *N. apis* did not exceed 1 million spore per bee (Bourgeois *et al.*, 2010), which means that it is not externally conditioned by transmission routes of the disease and infected colonies in the vicinity. Knowledge of the interactions between *A. mellifera* and *N. ceranae* may help identifying possible interspecific co-adaptations that may have been acquired through decades of parasitism (Botias *et al.*, 2012).

In conclusion, obtained data provide supportive information on the performance and the geographical distribution of the nosemosis in Algeria and showed that the levels of infections with the nosemosis in bee

colonies at the majority of the cases were found having weak or very weak infection levels. Different areas and the hives did not affect presence or absence of the infection with the nosemosis. Further study using genetic analyses is needed.

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