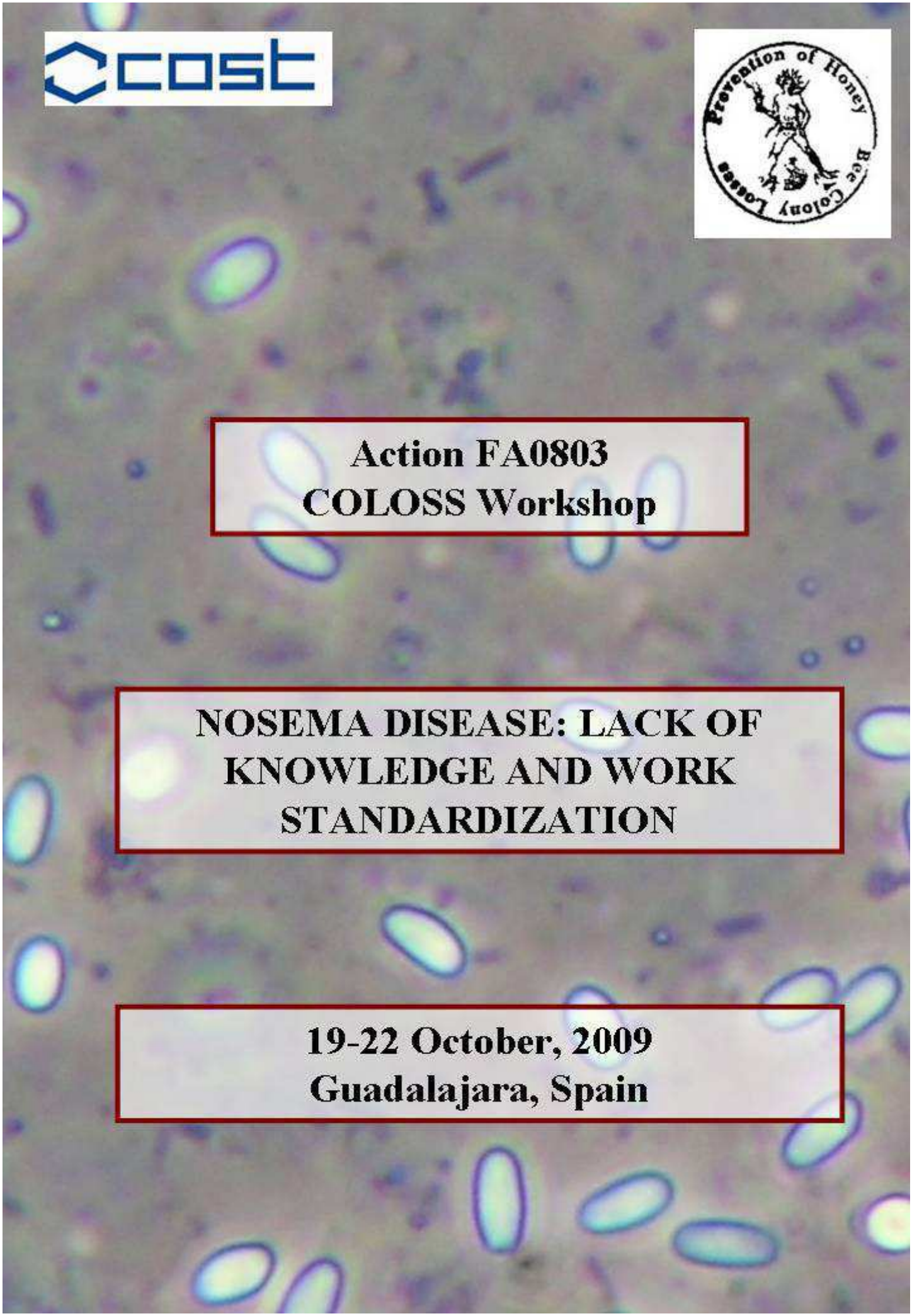

Action FA0803
COLOSS Workshop

NOSEMA DISEASE: LACK OF KNOWLEDGE AND WORK STANDARDIZATION

19-22 October, 2009
Guadalajara, Spain

NOSEMA DISEASE: LACK OF KNOWLEDGE AND WORK STANDARDIZATION

Scope of Workshop:

Up to the moment, two different microsporidia species have been shown to infect *Apis mellifera*: *Nosema apis* and *Nosema ceranae*. While the disease due to *N. apis* has been widely studied, those due to *N. ceranae* only has been studied in the last four years. Due to the recent description of this last agent, there are several question about the epidemiology, pathology, diagnostic and control that have to bee answered. The workshop **"Nosema disease: lack of knowledge and work standardization"** has been designed to put all the knowledge together and to standardize some specific aspects for researching. The workshop goal will be to answer the most of questions that the researchers have nowadays or to determine how that questions could be studied to get answered. With this objective, all the COLOSS member will be asked for questions that feel have to be answered in an open call (even in the case they are not interested to join to the workshop). Questions will be grouped by topic and speakers will describe what it is know and unknown in every topic and a discussion will be made afterwards.

Conclusions of Workshop:

1. The disease caused by *Nosema ceranae* is not similar to nosemosis by *Nosema apis*.
2. A proposal to differentiate nosemosis by *Nosema apis* as "nosemosis type A" and nosemosis by *Nosema ceranae* as "nosemosis type C".
3. Koch´s postulates have been confirmed in nosemosis by *Nosema ceranae.*
4. There is a relationship between *Nosema ceranae* and bee losses in some cases.
5. Standard protocol for experimental infection will be discussed by a specialist panel that has been formed during the Workshop: Marie Pierre Chauzat (Head), Violeta Santrac, Zachary Huang, Asli Özkirim, Raquel Martín-Hernández, Ulrike Hartmann, Claudia Dussabaut, Frederic Delbac, Antonio Nanetti.
6. There is a need of *Nosema apis* genome to compare both (*N. apis* and *N. ceranae*) to find differences that can explain observations
7. New genetic markers must be developed for monitoring the disease

8. New polymorphic markers are needed to define an isolate (to differentiate genotypes or strains).
9. *Nosema ceranae* shows different epidemiological patterns in Europe.
10. Management practices and beekeeping material have an important role in transmission.
11. *Nosema ceranae* is considered as an emerging pathogen in honeybee in the 21st century and can be found in other non-apis insects.
12. For *Nosema apis,* spore counts and clinical signs are considered as the best available diagnosis.
13. The development of standard protocol for sampling and diagnosis of nosemosis by *Nosema ceranae* will be discussed by a specialist panel that has been formed during the Workshop: Antonio Nanetti, Zachary Huang, Giovanni Formato, Mariano Higes, Anna Gajda, Marie-Pierre Chauzat, Asli Özkirim and Martin Kamler.
14. Nosemosis by *Nosema ceranae* can be controlled by fumagillin, but EU regulatory restraints are present. Furthermore, differences in fumagillin stability according to preparation, administration, conservation conditions may affect residues and efficacy.
15. There are some “alternative” possibilities to limit the development of *Nosema ceranae* with “natural” products (meeting requirements of organic beekeeping). Residue studies are required for consumer safety.
16. Beekeeping management such as renewal of combs or queens may play an important role in controlling nosemosis.

