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Short communication

A SCIENTIFIC NOTE ON INCIDENCE OF *NOSEMA APIS* AND *NOSEMA CERANAE* IN SLOVAKIA DURING THE YEARS 2009 AND 2010

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ABSTRACT

During the last few years a new kind of nosemosis was diagnosed in the bee colonies in the territory of Europe. In Slovakia the causative agent *Nosema ceranae* was confirmed in 2008.
The aim of our study was to monitor the prevalence of mono infection and co-infection of both species *N. apis* and *N. ceranae* by using polymerase chain reaction (PCR). The analysis was performed on 72 samples of dead bees, which were collected from bee colonies representing all regions of the country in the years of 2009 and 2010. Prior to PCR analyses positive samples were selected by microscopic examination, confirming the presence of *Nosema sp. spores*.
In the year 2009, *N. apis* mono infection was diagnosed overall in one sample. The *N. ceranae* mono infection was diagnosed in 16 samples, while *N. apis* + *N. ceranae* co-infection was diagnosed in 2 samples. In three samples causative agent was not identified by differential diagnostics and those were considered as *Nosema spp*. positive. The ascertained prevalence of *N. apis* and *N. ceranae* was 14.3 % and 85.7 %, respectively.
In the year 2010, the *N. apis* mono infection was not diagnosed, while *N. ceranae* mono infection was diagnosed in 27 samples and *N. apis* + *N. ceranae* co-infection was confirmed in 3 samples. The ascertained prevalence of *N. apis* and *N. ceranae* was 9.1 % and 90.9 %, respectively.
During the period of two years (2009 - 2010), a gradual increase in the prevalence of *Nosema ceranae* and decrease in the prevalence of *Nosema apis* was recorded in Slovakia.

Key words: *Nosema apis*; *Nosema ceranae*; prevalence; PCR

INTRODUCTION

The *Nosema apis* was the only diagnosed microsporidian intracellular parasite in the bee colonies in Slovakia until 2008. During its own life cycle this parasite directly damages epithelial cells of the ventriculus. After that the individual bee digests the carbohydrates and protein components of food imperfectly and causes the worker bee rectum to become overfilled, which is followed by diarrhoea. The aging process of worker bees goes faster because of the insufficient evolution of pharyngeal glands and also the egg production by queen bee decreases. After that, the bee colony is gradually weakened till its collapse. This is caused by decrease in fertility and by insufficient care for the bee brood (the lack of nurse bees for feeding larvae).

During the last few years, the presence of a new kind of nosemosis was confirmed in the territory of Europe. This new infection is caused by the *Nosema ceranae*. It was probably introduced to Europe by humans, but the European bee eater (*Merops apiaster*) could play some role in its introduction, too (Higes *et al.*, 2008). *Nosema ceranae* usually infects *Apis ceranae* in Asia. The presence of *N. ceranae* in Europe was

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confirmed for the first time in Spain in 2006 (Higes *et al.*, 2006). In Hungary it was confirmed in 2009 (Tapaszti *et al.*, 2009). In Slovakia the first presence of this parasite was observed and confirmed in the territory of Plavečské Podhradie in 2008 (Staroň, 2009).

Several researches present different observations about the virulence of *N. ceranae* . Some studies point out to the higher virulence of *N. ceranae* in comparison to *N. apis* (Paxton *et al.*, 2007) and its possible role in the CCD syndrome (Higes *et al*., 2009). On the other side, Forsgren and Fries (2010) showed comparable virulence of both species where in the process of co-infection *N. ceranae* does not have any competitive advantage. Opinions about virulence of both pathogens are in contradiction, because many environmental factors affect the course of infection (Fries, 2010). *N. ceranae* causes higher immunosuppression in comparison to *N. apis* (Antúnez *et al.*, 2009) and in combination with the impairment of bee colony by neonicotinoids. It also contributes to CCD syndrome (Alaux *et al.*, 2010). The *N. ceranae* infection is tolerated more when bee colonies have enough glycid and protein reserves, because the parasite increases the host energy intake and when the host does not have enough reserves, it undergoes energy stress (Mayack and Naug, 2009; Naug and Gibbs, 2009).

The objective of this study was to monitor the prevalence of mono infection and co-infection by *N. apis* and *N. ceranae* during the two year period of 2009 and 2010 in Slovakia. The aim was also to determine the participation of both pathogens in *Nosema spp.* positive samples, too as many authors observed that the *N. ceranae* is gradually replacing the original *N. apis* (Chen *et al*., 2009; Fries, 2010).

