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as the fat body were dissected from the infected bees 7 and 10 days post infection and examined by light and electron microscopy. Both *Nosema* species were found to infect epithelial and basal regenerative cells in the ventriculus, whereas ileum and rectum contained spores of the microsporidia but failed to show overt signs of infection. No stages of the parasites or tissue damage were detected in the other organs that were examined, confirming high tropism of both species for ventricular epithelium. Our direct histopathological observations do not support the hypothesis that the two *Nosema* species exhibit tropism for honey bee organs other than the ventriculus.

P031 Efficacy of Polyvar Yellow® for controlling *Varroa destructor* in Spanish honey bee colonies

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Varroa destructor mite is a serious and devastating ectoparasite that affects honeybee colonies worldwide. It is believed that this parasite is one of the drivers in the colony loss phenomenon suffered by beekeeping around the world in recent decades. To prevent the mite causing the death of colonies, it is necessary to systematically apply acaricide treatments. However, the number of acaricides registered as veterinary medicaments for their treatment is limited and their efficacy is not enough to control de mite. Last year, the Spanish regulatory agency approved a new medicine based on the pyrethroid flumethrin to control of varroosis (PolyVar Yellow®-flumethrin 275 mg).
To determine the efficacy of this product in the early autumn, we conducted a clinical trial following the requirements of the "Guidelines on veterinary medicinal products controlling Varroa destructor parasitosis in bees of EMEA". Twenty homogeneous *Apis mellifera iberiensis* colonies were naturally infested with *V. destructor*. They showed normal behaviour and no signs of other infectious diseases at the beginning of the trial. No acaricidal treatment was allowed for at least 6 months prior the experiment (last treatment coumaphos). Ten colonies were treated randomly with PolyVar Yellow® and the other ten received Apivar® (amitraz, 500mg/strip), both treatments lasted 56 days according to the standard procedure. The mean acaricidal efficacy recorded for Apivar® was 97% (range 93.2-99.8%) and 89.3 % (range 80.3-99%) for PolyVar Yellow®. A high throughput genotyping assay based on TaqMan® was conducted to determine whether the cause of the lower efficacy in the treatment with PolyVar Yellow® was correlated with the presence of pyrethroid-resistant mites in the population in some colonies of treated group. The results showed that indeed, the frequency of resistant mites were higher in colonies showing lower efficacy.
Overall, our data evidenced that PolyVar Yellow® can be an effective alternative to manage the parasitism in colonies where there is no resistant mites or where their frequency is very low. The implementation of an IPM strategy based on a strict rotation of different acaricides with other management approaches should be the key for a long-term control of the mite.

P032 BEEHEAL: standardization of laboratory methods for sample processing, nucleic acids extraction and PCR for microsporidia and viruses analysis

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BEEHEAL is a project designed to determine the phenology and interaction of *Nosema ceranae* and viruses in four Mediterranean countries: Spain, France, Portugal and Israel, including some territories where *Varroa destructor* is not present (Azores and Ouessant islands). This will allow us to study and compare the interactions between pathogens in a wide range of hosts, beekeeping and climatic conditions.
The honey bee samples collected along the year in the different countries will be analysed for pathogens in three laboratories. This requires a standardisation of methods to compare the results in order to assess the effect of every variable in a reliable way. To that end, the participating laboratories have been working together to establish the sampling methodology, the conservation of the samples, the nucleic acids extraction and the PCR analysis.
We analysed the nucleic acid extraction using different buffers or commercial kits. The incubation of sample in TE buffer at 95°C for 20 minutes showed a good sensibility level and good value for N. ceranae DNA extraction. The integrity of RNA was also evaluated to guaranty that the same sample (either individual bees or composite samples) can be analysed for Microsporidia and viruses detection.
A joint protocol for sample processing, DNA and RNA extraction and PCR analysis has been developed and adjusted to the particular conditions and equipment of each laboratory. The standardisation of methods to be implemented by each participating laboratory will avoid the biases on conclusions based on the diverse methods applied.
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P033 Infection levels of Nosema ceranae (fries et al., 1996) in honey bee colonies in Poland

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The epidemiology of honey bee transmissible diseases is influenced by several factors related to the host and parasite but also by environmental and beekeeping conditions.
While the epidemiology of the infection by *Nosema ceranae* in *Apis mellifera* is well characterized in Spain (Mediterranean country) and some other warm regions, there are some colder regions of Europe where the studies are limited to some specific moments of the year and the data on infection level expressed by a percentage of infected bees in colonies in those regions are scarce or hardly available.
In this study, the infection of *N. ceranae* was studied in hybrids of *Apis mellifera carnica* and *Apis mellifera ligustica* in Poland where different pathological repercussions of the infection than in Spain are reported. Interior bee samples were collected from 5 colonies located at the apiary in the Warsaw University of Life Sciences from October 2015 until March 2017. The number of bees infected per colony was analysed by PCR to determine the percentage of infection by *N. ceranae* and *Nosema apis*.
The infection by *N. ceranae* was detected in every sampled month, although the lower levels were found in July (2016) when it was on average only 0.8% and the higher in March (2017) when about 83.3% of bees were infected. Conversely, *N. apis* was only detected in August 2016 in a colony. This pattern shows differences with those described in Mediterranean areas where the lowest level of Microsporidia infection is usually found during the spring.