Time	Workshop Program
19.10.2009 (Monday)	
	Arrival and Hotel accommodation in Guadalajara city.
20:00	Social event
20.10.2009 (Tuesday)	
9:00-9:30	Registration
9:30-10:00	Official Welcome and presentations.
10:00-10:30	Plenary talks: Honeybee nosemosis. Disease or syndrome? Dr. Mariano Higes, Raquel Martín, Researcher from the Apicultural Center (CAR), JCCM, Spain
10:30-11:00	Coffee break
11:00-12:00	Plenary talks about aetiology and pathology: 1. How does *Nosema apis* make honey bees forage earlier? Dr. Zachary Huang. Associate Professor, Michigan State University, USA. 2. Physiological and behavioural changes in *Nosema* infected bees: A model to understand colony collapse. Dr. Dhruba Naug. Assistant professor Colorado State University, USA. 3. Histopathology of *Nosema* infected bees. Aránzazu Meana. Associate professor of Pathology, Veterinary Faculty, Complutense University of Madrid. Spain.
12:00-13:30	Presentations and Q&A about aetiology and pathology.. - **Preliminary results on cage experimentations of adult honey bees fed with spores of *N. ceranae.*** Chauzat MP. - ***Nosema spp.* infection in Spain: Consequences in colony productivity and vitality.** Cristina Botías. - **Interactive effects between *Nosema* microspores and a neonicotinoid in honeybees.** Dussaubat, C. - **To bee or not to bee: differential mortality induced by *Nosema ceranae*?** Ulrike Hartmann. Open discussion
14:00-15:30	Lunch
15:30-16:30	Plenary talks about Nosema genome and molecular detection. 1. A comparative genomic approach to the study of microsporidian parasite, Nosema. Judy Chen, Research Entomologist. Agricultural Research Services, United States Department of Agriculture, USA. 2. Diversity and recombination of rDNA in the microsporidian Nosema ceranae: how reliable is the genotyping? Nuno Henriques-Gil, Genetics Laboratory, San Pablo-CEU University, Madrid. Spain.
16:30-17:00 17:00-18:00	Coffee break Presentations and Q&A about *Nosema* genome and molecular detection. - **Molecular basis of genome interaction of the honeybee *Apis mellifera* with an evolutionary old and novel introduced Nosema species.** *Matthias Müller*. - **Genetic variation in resistance to *Nosema* infection within honeybee colonies.** Katherine Roberts. - **New tools for epidemiological and pathogenicity studies of *Nosema ceranae*.** Frédéric Delbac. - **Molecular diagnosis of *Nosema* – what's the limit of detection?** S. Erler. Open discussion.
20:30-Open	Social Dinner
21.10.2009 (Wednesday)	
9:00-10:00	Plenary talks about control (epidemiology and diagnostic) 1. Epidemiology of *Nosema ceranae*. Dr. Aránzazu Meana, Associate professor of Animal Health, Veterinary Faculty, Complutense University of Madrid. Spain. 2. Epidemiology of *Nosema ceranae* in the Netherlands. Methodology and Complexities. Romee Van der Zee. Head of Netherlands Centre for Bee Research, NCB. Netherland. 3. *Nosema* Diagnostic. Dr. Raquel Martín, Researcher from the Apicultural Center (CAR), JCCM, Spain
10:00-11:00 11:00-11:30	Pesentations and Q&A about epidemiology and diagnostic. - ***Nosema* situation in the Czech Republic.** Martin Kamler. - **Incidence of *Nosema spp.* and colony performance in Austria 2006–2008**. Derakhshifar, I.. - **Presence of *Nosema apis* and *Nosema ceranae* in Italian apiaries.** Franco Mutinelli. - **First detection of *Nosema ceranae* in *Apis mellifera* from Bosnia and Herzegovina.** Violeta Santrac - **Two Diagnostic Methods of Nosemosis in Turkey.** Aygun Yalçınkaya. - **The size of bee sample for investigation of *Nosema sp*. infection level in honey bee colony.** Anna Gajda. - **Parasite infections of pollinator communities.** Sophie Evison. Open discussion. Coffee break
11:30-12:30	Plenary talks about control (treatment and profilaxis) 1. ApiHerb as an alternative product to treat *Nosema* infection. Dr. Antonio Nanetti. Researcher, CRA- Unità di Ricerca di Apicoltura e Bachicoltura. Bologna. Italy 2. Fumagillin stability. Dr. Jose Luis Bernal, Professor of Analytical Chemistry. Faculty of Chemistry, Valladolid University. Spain 3. Thymol: an alternative treatment for control of *Nosema ceranae* ? Dr. Cecilia Costa. Researcher, CRA- Unità di Ricerca di Apicoltura e Bachicoltura. Bologna. Italy
12:30-13:30	Presentations and Q&A about treatment and profilaxis. - **Medication possibilities against new age-nosemosis.** Békési László, Szalai Enikő. - **Potential of microalgae and plant extracts for the control of nosemosis in honeybees.** Frédéric Delbac. - **Effectiveness in reducing the number of *Nosema* spores of Api Herb and Vita Feed Gold.** Giovanni Formato. - **Epidemyology and Treatment of Nosemosis in Turkey.** Aslı Özkırım. - **Rare *Nosema* infections in Denmark.** Per Kryger Open discussion.
13:30-14:00	Elaboration of conclusions
14:00-15:30	Lunch and end of workshop
20:30-Open	Social Dinner - Optional only for those interested to stay one day more for practical work in Centro Apícola Regional (CAR)
22.10.2009 (Thursday) Optional	
9:00-9:30	Transport Hotel to Centro Apícola Regional (CAR)
9:30-14:00	Practical work in field and laboratory
13:00-14:00	Lunch and end of workshop

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COST Action FA0803 - Prevention of honeybee COlony LOSSes
Nosema disease: lack of knowledge and work standardization
Abstract Submittal Form

Title: Please limit your title to 150 characters.

