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ABSTRACT BOOK

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OP-122

Frequent parasitism of *Apis mellifera* by trypanosomatids in geographically isolated areas with restricted beekeeping movements

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Trypanosomatids form a group of high prevalence protozoa that parasitise honey bees, with *Lotmaria passim* as the predominant species worldwide. However, the knowledge about the ecology of trypanosomatids in isolated areas is limited. The Portuguese archipelagos of Madeira and Azores provide an interesting setting to investigate these parasites because of their geographic isolation, and because they harbour honey bee populations devoid of two major enemies: *Varroa destructor* and *Nosema ceranae*. Hence, a total of 661 honey bee colonies from Madeira and the Azores were analysed using different molecular techniques, through which we found a high prevalence of trypanosomatids despite the isolation of these islands. *L. passim* was the predominant species and, in most colonies, was the only one found, even on islands free of *V. destructor* and/or *N. ceranae* with severe restrictions on colony movements to prevent the spread of them. However, islands with *V. destructor* had a significantly higher prevalence of *L. passim* and, conversely, islands with *N. ceranae* had a significantly lower prevalence of the trypanosomatid. *Crithidia bombi* was detected in Madeira and on three islands of the Azores, almost always coincident with *L. passim*. By contrast, *Crithidia mellificae* was not detected in any sample. A High-Throughput Sequencing analysis distinguished two main haplotypes of *L. passim*, which accounted for 98% of the total sequence reads. This work suggests that *L. passim* and *C. bombi* are parasites that have been associated with honey bees predating the spread of *V. destructor* and *N. ceranae*.

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OP-123

Effects of a diet containing 7 probiotic strains (Honeybeeotic™) on commercial honey bee colonies in terms of haemolymph cytology and phenoloxidase activity

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Starting from 2005, in Roti Abbey area, Matelica, Marche Region, Italy, was identified an honey bee population, isolated from several decades by other populations of *Apis mellifera ligustica*, in an area far from any human activities, without pollutants, and away from "genetic contamination" by other honey bees' populations. We isolated and characterized 7 strains of probiotic bacteria by quantitative PCR along with deep sequencing of amplicons of the V4 region of the bacterial 16S rRNA gene. Through this analysis we observed some important differences regarding both the size and the composition of the microbiome of Roti's bee workers, compared to the common honeybee (*Apis mellifera ligustica*). To investigate the innate immune response of honeybees to microbiota modification, by integration with these 7 probiotic strains of Roti's bees origin (Honeybeeotic™: *Lactiplantibacillus plantarum* DSM 33923; *Apilactobacillus apinorum* DSM 34547; *Lactiplantibacillus fabifermentas* DSM 34546; *Lactiplantibacillus fabifermentas* DSM 34454; *Lactiplantibacillus plantarum* DSM 34542; *Lactiplantibacillus plantarum* DSM 34500, and *Lactiplantibacillus plantarum* DSM 34499), 4 hives received a beekeeper-formulated diets (A = 50% glucose syrup, B = 50% glucose syrup supplemented with probiotics, C = Apifonda®, and D = organic molasses) for one month. The diet B was integrated with the addition of Honeybeeotic™ 2×10^{11} bacterial blend daily for 30 days. Phenoloxidase activity and haemolymph cellular subtype count were investigated. Phenoloxidase activity was lowest ($P < 0.05$) in group B (5.59 ± 0.61), with higher activity (9.72 ± 1.54) in group C, and the haemocyte population differed within the four observed groups, with the highest number of oenocytoids ($\times 8$ cells [4-15]) and granulocytes ($\times 17$ cells [5-33]) in group C, followed by groups D and A. Although, immune responses following probiotic diet-integration have still not been completely clarified, this investigation demonstrates that, regardless of the type of artificial diet administered, an association between cell immunity and the phenoloxidase activity decrease is observable in bees supplemented with probiotics (Honeybeeotic™) compared to other unintegrated groups of bees. Modulation of the innate immune response of bees through the use of highly specific probiotics could improve feed sustainability and agricultural pollination efficiency by supporting larger, healthier honey bee colonies.

