

Nosema Diagnosis in Romanian Bee Colonies

Gabriela Chioveanu*, Raluca Cioranu*, Handan Coste*

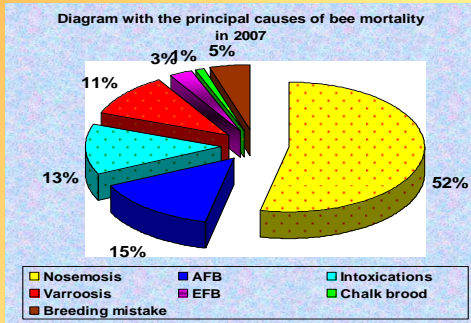
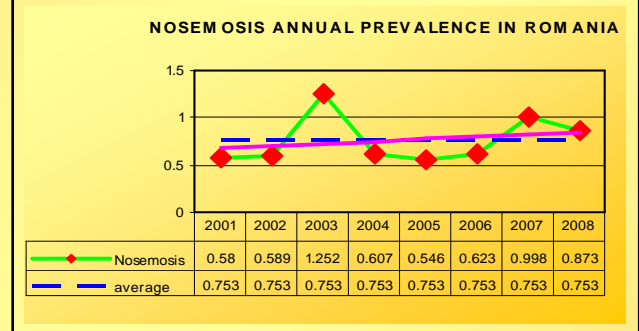
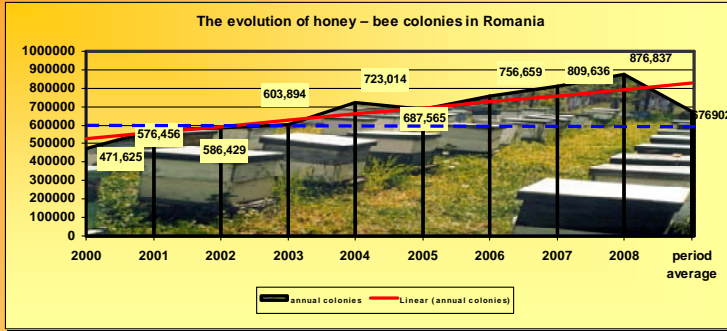
*Institute for Diagnosis and Animal Health, Bucharest, Romania
gabriela.chioveanu@idah.org



INTRODUCTION

Nosema disease is common and wide spread in honey bees *Apis mellifera*. Recently it was detected both *Nosema apis* and *Nosema ceranae* species in many bee colonies, from different European countries.

Romania is one of the most important honey producers from Europe and it has many melliferous zones of acacia, lime and sun flower plantations, all over the country. In 2008, there were registered 876,837 bee colonies of *Apis mellifera carpatica* species. The surveillance of health and the prevention of diseases at bee colonies represent a permanent concern of the veterinary services in Romania. During last two years, an increase infection by microsporidian parasites in honey bee has been detected in different regions from Romania, while apparent healthy and strong colonies become weak and finally died.



MATERIALS AND METHODS

131 bee samples were collected from Romanian apiaries that displayed severe losses and mortality during March 2007 up to September 2008.

There were analyzed for the identification of *Nosema spp.* spores and for establish the level of infection using microscopy qualitative and quantitative methods (OIE) and conventional PCR reaction followed by with RFLP technique (after Klee adapted in I.D.A.H.).

Infection level	No. spores	No. samples
0	No spores	71
1	10 000 – 490 000	10
2	500 000 – 1 000 000	34
3	1 000 000 – 2 400 000	12
4	>2 500 000	4
Total		131

RESULTS AND DISCUSSIONS

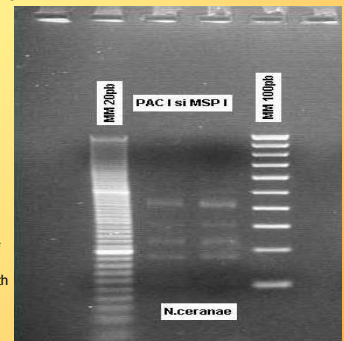
From a total of 131 bee samples analyzed, a number of 60 were found positive at microscopy test, but from these only 38 were found positive in PCR tests. In 93 samples, the *Nosema* spores have not been detected by molecular biology tests, and by microscopy methods in 71 samples.



Figure 1 (Cioranu): Ethidium bromide stained agarose gel showing PCR products for SSU rRNA. Lanes 1 - 4, positive samples; lanes 5-6, negative samples; Lanes 7-8, positive samples; lanes 9-12, negative samples; lane 13 – positive control; Lane 14 – negative template control.

In figure no 1, the bands specific for *Nosema spp.* can be visualized at approximately 400bp. We have obtained bands for 63% samples found with clinical signs and positive at microscopic examination. The positive samples were characterized by RFLP. The electrophoresis profile of the RFLP can be visualized in figure no 2.

Fig 2 (Cioranu): Ethidium Bromide stained agarose gel showing examples of RFLP analysis of SSU rRNA of *N. ceranae* digested with the enzymes *Msp I*, *Pac I*, *Nde I*. The samples were flanked with 20 bp ladder. Fig 2: Ethidium Bromide stained agarose gel showing examples of RFLP analysis of SSU rRNA of *N. ceranae* digested with the enzymes *Msp I*, *Pac I*, *Nde I*. The samples were flanked with 20 bp ladder.



The RFLP pattern showed only *N. ceranae* in all positive samples analyzed. To confirm the presence of *N. ceranae* in samples, molecular biology laboratory select 3 samples for direct sequencing on 3130 instrument. The sequences obtained were blasted in Genbank certifying that the amplicons from RFLP were of *N. ceranae*. Using Mega Software, Neighbor Joining algorithm method, a phylogenetic tree was constructed with bootstrap value of 5000 replicates (figure 3).

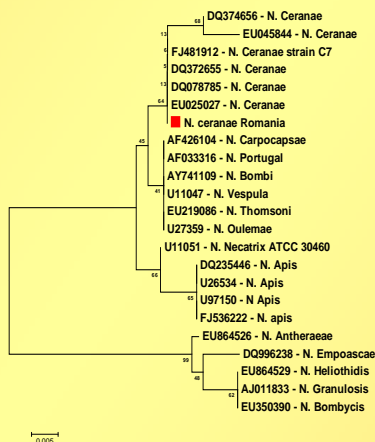


Fig.3 (Cioranu and Coste) Phylogenetic tree – presence of *N. ceranae* in Romania (red) and other *Nosema* species

CONCLUSIONS

>In 2007-2008 the number of *Nosema* outbreaks increased, compared with 2004-2006.

>In 2008, the presence of *N. ceranae* was confirmed for the first time in Romania using molecular biology techniques in the samples that first were analyzed by microscopy methods.

>The molecular biology techniques provide a precise tool for an exact identification of the species and furthermore, characterization of *Nosema* isolates.

>The researches will continue for establish if in Romania exists only *N. ceranae* species in all 41 districts and for optimize molecular tests.



Zones where were identified apiaries infected with *N. ceranae*