Two Diagnostic Methods of Nosemosis in Turkey

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

Nosemosis is a common honeybee disease in Turkey. Generally, honeybee gut is used for diagnosis of Nosemosis in honeybees. In some laboratories, honeybee gut is taken out and put on slides. After that it is diluted by saline solution and investigated for occurrence of Nosema spp. spores under the light microscope. By this method, it is not considered the infection level. So, It couldn't be possible to apply proflactic treatment of colonies. Other laboratories not only detect Nosema spp. Spores, but also infection level in Turkey. In our laboratory, all samples from different regions of Turkey are collected in spring and autumun. They are registered and classified according to the local region. At least 30 honeybee samples from each colonies are collected. Honeybees' gut are taken out via forceps and collected in a dish then diluted 1 ml of saline solution per bee. 0.1 ml of the solution are put on heamocytometer and counted Nosema spp. spores under the light microscope. It is calculated the number of Nosema spores per bee. By this method, it is possible to determine infection level of Nosemosis. Because it is also known that infection level is the most important thing for evaluating the distribution of Nosema disease and the kind of treatment of Nosemosis.
In order to distinguish the species of Nosema (apis/cerena), molecular methods have been just applied in one laboratory in Turkey, but there isn't any publication about this research yet.

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Medication possibilities against new age-nosemosis

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

Though nosemosis caused by *Nosema apis* has been a well known bee disease for a long time, questions of the prevention and control are intensively discussed by beekeepers all over the world again. The uncertainty of its epidemiology increases the anxiety induced both by colony collapse disorder in many places, and by the presence of the newly introduced species *N. ceranae*. In our investigations the efficacy of the well known fumagillin antibiotics has been compared with another preparation of different structure (Nonosz®.)

Although the registration of fumagillin has been withdrawn in EU countries a great many beekeepers are using it all over the world.

Since the utilization of antibiotics has more and more limitation in food stuff producing animals, finding an appropriate (non-antibiotic) replacement is a recurring problem.

Nonosz® is a Hungarian made preparation of curative effect (sodium ortho-hydroxy-carbonic acid and Beta vulgaris cv.- Chemor Kft) that entered into the market on the basis of preliminary positive results against nosemosis.

Both in laboratory induced infections and in experiments on infected honeybee colonies the efficacy of the two products gave similar results in decreasing the spore production.

According to the results of the laboratory tests with *N. ceranae* delayed effect of the Nonosz was detected. Some loss occurred in the first days, but the spore production remained much below the control.

Our experiments seem to be evidence about the efficacy of fumagillin against *N. ceranae* and also demonstrating that alternatives may exist for the medication of new-age nosemosis.

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Fumagillin stability in different media

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

The stability while using a commercial formulation, Fumidil B, which contains Fumagillin dicyclohexilamine, is affected by several factors. The water to dissolve the product has some influence so it must be neutral and of mild hardness. Fresh solutions stored in amber containers and kept in a fridge can hold out for a month. The use of syrup or honey-sugar patty, as a medium to apply the product, favours the stability of the compound. Temperature has a slow effect on the degradation, whereas sunlight or UV exposure reduces drastically, in a few hours, the initial concentration of fumagillin. Laboratory tests suggest that the mixture of honey-powdered sugar is the best option to apply the formulation.

COST Action FA0803 - Prevention of honeybee COlony LOSSes
Nosema disease: lack of knowledge and work standardization
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Title: Please limit your title to 150 characters.

***NOSEMA SPP.* INFECTION IN SPAIN: CONSEQUENCES IN COLONY PRODUCTIVITY AND VITALITY**

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

During the last years, Nosemosis has shown to be an increasing beekeeping problem worldwide. Nowadays, two different *Nosema* species have been identified in *Apis mellifera*: *Nosema apis* and *Nosema ceranae*.
A long asymptomatic incubation period in the *N. ceranae* disease has been described (Higes et al., 2008), involving continuous death of adult bees, non-stop brood rearing by bees and colony loss in winter or early spring despite the presence of sufficient remaining pollen and honey.
Evolution of 50 naturally infected colonies of *Apis mellifera iberiensis* during one year is reported. In September 2007 the 50 colonies were infected by *N. ceranae*, and 26 of them were infected by *N. apis* too (PCR tested). The colonies were divided into 5 groups of 10 colonies each: (C) Control (5 unmanaged colonies); (CS) Control with Syrup (5 colonies supplied with syrup every season); (1T) One treatment in a year (5 colonies provided with Fumagillin in Autumn); (2T) Two treatments in a year (5 colonies treated with Fumagillin in Autumn and Spring); (4T) Four treatments in a year (5 colonies provided with Fumagillin every season).
The group of colonies with 2 treatments in a year was the most productive (5 times more honey than the control group unmanaged) and showed a high level of vitality, considering the amount of brood cells and the population of adult bees as a sign of it. The group with 4 treatments showed similar results.
The control groups, in spite of being asymptomatic, were less productive and less populated. The unmanaged control colonies registered the highest number of dead colonies.

COST Action FA0803 - Prevention of honeybee COlony LOSSes
Nosema disease: lack of knowledge and work standardization
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Preliminary results on cage experimentations of adult honey bees fed with spores of *N. ceranae*

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Infections with *Nosema apis* spores have been largely reported in the literature. A new species of *Nosema* has been recently identified on the honey bees. Caged experiments have shown high and rapid mortality of adult bees when they were fed with spores of *Nosema ceranae*.
Adult honey bees have been caged in order to study the mortality induced by the feeding of syrup supplemented with *Nosema ceranae* spores collected in France.
Various factors have been studied in order to better define the optimum experimental conditions : spores conservation, number of spores in the inoculums, age of honey bees, quality of food given to caged insects, temperature.
Results will be given en multiplication on spores and mortality of honey bees.
Experimental conditions will be discussed.

