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Prevalence of *Nosema ceranae* in North and South Regions of Azerbaijan

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Abstract

Nosemosis is one of the most dangerous infectious diseases of honeybees in Azerbaijan and the world. *Nosema ceranae* is the dominant species in Azerbaijan, and this study aimed to detect the prevalence of the infection in the country. For this purpose, an average of 100 honeybee samples were collected from 64 hives, 24 from three regions in the north and 40 from five areas in the south. In the lab, the abdomens of 50 bees from each group were dissected and crushed in a container, adding 50 ml of distilled water. According to obtained data after microscopic examination, the *N. ceranae* spores were found to have a high-level prevalence in northern regions (45.8% average) than in the southern areas (22.5% average) in Azerbaijan. Molecular diagnoses of Nosema-positive samples have been performed with PCR and *N. ceranae* has been detected from all regions. Data show us that the Nosemosis is common in Azerbaijan and is a significant threat in the beekeeping industry.

Keywords: *Nosema ceranae*, prevalence, honeybee, Azerbaijan

Introduction

Beekeeping is one of the important economic sectors in the world, as well as in Azerbaijan. In recent years, the country has been making significant progress with approximately 500.000 colonies and annual honey production of 6.000 tons[1].

The productivity of the bees depends on both physiological characteristics and health levels. In case the disease occurs in the bee colonies, the functional ability of the bees falls, and the losses become happening in the bee colonies. Nosema is one of the microsporidian species and is becoming a dangerous honeybee pathogen with its two species: *Nosema apis* and *N. ceranae*[2,3]. These parasites infect the digestive system of adult bees and cause significant bee losses worldwide[4-7]. The first reported experimental infection of *A. mellifera* by *N. ceranae* clearly showed that this parasite was highly pathogenic to its new host[2]. It induced significantly higher bee mortality than *N. apis*[8].

The existence of *Nosema apis* in Azerbaijan has been noted in many scientific publications and conferences, and reported one the most prevalent diseases among the honeybees[9,10]. Recently, the existence of *N. ceranae* has been recorded in our preliminary research before[11].

This study aimed to determine the prevalence of *N. ceranae* infection of honeybees in southern and northern regions in Azerbaijan.

Material and Method

The study was conducted in May-June 2019. Since honeybees are invertebrates, there was no need for an ethics

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committee report. An average of 100 worker bee samples per hive was collected from three regions in the north of Azerbaijan (Guba, Gusar, Shabran) and five in the south (Astara, Lankaran, Masalli, Jalilabad, Bilasuvar). Samples were collected from 64 hives, eight from each region in different places. The samples were put into specific plastic boxes and were brought to the laboratory to keep cold.

To determine the Nosema spores in the sampled bees, 50 bees were dissected from each group. The abdominal parts of the bees were cut and crumbled by adding 50 ml of distilled water in a large tube. After the resulting suspension was filtered through the filter, 0.1 ml of liquid was taken, dripped on a hemocytometric slide, covered with a cover glass and examined under an ×400 magnified light microscope. The total number of spores (N) per bee is found with this formula: $N= S\times4\times10^6 / 80$; where 'S' is the total spores in five cytometric areas on the slide[12,13]. The average number of spores was determined in these areas, and the total number of spores for an individual was estimated.
PCR analyses of Nosema-positive samples were conducted in the preliminary study before this study[11], in which the samples were taken at the same time. A multiplex-PCR method and 16S rRNA genes have been used[11].

Results and Discussion

As a result of PCR analysis on samples in our previous study, all species have been identified as Nosema ceranae[11]. It was found that it had a high-level prevalence in northern (12.5-62.5%) than in southern in Azerbaijan (0-37.5%).
The study examined 64 samples from eight hives of eight sites, finding 20 (31.25%) hives Nosema-positive in total. According to the data in the Table 1, the highest infection rate of Nosema was detected in Quba and Qusar localities (62.5%), the least in Bilasuvar and Shabran localities (12.5%). No infection was seen in the Masalli area. If reviewed by data, the infection in the northern region of the country (45.8% average) appears to be greater than in the south (22.5% average).

