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International trade and diseases: potential of different bee products for pathogen spread

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Key words
Honey bee pathogens, honey bee products, honey, pollen, wax, international trade, deformed wing virus, *Nosema ceranae*, *Crithidia mellificae*, *Tropilaelaps mercedesae*

Aim of the study
The main objective of this project is to assess the viability of honey bee products, such as honey, pollen and wax, to produce infection in exposed honey bees using a honey bee virus (Deformed wing virus, DWV), and two intestinal parasites (the microsporidia *Nosema ceranae* and the trypanosomatid *Crithidia mellificae*). The project also considered the development of a standardized approach for visual inspection of honeycomb and dry pollen imports for *Tropilaelaps* spp. mites

Material and methods
In vitro assays using hoarding cages were performed as follows: recently emerged adult worker bees from 3 queen-right colonies were pooled together. 30 bees were allocated in each cage. Two groups were evaluated, cages containing spiked products with three different concentrations of fresh-prepared pathogens and non-contaminated products. Bees were fed with 50% (v/v) sugar solution and pollen paste (6:3:1, pollen:sugar:water) for 10 days for DWV and 13 days for the intestinal parasites. Each assay consisted of 5 replications. Bees were kept at 34.5°C and 70% RH. Moreover, in the case of *N. ceranae* similar assays were performed using one month old spores. The pathogens were detected using RT-qPCR or qPCR after RNA and DNA extraction of the exposed bees, respectively. In parallel to the *in vitro* assays, a survey of DWV in commercial honey and pollen products were performed in products acquired from a variety of local Swiss grocery stores. The honeys (N=34) originated from different continents and the pollen (N=5) originated from Spain. Regarding the development of a standardized approach for visual inspection of *Tropilaelaps* spp mites for honeycomb and dry pollen imports, the study was conducted in Chiang Mai, Thailand. The genotypic identity of *Tropilaelaps* mites was confirmed by DNA analysis as *T. mercedesae*. Dark honey combs (6x13 cm) and dry pollen (25 gr) were assigned to one of two experimental groups (N=5 per honeybee product) that consisted of: (1) 20 female *T. mercedesae* were introduced and (spiked group) (2) none of mites were introduced (control group). In order to test the mite inspection protocol and the mite survival periods, all the packages (spiked and control groups) from each product were randomly allocated in an incubator and kept at at 25°C and 70% RH. The inspection of *T. mercedesae* mites in packages of empty honey comb and dry pollen was performed daily on a white paper sheet (30x30 cm) with the light source. The numbers of live and dead mites were recorded.

Results and significance

Regarding DWV, our results showed that all bees which were provided with low DWV concentration (10^5 virus copies/bee), irrespective of the bee product used for appliance, produce infections in the some of the exposed honey bees. In addition, a screening of commercial for DWV resulting in 3 samples of honey and 2 of pollen reaching the threshold of our lower experimental concentration, meaning that they potentially could transmit the virus to exposed honey bees.

Regarding *C. mellificae*, honey was the only product that produces infection in the exposed honey bees regardless of the experimental concentrations (1.5×10^5, 1.5×10^4 and $a.5 \times 10^3$ cells/bee).

Concerning *N. ceranae*, the three products produce infection on the exposed bees regardless of our experimental spore concentrations (1×10^3, 1×10^4 and 1×10^5 spores/bee). However, at the lowest concentration, transmission by pollen produces the highest amount of spores (1.3×10^9) in the exposed bees than honey and wax (2×10^8 and 5×10^7, respectively). In addition, when testing the viability of *N. ceranae* after been kept for one month in the products at room temperature, pollen was the only product that remains highly infectious at the middle and lower experimental concentration. The results show important data about the potential of transmission of common bee pathogen through certain honey bee products. Further research is needed to evaluate the pathogen's viability after long term storage honey bee products.

Regarding the standardized approach for visual inspection of *Tropilaelaps* spp mites Our tested protocol allowed for the detection of *Tropilaelaps* mites in the spiked products using a blind test. Also, all non-spiked products were recorded as mite-free during all the experiment which advocate for the effectivity of the protocol on the detection of mites. In sight of these results, it is a proposed tool for the detection of *Tropilaelap*s mites during the inspection of small amount of honey bee products as honey comb and pollen. It is a rapid and easy protocol, with minimum training requirements, that can be implemented in travel check points.

Publications, posters and presentations

Schittny Dominik (2016) Oral transmission of Deformed wing virus. BSc thesis, University of Bern (Accepted)

Schittny Dominik (2017) Hive Products as Carriers for Horizontal DWV Transmission. Presentation at the AG-Tagung, Celle, Germany. This talk was **awarded** with the **"Evenius-Preis"** for the best student talk

Schittny Dominik (2018) Transmission via hive products: Globalization of the honey bee virosphere? Poster at the EurBee Conference, Ghent, Belgium. This poster was **awarded** with the **Ingemar-Fries award** for the best student poster.

Martin Anna (2017) Hive products as vector for Trypanosomatids. MSc thesis, University of Zurich

Roth Kaspar (2017) Honig, Pollen und Wachs als Matrices für orale Übertragung von Darmparasiten der Honigbienen (*Apis mellifera*). Diplomarbeit für Naturwissenschaftliche/r Labortechniker/-in mit eidg. Diplom

Neumann Peter (2017) Internationaler Handel und Krankheiten: Potential verschiedener Bienenprodukte für die Verbreitung von Krankheiten" VPH seminar talk, Bern, CH

Khongphinitbunjong Kitiphong, Chantawannakul Panuwan, Neumann Peter, Yañez Orlando (2018). Survival of ectoparasitic mites *Tropilaelaps mercedesae* in association with honey bee hive products. Manuscript in prep. (aiming at *Journal of Invertebrate Pathology*)

Schittny Dominik, Yañez Orlando, Neumann Peter (2018). Honey bee virus transmission via hive products. Manuscript in prep. (aiming at *Journal of Economic Entomology*)

Yañez Orlando, Martin Anna, Neumann Peter (2018) Oral transmission of *Crithidia mellificae* and *Nosema* spp. via honey bee hive products. Manuscript in prep. (aiming at *Apidologie*)

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***Project duration** April.2015 - March 2018*