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A comparative genomic approach to the study of microsporidian parasite, Nosema

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

Nosema ceranae, originally considered a microsporidian parasite of Eastern honey bees, *Apis cerana*, is, along with the long known resident species, *N. apis*, a disease agent in European honey bees, *A. mellifera.* In order to gain more insight into the epidemiology, pathogenicity, and evolution of Nosema parasitism in bees, we have used a genomic approach to determine 1) the historical occurrence of two *Nosema* species in both honey bee hosts, 2) the tissue tropism, secondary structures of rRNA, and phylogenesis of two *Nosema* species, 3) the complete sequences of the *N. ceranae* genome and nearly completed sequences of *N. apis* genome. Our results showed that both *Nosema* species produced single and mixed infections in European and Asian honey bees and that *N. ceranae* is much more invasive in both host species than *N. apis.* While ultrastructural features showed that both species possess all of the characteristics of the genus *Nosema*, the tissue tropism was species specific which may lead to the difference of two *Nosema* species in host pathogenicity. The 454 pyrosequence of *N. ceranae* lead to a draft assembly (7.86 MB) and annotated genome, showing that the genome was highly AT-biased. Of 2,614 predicted protein-coding sequences, the genes conserved among microsporidia lack clear homology outside this group. Future comparisons of the genes conserved among microsporidia in two Nosema species will provide valuable insights for identifying virulence factors of parasites and in turn should translate into new strategies for diminishing the effects of parasites and improving honey bee health.

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Title: Please limit your title to 150 characters.

Interactive effects between *Nosema* microspores and a neonicotinoid in honeybees

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

In the past years honeybee decline has been reported almost worldwide. Many single factors like parasites and pesticides have been identified as a potential cause; however, a combination of these agents is more likely to contribute to honeybee losses. Consequently, we tested the hypotheses describing honeybee losses as a multifactorial syndrome by investigating the interactive effects of *Nosema* microspores and an insecticide neonicotinoid on honeybee health in laboratory and field conditions. In order to measure mortality and energetic demands during the first 10 days of honeybee life, honeybees were artificially infected with 200,000 spores/bee of *Nosema,* and held in laboratory conditions. Then, they were exposed chronically to the neonicotinoid at three different concentrations encountered in nature. In parallel, immune parameters were studied in bees infected with the same *Nosema* dose in combination with one dose of the neonicotinoid. Furthermore, a similar experiment was conducted in the field over 30 days. Mortality and foraging behavior were recorded. In addition, spore transfer between infected and non-infected honeybees within hive was studied. Concerning the laboratory experiment, the results of honeybee mortality and energetic demands will be shown.

COST Action FA0803 - Prevention of honeybee COlony LOSSes
Nosema disease: lack of knowledge and work standardization
Abstract Submittal Form

Title: Please limit your title to 150 characters.

Thymol: an alternative treatment for control of *Nosema ceranae* ?

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

In a first series of trials the natural compounds thymol, resveratrol, vetiver essential oil and lysozyme were assessed for potential use in control of *Nosema ceranae* infection of honey bees. None of the substances showed an increased bee mortality or decreased dietary preference. Adult worker bees from a nosema-free apiary were individually infected with 1 µl of sucrose syrup containing 18000 *N. ceranae* spores, placed in cages and fed with candies cpntaining the screened substances. Infection levels were monitored over 25 days, by removal and dissection of 2 live bees per cage. On day 25 post-infection bees fed with candies containing thymol and resveratrol had significantly lower infection rates, and bees supplied with resveratrol candy also lived significantly longer.
In a second set of trials the two most promising active ingredients, thymol (100 ppm) and resveratrol (10 ppm), were supplied to artificially infected bees in candy or in syrup, with the same procedure used in the first trials. On day 25 post-infection bees fed with thymol syrup had significantly lower levels of infection compared to control bees. Bees fed with thymol and resveratrol syrup lived significantly longer than bees fed with control syrup.
Following the promising results, field tests were performed by feeding naturally nosema infected hives with syrup containing thymol during two consecutive springs. In the first year no significant differences in infection levels were observed, while in the second year the hives treated with thymol had a significantly greater decrease in infection compared to the control group.

COST Action FA0803 - Prevention of honeybee COlony LOSSes
Nosema disease: lack of knowledge and work standardization
Abstract Submittal Form

Title: Please limit your title to 150 characters.

Potential of microalgae and plant extracts for the control of nosemosis in honeybees

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

Nosemosis is one of the most frequently observed parasitic pathologies affecting adult honeybees. This disease is due to Microsporidia, some obligate intracellular fungi-related parasites. *Nosema apis* was the historically described microsporidian species in *Apis mellifera*. This species can invade and proliferate in the ventriculus and midgut epithelial cells causing digestive disorders including diarrhea. More recently another species named *Nosema ceranae* has been also shown to colonize honeybees and seems to be now the predominant microsporidian infection in *A. mellifera*. The treatment of *Nosema* diseases was long-time done using Fumidil-B, a water soluble form of fumagillin. However, as this antibiotic is no longer licensed in the majority of EU member states, new molecules need to be identified. The aim of our study is to screen food additives like microalgae and plant extracts to control the development and spread of *Nosema* parasites and thus prevent this infectious disease. As no *in vitro* culture was available for both *N. apis* and *N. ceranae*, the efficacy of the natural extracts has been first investigated by ELISA using human cells infected by the species *Encephalitozoon cuniculi* as microsporidian model. Antiparasitic activity of more than 300 microalgae and 2200 plant extracts (collaboration with UMR CNRS-Pierre Fabre, Toulouse, France) has been evaluated. Currently, 20 extracts showed a 80% inhibition of parasite growth using this *in vitro* drug screening assay. The next step will consist in the validation of these *in vitro* effective extracts in experimentally infected caged-bees.

COST Action FA0803 - Prevention of honeybee COlony LOSSes
Nosema disease: lack of knowledge and work standardization
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New tools for epidemiological and pathogenicity studies of *Nosema ceranae*

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Two microsporidian species were characterized in *Apis mellifera*: *Nosema apis* and the more recently described *Nosema ceranae*. Spores of both species can be obtained from naturally and/or experimentally infected bees. Our main objectives are to study the epidemiology of both species and to better understand the host-parasite interactions. Thus, we are developing some culture models for the *in vitro* proliferation of these microsporidian species. Preliminary results indicate that human fibroblasts could be useful for propagation of *N. ceranae*.

Another project concerns the identification of new markers as tools for a more precise molecular epidemiology. We recently selected some genes coding for potential polar tube (PTPs) and spore wall (SWPs) proteins in the genome database of *N. ceranae*. Such components have been previously demonstrated to be interesting markers for mammal microsporidiosis diagnostic. Analysis of the genetic diversity of these markers in *N. apis* and in different *N. apis* and *N. ceranae* strains will be then undertaken. In order to raise specific antibodies we also recently produced some *N. ceranae* PTPs and SWPs in *Escherichia coli* and injected these recombinant proteins in mice. Some comparative genomic and proteomic approaches will help us to characterize microsporidia-specific protein coding genes that could be related to pathogenicity and to elucidate some metabolic pathways that could be potential targets for anti-*Nosema* drugs.