Table 1. Prevalence of N. ceranae according to regions in Azerbaijan

Regions	Number of Colonies	Positive colonies	Minimum number of spores	Maximum number of spores	Percentage (%)
Astara	8	3	100.000	800.000	37.5
Lankaran	8	3	250.000	5.150.000	37.5
Masallı	8	0	0	0	0
Jalilabad	8	2	50.000	1.200.000	25
Bilasuvar	8	1	50.000	750.000	12.5
Shabran	8	1	50.000	200.000	12.5%
Quba	8	5	500.000	5.700.000	62.5%
Qusar	8	5	50.000	1.800.000	62.5%
Total	**64**	**20**	**1.050.000**	**15.400.000**	**31.25%**

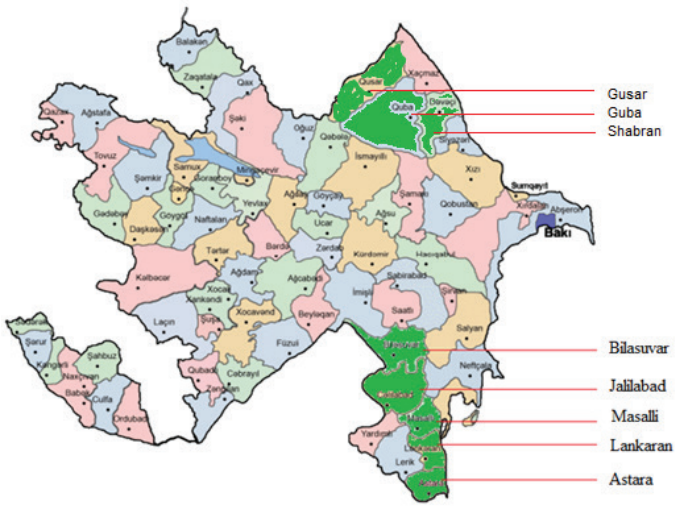

Figure 1. The location of the Azerbaijani regions in the map from which the samples were taken.

Nowadays, infection with *N. ceranae* has been proved almost on all continents. Recent reports have confirmed changes in the clinical and epidemiological patterns of Nosemosis, suggesting that *N. ceranae* has been one of the most prevalent pathogens in honeybees around the world[5]. The prevalence of *N. ceranae* in Iran's Azerbaijan climatic zones was significantly different, and the highest majority was found in the semi-humid climate (71.00%), followed by very humid (68.10%) and humid (53.80%) climates[14]. The number of Nosema spores per bee and climatic conditions seems to be related to the clinical signs and mortality. Obvious clinical signs have been higher during the rainy months, which we also observed[15]. As a result of the laboratory studies, it was defined that the prevalence level of the disease is higher in the districts of the region in which the humidity is relatively high. Based on these results, it should be noted that there is a proportion between the moisture and the Nosema disease. However, not only the humidity factor but also the other influential factors should be indicated as the causes of the disease. These possible factors include the lack of proper care for the bees, dirty water feeding, excessive treatment with a high dose of Varroa mite, and other environmental factors. It appears that *N. ceranae* is better adapted to complete its endogenous cycle with a higher biotic index at different temperatures reflecting the epidemiological differences between both microsporidian species in field conditions and at the colony level[16].

The highest indicators in the northern region were mentioned in Quba and Qusar regions. During the collection of material from those regions, pesticides have been used in the surrounding areas, especially in the apple and cherry gardens. In the Southern part, the highest indicators of the disease belong to the Astara region. The high humidity level in that region possibly leads to making available conditions for creating this disease.

In previous years, during the studies of the national scholars, the existence of *N. apis* species has been detected[9]. Tahirov and Hüseynov[17] reported that the Nosemosis is a widespread disease, with the prevalence of *N. apis* at 24.9-48.3% in low coastal areas, 29.8-63.2% in a mountainous region and 58.2-87.0% in Zengezur mountainous region of Nakhichevan Autonomous Republic, only via microscopic examination. Although there was no molecular diagnosis in those studies, it was possible to see such species of this disease in many regions of Azerbaijan. The presence of *N. ceranae* in the literal materials was noted in previous studies in the northwest part of Iran (close to the Azerbaijan border) as 48.2%[18]. In our survey conducted in 2019, the proliferation of *N. ceranae* in the southern part of Azerbaijan was described at the molecular level[11].

This study showed that *N. ceranae* is a severe and widespread problem in Azerbaijani honeybees. *Nosema apis* has not been detected in the samples taken from all eight provinces. Whether in the north or south of the country, the disease is common in the apiaries and necessary measures should be taken about the disease nationwide.

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