MATERIAL AND METHODS

The tested samples originated from the queen breeders. They were brought to the laboratory to perform the periodical diagnostics of *Nosema spp*. Half of the sample was separated and the light microscopy examination was performed to identify *Nosema spp.* spores. The other half of dead bee bodies was left to perform PCR analysis. The selected samples, which showed medium and strong positivity of *Nosema spp*. spores during the light microscopy, were differentiated by PCR analysis. The reference material (*Nosema ceranae*, *Nosema apis*) was provided to us by Dr. Mariano Higes, Bee Pathology laboratory, Centro Apícola Regional, Spain. Sample for PCR analysis consisted of samples of abdomens of approximately 10 adult bees. Extracted abdomens were homogenized; afterwards the germinative buffer solution was added to suspension to ensure the swelling of the spores. The nucleic acid (DNA) was isolated from suspension by commercial kit Dneasy® Plant Mini Kit (QIAGEN). The extracted DNA was stored at -20°C temperature till PCR analysis was carried out. During the PCR analysis the required thermal conditions and the reaction mixture including the Taq DNA polymerase as well as the specific primers were adequately maintained. The sections that are unique for *Nosema* genus and for individual species of this genus were amplified. Agarose gel electrophoresis was used to evaluate the results. The detected fragment size of 122 bp appeared on the gel in case of presence of a representative genus *Nosema*, while the fragment size of 219 bp was observed in case of *Nosema ceranae* and the fragment size of 321 bp in case of *Nosema apis* (Martín-Hernández *et al.*, 2007).

RESULTS AND DISCUSSION

In 2009, twenty-nine samples were examined according to the described methodology. *N. apis* mono infection was diagnosed overall in one sample (3.45 %), the *N. ceranae* mono infection was diagnosed in 16 samples (55.17 %), while *N. apis* + *N. ceranae* co-infection was diagnosed in 2 samples (6.9 %). There were 7 (24.14 %) *Nosema spp*. negative samples. Three samples (10.34 %) were not distinguished by differential diagnostics; they were set only as *Nosema spp*. positive. These were observed because the extremely sensitive primers were purposefully used for detection of the genus *Nosema* (common for *Nosema ceranae*, *Nosema apis* and *Nosema bombi*); those were able to detect pathogens from 10 spores already. However, the primers that are specific only for *Nosema ceranae* or *Nosema apis* can detect from 10^3 spores, therefore the sample with lower infection level seems to be negative (Klee *et al*., 2006). The ascertained prevalence of *N. apis* and *N. ceranae* was 14.3 % and 85.7 %, respectively.

In the year 2010, forty-three samples were examined with this methodology. The *N. apis* mono infection was not diagnosed, *N. ceranae* mono infection was diagnosed in 27 samples (62.79 %), while *N. apis* + *N. ceranae* co-infection was confirmed in 3 samples (6.98 %). There were 13 (30.23 %) *Nosema spp*. negative samples. The ascertained prevalence *N. apis* and *N. ceranae* was 9.1 % and 90.9 %, respectively.

The negative results of PCR analysis were probably caused by the separation of mixed sample and by the presence of artefacts during the light microscopy (mouldy samples of dead bee bodies).

In Hungary, differentiation between *N. ceranae* and *N. apis* spores by PCR analysis from 38 samples also showed similar results. Only one sample contained *N. apis*, and in the other 37 samples *N. ceranae* was detected, which indicates the dominance of *N. ceranae* in Hungarian apiaries (Tapaszti *et al*., 2009). Results from Croatia showed that *N. ceranae* is the only *Nosema*

species found to infect honey bees in the geographic territory of Croatia (Tlak Gajger *et al*., 2010).

Our two-year observation in Slovakia points out that the *N. ceranae* prevalence at the expense of *N. apis* is rising. We also found a possible co-infection of *N. apis* and *N. ceranae* of some bee colonies *Apis mellifera.*

CONCLUSION

During the two year period 2009 and 2010, a gradual increase was recorded in the prevalence of *Nosema ceranae* and decrease in the prevalence of *Nosema apis* using polymerase chain reaction (PCR) analysis of bees (*Apis mellifera*) in Slovakia.

Abnormal failure of the bee colonies leading to nosemosis is considered as the only reason of death of bee colonies in Slovakia. For strong bee colonies with enough food supplies, the virulence of *N. ceranae* against *N. apis* is probably not higher. It is necessary to monitor the prevalence of both species of *Nosema* in Slovakia in the forthcoming beekeeping seasons.

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