COST Action FA0803 - Prevention of honeybee COlony LOSSes
Nosema disease: lack of knowledge and work standardization
Abstract Submittal Form

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Incidence of *Nosema spp.* and colony performance in Austria 2006–2008

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

During the years 2006 to 2008 465 dead bee samples from dead or weakened colonies or from colonies with noticeable bee mortality or colonies with suspected poisoning were investigated for Nosema infection. By light microscopy 85 samples (=18 %) were rated as Nosema spore positive in total. Bees from the group with suspected poisoning had the highest Nosema spp. incidence (37 %), followed by samples collected from spring to autumn (18 %) and during the winter period (16 %), respectively.
Nosema incidence (%) in bee samples from (A) winter period and from (B) spring to autumn was considerably higher in dead than in living colonies (A: dead colonies: 21 %; living colonies: 13 %; B: dead colonies: 22 %; living colonies: 14 %, respectively). These results indicate that an infestation by Nosema spp. increases colony mortality during the winter period and the active season.
The contrary was the case in bee samples from colonies with suspected poisoning (dead colonies: 29 %; living colonies: 38 %). This could mean that the higher losses of colonies were partly caused by other factors than Nosema.

For species differentiation 126 samples from the total pool were analyzed subsequently by PCR. 67 of these samples (53 %) were tested negative for Nosema and 59 samples positive (47 %). *N. ceranae* was confirmed in 30 % of the positive samples and *N. apis* in 10 %. 6 % of samples were infested by both Nosema species.
Both *Nosema*-species were present in all federal provinces of Austria, except in Vorarlberg (adjacent to Switzerland), where only *N. ceranae* was detected.

COST Action FA0803 - Prevention of honeybee COlony LOSSes
Nosema disease: lack of knowledge and work standardization
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Title: Please limit your title to 150 characters.

Molecular diagnosis of *Nosema* – what's the limit of detection?

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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During last years different methods for the diagnosis of *Nosema* disease in bees have been developed. Molecular tools might have a strong advantage compared to microscopic methods – higher sensitivity. However, currently very little is known about the limit of detection of different molecular methods. The limits of these methods strongly depend on sample preparation and assay development. The evaluation of detection limits might influence the outcome of molecular diagnostics, especially as the rate of false negatives might be influenced.
Here we present a comparative study on *Nosema* detection methods in different bees (honey bees and bumble bees) focussing on the detection limits. Different published and unpublished primers for PCR based screening methods were analysed for their sensitivity in molecular assays utilizing three different *Nosema* species (*N. apis, N. ceranae* and *N. bombi*).
We found high differences in detection levels for the different *Nosema* diagnosis methods depending on the *Nosema* species and the primers based on the similar spore loads and dilution series.

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Title: Please limit your title to 150 characters.

Parasite infections of pollinator communities

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

The pollinator community in the UK has been steadily in decline due to a variety of reasons including monoculture, climate change, pesticides and diseases. Several honey bee diseases have been well studied, although interspecific transmission between pollinators has received little attention. In order to assess the occurrence and spill over of cryptic parasites in pollinators, we screened pollinators using PCR that were collected foraging in and around the Leeds and North Yorkshire area. We screened bumblebees, honeybees, wasps, hoverflies and solitary bees. We found several common honeybee infections in all pollinators, including Nosema, Wolbachia, and Chalkbrood. We found a Nosema infection rate of approximately 7%, with the majority of infections being N. bombi, found in the bumble bee Bombus pascuorum, but also across all groups screened. These results highlight the need for community level, rather than species level studies when considering issues such as pollinator declines.

COST Action FA0803 - Prevention of honeybee COlony LOSSes
Nosema disease: lack of knowledge and work standardization
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Title: Please limit your title to 150 characters.

EFFECTIVENESS IN REDUCING THE NUMBER OF *NOSEMA* SPORES OF API HERB AND VITA FEED GOLD

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

Api Herb and Vita Feed Gold are liquid feeds that are on sale in EU for the prevention and control of Nosemosis. While Api Herb is based on vegetable essences and vitamins, Vita Feed gold is based on natural beet extract and molasses.
In this work we report the results of the effectiveness of the two mentioned products in reducing the number of spores of *Nosema*, after six weeks of treatments.
Both Api Herb and Vita Feed Gold resulted able to control the *Nosema* infection, as it proven by a statistically significant difference between the untreated group and the two treated groups (Bonferroni post-hoc test for multiple comparisons).
For the first three weeks of treatments, Api Herb and Vita Feed Gold showed a similar ability to control the Nosema infection, but from the 3rd week ahead, the Vita Feed Gold treatment seemed to better control the number of spores of Nosema.

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Title: Please limit your title to 150 characters.

The size of bee sample for investigation of *Nosema sp*. infection level in honey bee colony.

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

OIE recommends two methods for microscopic diagnosis of *Nosema* infection: non-quantitative and standardised. In the first method 60 forager bees should be investigated, in the second - 10 bees. Higes with co-authors pointed out that the number of *N. ceranae* spores per bee does not reflect the level of infection because midgut epithelial cells contain many more developmental stages of these spores than is the case in *N. apis* infection; therefore real-time PCR or counting infected bees should be performed. For this purpose 30 forager bees should be examined. However, in Poland collecting 30 foragers at some times of the season is often very difficult or almost impossible, e.g. between the middle of July and the middle of August, or in early spring if the colony is weak. In such cases alternative sampling should be proposed.
A three stage investigation of samples (composed of 30 forager bees) performed by us showed, that if only 20 bees were investigated 7 out of 44 positive samples could be found as negative. In 17 out of the 37 remaining samples the percentage of infected bees was at least twice as low in one batch of ten bees as in the other batch. Our conclusion is that also in Poland 30 foragers should be investigated.
Examination of samples composed of 20 foragers and samples of 2 x 20 interior bees (from the last comb) showed that although the number of spores per bee was much higher in the samples of foragers, the percentage of infected bees was almost the same in both kinds of samples if we chose the more infected batch of twenty interior bees for comparison.

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Title: Please limit your title to 150 characters.

To bee or not to bee: differential mortality induced by *Nosema ceranae*?

