Nosema ceranae has been present in Poland since at least 1995

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Introduction

Nosema ceranae infection is widely present in apiaries all over the world. It was commonly believed that this parasite had infected *Apis mellifera* only in recent years. However, lately it was proved that in the US it had been present in apiaries since at least 1996 (Chen et al. 2007) and in Finland since 1998 (Paxton et al. 2007). The team of Professor Aranzazu Meana and Dr Mariano Higes confirmed (using PCR analysis) the presence of *Nosema ceranae* in each of ten *Nosema* positive dead bee samples (from four different Polish apiaries) sent to them in 2007. In this work historical samples and present samples from Poland were investigated for the presence of *Nosema ceranae*.

Materials and methods

The following samples were examined:

- 25 dead bee samples collected from apparently healthy colonies in the years 1994-1996 (3 - in 1994, 16 - in 1995, 6 – in 1996).
- 432 samples, collected between 1st of December 2007 and 15th of March 2009; these samples were from colonies in which increased mortality of bees or disappearing of bees was observed (apiaries from different parts of Poland).
- additional 160 samples collected between 16th of March and 26th of July 2009

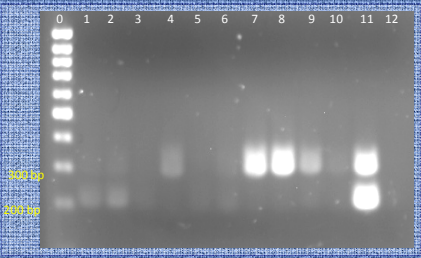
The samples were investigated microscopically (Topolska G., Kaprzak S. 2007; Topolska et al 2008) and by PCR (OIE Manual For Terrestrial Animals; Higes et al. 2006). The amplicon sizes for *Nosema apis* and *Nosema ceranae* were 321 and 218-219 bp respectively.

Results

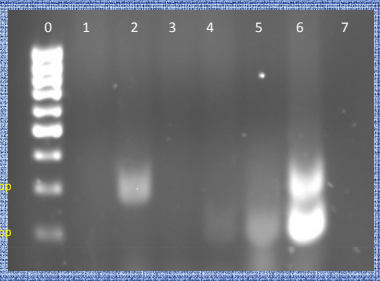
The sequences characteristic for *N. ceranae* were found in two samples from 1995 - from a Warsaw apiary (Fig. 1a) and one sample from 1996 – from Biała Podlaska (Fig. 1b). All bee colonies from the first apiary died because of Nosema infection at the end of 1995.

A weak signal was obtained in the case of 4 other samples from 1995 and 2 samples from 1996 (Fig. 1a). A very weak signal was obtained also in the case of two samples from 1994, however a more sensitive method should be used to confirm the presence of *N. ceranae* in these samples. In 306 (68%) samples, collected between 1st of December 2007 and 15th of March 2009, *Nosema* spores were detected. In 250 (82%) of these samples *N. ceranae* was present, 49% were positive only for *N. ceranae*, 18% only for *N. apis* and 33% were positive for both pathogens. A higher percentage of *Nosema* positive samples contained *N. ceranae* in 2009 than in 2008 (tab. 1).

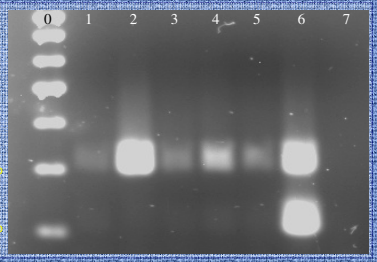
Fig. 1) Results of the electrophoresis of the amplicons produced by PCR of samples from 1994 -1996. lane 0 – 100 bp DNA ladder



a) lanes: 1, 2, 3, 4, 5, 6 and 9 – samples from 1995; lanes: 7, 8 and 10 – samples from 1996; lane 11 – positive control (*Nosema apis* – 321 bp and *Nosema ceranae*- 218 bp); lane 12 – negative control

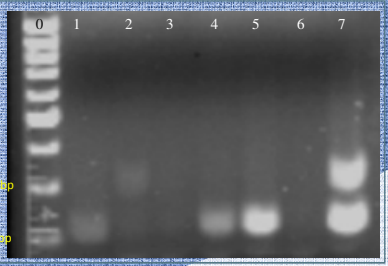


b) lanes: 1, 2 and 3 – samples from 1995; lanes 4 and 5 – samples from 1996; lane 6 – positive control (*Nosema apis*- 321 bp and *Nosema ceranae*-218 bp); lane 7 - negative control



c) lanes: 1, 2 and 3 – samples from 1994; lane 4 – sample from 1995; lane 5 – sample from 1996; lane 6 – positive control (*Nosema apis*- 321 bp and *Nosema ceranae*- 218 bp); lane 7 – negative control.

Fig. 2) Result of the electrophoresis of the amplicons produced by PCR of the samples collected between 1st of December 2007 and 15th of March 2009.



Lane 0 – 100 bp molecular marker; lanes 1, 2, 3, 4 and 5 – samples; lane 6 – negative control; lane 7– positive control (*Nosema apis* – 321 bp and *Nosema ceranae* – 218 bp).

Fig. 3) Cities from which the samples were collected (from 1994 to 1996) and examined by PCR for *Nosema ceranae*. Stars indicate the results obtained during electrophoresis of PCR amplicons. Numbers in brackets indicate the year when samples were collected.

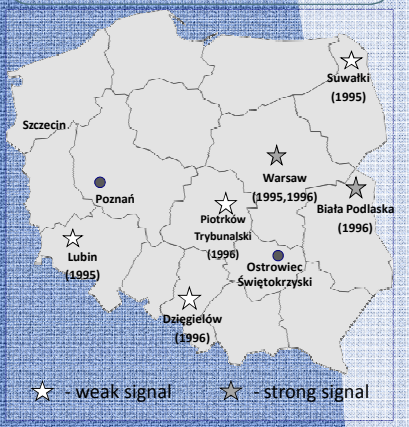


Table 1. *Nosema ceranae* detection in samples from 2008 and 2009

Year	Number of samples		
	Investigated	*Nosema* positive	*N. ceranae* positive (% of *Nosema* positive)
2008	261	170	137 (80.6)
2009	215	166	155 (93.4)

Conclusions

1. *Nosema ceranae* was already present in Polish apiaries in 1995 and 1996.
2. We were not able to show the presence of *Nosema ceranae* in Poland in 1994, however the very weak signal obtained during electrophoresis of PCR products suggests that that *Nosema ceranae* might have been present in Poland before 1995.
3. The examination of recent samples showed that *Nosema ceranae* is present in most colonies with *Nosema* infection in Poland

References

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