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

The endoparasitic microsporidian *Nosema ceranae* is a major suspect for the recent honeybee colony losses but there may be differences because both host and parasite are genetically diverse. Therefore, we here studied mortality of freshly emerged honeybee workers individually infested with Italian *N. ceranae* (10^5 spores per bee) using standard hoarding cage experiments over a period of 15 days. We compared our data with the literature on Chinese *N. ceranae* (Paxton et al. 2007). Our data revealed neither significant differences between the controls nor between *N. apis* (Paxton et al. 2007) and *N. ceranae* (this study). However, we found significantly lower mortality induced by *N. ceranae* compared to Paxton et al. (2007). Our results therefore suggest that there may be differences in host susceptibility and/or *N. ceranae* virulence, which should be tested using standardized COLOSS ring tests.

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Nosema disease: lack of knowledge and work standardization
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Title: Please limit your title to 150 characters.

Mechanisms through which Nosema apis affects onset of foraging

in worker honeybees *(Apis mellifera* L.)

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

It is known that Nosema apis accelerates the rate of behavioural development in honeybee workers, but the underlying mechanisms are unknown. We first confirmed that juvenile hormone titers were higher in Nosema infected bees that were of preforaging age. The higher JH titers can be achieved by several alternative mechanisms: enhanced JH production by host corpora allata (CA), reduced JH degradation, or JH production by *Nosema* directly. Three experiments were conducted to further study the mode of action of *Nosema*. *Nosema* infected workers had higher rates of JH biosynthesis than control bees in 4 of 4 colonies when workers were 6-8 days old. Rates of *in vivo* JH biosynthesis were also higher in *Nosema* infected bees than control bees. Allatectomized workers fed *Nosema* had no detectable levels of juvenile hormone in hemolymph. The majority of these workers (two out of three colonies) did not show earlier foraging compared to the control group (allatectomized bees with no *Nosema*). Finally, nosema-infected workers also showed higher JH degradation compared to control bees. These results suggest that *Nosema*-infected workers forage at an earlier age than control bees due to higher JH titers, which come about through increased JH production, and despite of the increased JH degradation in infected bees. Our results also suggest that *Nosema apis* does not produce JH directly.

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Nosema disease: lack of knowledge and work standardization
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***Nosema* situation in the Czech republic**

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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The status of nosemosis in honeybee colonies in the Czech Republic is traditionally demanded by State Veterinary Administration and investigated in Bee Research Institute at Dol (BRI) for many years. The *Nosema, Acarapis, Varroa and Paenibacillus* investigation is obligatory for all commercial queen breeders.
Method of examination is conducted microscopically, according to OIE recommended diagnostic techniques (version 2008) adapted by BRI. Positive result is finding of $>10^6$ *Nosema* spores in disintegrated sample of 20 bees. This finding corresponds to 5 % ill bees.
We summarized results for nosemosis monitoring from the period 2000 – 2009. In this period the accredited laboratory of BRI received samples from 76 to 114 queen breeders every year. Average number of samples was 3945 per year (minimum 2771 and maximum 6142). Microscopically, the mean prevalence of *Nosema* sp. spores was 29 % (24 % - 44 %) in individual year. Because of worldwide occurrence of microsporidian pathogens *Nosema ceranae* together with *N. apis*, we analyzed 92 *Nosema* positive samples specifically by molecular probes. DNA of *N. ceranae* was confirmed in 7 samples (6 %). Occurrence of *N. ceranae* in honeybee colonies in the Czech Republic was confirmed for the first time. However, the role of *N. ceranae* in colony collapse disorder in our apiaries is not known.

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NOSEMA DIAGNOSTIC

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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In order to make a good diagnostic, it is very important to do a good selection of sample to analyze, a good establishment of parameters to determine disease or not and finally use good detection methods.

For *Nosema apis*, the mean spore count per bee has been used to determine the extent of infection in a colony (Furgala & Hyser, 1969; Cornejo and Rossi, 1975) and this parameter has been even used to evaluate the need to apply treatment in infected colonies and apiaries (Sota and Bacci, 2004). However, some authors established that there is not a close relationship between this measure and the colony health status (Doull, 1965, El Shemy & Pickard, 1989).

Due to the scarce information for *N. ceranae*, different trials were made on natural infected colonies. These works demonstrate that the election-sample should be exterior-bees collected at noon at the hive entrance. On the other hand, spore count is not related with parasitic burden and health status of colonies as same authors described previously for *N. apis* (Doull, 1965; El Shemy and Pickard, 1989). Therefore, the best parameter to determine the health status of a colony infected with *N. ceranae* is the percentage of infected bees. The detection of infection has been shown with a high influence of the size sample that is get from the colony.

Finally, the use of triplex PCR technique to detect *N. apis* and *N. ceranae* infection in honeybees is improved by using COI (*A. mellifera)* as a target to control the DNA extraction.

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NOSEMA DIAGNOSTIC

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Histopathology of Nosema disease.

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

Data presented have been originated in several trials based either in experimental infections (nurse honey bee with spores or queens with infected nurses), or in natural infection in CAR apiaries and professional apiaries (interior and exterior bees).
Once refreshed the histology of digestive epithelium of honey bee, description of infected cells will be explained based on endogenous biological cycle of *Nosema* spp. divided in phases.
Phase I is the extracellular stage of *Nosema ceranae* includes mature spores. Under appropriate conditions, the spore is activated in bee gut, its polar filament everts and pierces a bee intestinal cell, injecting the sporoplasm into it. **Phase II** is the first phase of intracellular development. *Nosema ceranae* maintains direct contact between plasmalemma and host cell cytoplasm lacking any type of interfacial envelope. Once injected, the sporoplasm forms an elongated multinucleate cell that divides by multiple fission. **Phase III** or the sporogonic phase. Material secreted by the parasite forms an electro-dense addition to the plasmalemma. Then cell undergoes one nuclear division process by binary fission producing two sporoblast cells that mature to produce spores
Conclusions: All the life cycle stages of *Nosema* are diplokariotics. All parasitic stages are in direct contact with the host cell cytoplasm. Emptied spores probably indicate intracellular germination of spores and a quick spreading through epithelial layer. Regenerative cells are not activated, alterations are irreversible and a death cause.
Some of the images to be seen:
Experimental infection day 3 p.i. A few epithelial cells at the tip of the folds presenting intracellular parasite stages, mature spores present.
Experimental infection day 6 p.i. Heavily infected tissue with tightly packed parasites. Basophilic mature spores in apical region of epithelial cells.
Large heterogeneously stained parasitic structures at the bottom of the folds.Parasitic epithelial cells and perithrophic membranes fragments in the gut lumen. Cell nuclei are displaced to an apical position. Empty spores clearly indicate horizontal transmission. Cellular membrane appears disrupted. Regeneration crypts are not activated.

COST Action FA0803 - Prevention of honeybee COlony LOSSes
Nosema disease: lack of knowledge and work standardization
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Title: Please limit your title to 150 characters.

Epidemiology of *Nosema* disease.

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

Nosemosis is a common worldwide disease of adult honey bees (*Apis mellifera*) that is caused by microsporidia. *Nosema apis* infecting *Apis mellifera* was described more than one hundred years ago and is one of the first microsporidia to be described. Recently a second microsporidian, *Nosema ceranae*, infecting the same host has been reported in Europe and Asia, being considered nowadays also a pandemia. The relative risk obtained in colonies with either both species or only *N. ceranae,* for suffering bee depopulation is almost six times greater than those with negative PCR results. Epidemiological patterns in Spain for *N. ceranae* include a long asymptomatic phase previous to cold month's depopulation and colony death. Different factors relate with disease such as type of host, temperature influence on parasite biotics and biological effects, or immune deflection of *N. ceranae* infected honeybees compared with N. *apis* will be presented.

While the disease caused by *N.apis* is accepted to be transmitted among bees via ingestion being the reservoirs live infected bees that contaminated the combs and deposits of viable spores on or in wax, honey or any hive surface, the same is suspected for *N. ceranae*. The rapid, long distance dispersal of *N. ceranae* has been attributed to the transport of infected honey bees by commercial or hobbyist bee keepers but there may be other alternatives. Hive structures can transmit the infection. The transmission by means of frames and other materials from the continent to an island of the Netherlands has been confirmed. The presence of viable spores in corbicular pollen can be considered a high spreading factor. The demonstrated viability of spores inside the regurgitated pellets of bee-eaters indicates that they can act as fomites of infective spores. The flying behaviour of bee-eaters can spread them all over long distances. Mortality and morbility rates are discussed while considering a dead colony the absence of a queen and the need to establish new parameters to identify nosemosis by *N. ceranae* due to absence of clinical signs related with *N. apis*. Percentage of forager's infection is more reliable to show a relationship with the impact of infection than mean spore count in a colony infected by *N. ceranae*.

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Title: Please limit your title to 150 characters.
Molecular basis of genome interaction of the honeybee *Apis mellifera* with an evolutionary old and novel introduced Nosema species
Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.
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Text of Abstract: Please limit abstract text to 250 words.
The honeybee, *Apis mellifera*, and two Microsporidian parasites, *Nosema apis* and *N. ceranae* will be used to study coevolutionary mechanisms of host resistance and parasite virulence. *N. apis* is an evolutionary old and well adapted parasite of the honeybee whereas *N. ceranae* is a novel, highly virulent, and potentially mal-adapted parasite of *A. mellifera* that recently swapped hosts from *A. cerana*. We will use the sequenced genome of *A. mellifera* and genomic tools to take advantage of the haploid drones as simple genetic test organisms in evolutionary genetics studies. We will identify major quantitative trait loci responsible for resistance to *N. ceranae*, *N. apis* and mixed infections testing drones, which are offspring of hybrid queens of susceptible and more resistant lineages. We will compare the transcriptome of drones and workers to asses the impact of heterozygosity on parasite resistance, and test for the selection potential of virulence and its trade off with transmission ability in *Nosema*. Finally, we will identify between parasite selection within the honeybee host.

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Nosema disease: lack of knowledge and work standardization
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Title: Please limit your title to 150 characters.

Presence of *Nosema apis* and *Nosema ceranae* in Italian apiaries

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

The present study describes the results of a preliminary screening to assess the presence of *Nosema apis*/*Nosema ceranae* in Italian apiaries.
238 samples of adult honey bees from 19 of the 20 Italian regions (Valle d'Aosta was not included) were submitted to the Istituto Zooprofilattico Sperimentale delle Venezie for diagnosis of *N. apis*/*N. ceranae* infection. Honey bee crushings were subjected to light microscope examination (400X) to detect the presence of *Nosema* spp. spores and to DNA extraction by QIAamp DNA mini kit (Qiagen Gmb, Hilden Germany) with a pre-incubation step with lysozime. DNA was amplified by using specific primers for a region of 16S rRNA gene of *Nosema* spp (Higes *et al*, 2006) and the samples were tested in duplicate. For species identification, the PCR products were sequenced and the sequence similarity analysis was performed using BLAST database search.
Of 238 analysed samples, 95 were negative and 143 produced a PCR product of expected size, approximately 240-252 bp. After PCR product sequencing, 136/143 samples were positive for *N. ceranae* and in one sample both *N. apis* and *N. ceranae* were present. The sequence analysis of the other 7 samples identified different species of *Nosema*.
Our results confirmed the preliminary findings of Klee *et al* (2007) on Italian apiaries and demonstrated that *N. ceranae* is a well established parasite of *Apis mellifera* in all 19 investigated Italian regions. Furthermore, in only one apiary in Trentino Alto Adige region (northern Italy) a co-infection *N. apis*/*N. ceranae* was present.

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ApiHerb as an alternative product to treat nosema infection.

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

Nosema apis and *N. ceranae* are causative agents of two different forms of nosema disease. In particular, the latter microorganism is claimed as a major factor of depopulation and death in *Apis mellifera* colonies in regions where beekeeping is performed at economic level.
Fumagillin was once generally available as an effective medication, but regulatory restrictions banned it from several countries where, aside illegal import and use, the control means are restricted to higienic practices, usually of unadequate efficacy.

In this context, the product ApiHerb (Chemicals Laif, Padua, Italy), based on herbal extracts, was developed and tested.

In laboratory, uninfected caged honey bees were fed with the substance as a syrup suspension (*ad libitum* for one day) and artificially infected with *N. ceranae* spores. The individual spore load of the bees was evaluated 10 days post-infection, recording significantly milder infections if compared to untreated controls.

Free flying colonies of a severely hit apiary received three weekly administrations of the product by trickling. The pre - post treatment difference of luminal spores was measured in bee samples, resulting in a significant decrease (-46%) if compared to negative controls. The decrease was not significantly different to the one recorded in positive controls (-60%) receiving fumagillin two times, one week apart.

The experiments show a distinct effect of ApiHerb in limiting the development of *N. ceranae* infection of the honey bees. Due to its herbal composition, the product may suit the restrictions of the organic beekeeping also.

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Title: Please limit your title to 150 characters.

Physiological and behavioural changes in *Nosema* infected bees: A model to understand colony collapse.

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

In spite of the tremendous interest in the recent large honeybee losses attributed to colony collapse disorder, there is still no definitive explanation for the phenomenon. A host of pathogens has been implicated in the process including the newly discovered microsporidian species, *Nosema ceranae*. Results from our experiments show that *N. ceranae* exerts a significant energetic stress on its host. This energetic stress leads to a number of physiological effects in the host such as an increased hunger level and an inability to thermoregulate, which in turn lead to behavioral changes such as a lower inclination to share food, a higher propensity to forage and a preference for inhabiting the central regions of the colony. These changes have a profound effect on the transmission of the disease inside the colony as well as the mortality of bees outside. I argue that energetic stress in the host is a general physiological effect of a number of pathogenic infections and that energetic stress exacerbated by habitat loss has played an important role in the recent colony collapses.

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Title: Please limit your title to 150 characters.

Diversity and recombination of rDNA in the microsporidian *Nosema ceranae*: how reliable is the genotyping?

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

The rDNA sequences are usually one of the first options for genetic markers in a wide range of materials. However, in *Nosema ceranae* overlapping sequences are systematically obtained, precluding clear genotyping of different isolates. The results suggest that the repeats of the rDNA are not identical within a given strain. We cloned rDNA fragments from *N. ceranae* of different geographical origins in bacterial plasmids. A high diversity was obtained among the 105 sequences analysed, that could be grouped into 79 haplotypes. While a same haplotype can appear from different isolates (even from samples as far as Guadalajara and Kyrgyzstan), two different clones from a same isolate may be deeply different. Therefore epidemiological or phylogenetic relationships among isolates of *N. ceranae*, based on repeated sequences such as rDNA, have to be taken with caution. Additionally, the comparison of sequences indicates that recombination is generating new variants

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Epidemyology and Treatment of Nosemosis in Turkey

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

Nosemosis is a common honeybee disease in Turkey. Even though it is very simple to determine the occurrence of the disease in every region of Turkey, infection level is very different in some regions. Turkey has 7 geographical regions have different climatic and physical conditions. Several samples are collected twice from each region of Turkey every year for monitoring Nosema situation in Turkey. Our results show that Nosema spores can be detected in all regions but the main difference is infection level of this disease. When comparing of the graphs of infection level especially with climatic conditions, the maximum number of nosema spores can be found the north of Turkey; Black Sea region. (The situation of Nosemosis in other regions will be explained in the presentation)

For treatment, Fumagilline shouldn't be used legally but most of the beekeepers still use this compound by informal way. Depend on infection level, they prefer to apply cultural methods to prevent spreading of the disease or to protect their colonies. On the other hand, our reseachs are concentrated on botanical compounds for treatment of Nosemosis.

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Title: Please limit your title to 150 characters.

Rare Nosema infections in Denmark

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

In Denmark beekeepers have been trying to produce Nosema resistant bees for the past nearly 20 years. Today we have far less problems with Nosema, compared to when we started. In general beekeepers think the program has been a success. There might be other explanations for the decrease in Nosema cases. Using more hygienic methods when cleaning hives the beekeepers, having isolated and ventilated hives, and working mainly from stock that is free of Nosema when splitting colonies all contributes to reduce Nosema in apiaries. Still, the queen breeders of Denmark would be very keen to have their bees tested, both for Nosema apis and Nosema ceranae. They presently rely on the counting Nosema spores from breeder colonies in early spring only. The better way would be to expose bees to Nosema infections in lab, and see if indeed there is a difference.
The Danish work has been undertaken entirely by private breeders. The beekeepers have been rather smart, and already nearly 20 years ago developed a computer assisted Nosema spore counting system, which seems to work very well for them. I will give an overview of the methodology used, and the results achieved. The first record of Nosema ceranae is from 2005, but probably the new Nosema arrived well before that in Denmark given the place of the first discovery.

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Title: Please limit your title to 150 characters.

Genetic variation in resistance to *Nosema* infection within honeybee colonies

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

In the UK, current nosemosis infection is due to both *Nosema apis* and *Nosema ceranae.* Originally *N. apis* was the sole causative agent of the disease, however increasingly *N. ceranae* is found to be co-infecting or displacing *N. apis.* Honeybee colonies are genetically diverse due to the multiple mating of queens, leading to genetic variation in the workers making up a colony. This variation may also lead to variation in resistance to parasites, and further complicate the host-parasite interaction of *Nosema* with differing host genotypes. Understanding the host-parasite dynamics of infections is crucial to detangling the pathology and control of both *Nosema* species. In this study we are examining the effect of host genotype and parasite species on nosemosis. We found using quantitative PCR that most natural infections were mixed, meaning production of pure *Nosema* cultures was needed for controlled experimentation. We then used these pure cultures of both *Nosema* species to experimentally infect individual workers of *Nosema* free colonies. These individuals were then checked for infection using both spore counts and quantitative real-time PCR, and later genotyped, in order to look for variation in infection of workers based on their patriline. This study determines if honeybees genotypically vary in resistance to both *Nosema* species, and could feed back into the apicultural industry to help breed more resistant lines of honeybee.

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Title: Please limit your title to 150 characters.

First detection of *Nosema ceranae* in *Apis mellifera* from Bosnia and Herzegovina

Authors and Affiliations: Please list all authors and their affiliations. For the contact author only (typically the senior author), include email address, mailing address, and phone number. Denote contact author with an asterisk = *.

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Text of Abstract: Please limit abstract text to 250 words.

Many neighbouring countries have reported high prevalence of *Microsporidia* and its different epidemiological patterns.

The aim of this work was to gain knowledge about the presence of *N. ceranae* in Bosnia and Herzegovina's honey bees, its implication on bee pathology, and future needs for the more efficient diagnostic molecular tools.

- Fifteen samples of dead honey bees were analyzed according to the OIE Manual (OIE, 2008) for *Nosema* disease. Levels of spores in naturally infected bees were different and scored as low, middle, and very high spore count.

Samples were collected during February and March of 2008 and 2009 from randomly selected areas of seven epizootiological units in Bosnia and Herzegovina (Prijedor, Zvornik, Gradiska, Doboj, Banja Luka, Modrica, Dubica
Since molecular diagnostic of *Nosema* species does not currently exist in Bosnia and Herzegovina, we asked the Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (Padova, Italy) to perform this diagnostics as part of COLOSS project cooperation.

Results

1. All fifteen samples were positive for *N. ceranae*
2. Neither *N. apis* alone nor *N. apis/N. ceranae* co-infection were detected