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Letter to the Editor

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Varroa destructor mite infestations in capped brood cells of honeybee workers affect emergence development and adult foraging ability

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Given recent higher declines in managed *Apis mellifera* honeybees, which are the most commonly managed bee species around the world (Garibaldi et al. 2013), numerous health threats involved in the losses have been received great attention. To date, *Varroa destructor* mite infestation has become the biggest challenge in commercial beekeeping, which is considered as a thorn stuck in the throat of beekeepers. The *Varroa* mite threatens bee health by absorbing the fat body and hemolymph of immature and mature bees (Ramsey et al. 2019). *Varroa* mite infestation during the development of worker bees was found to reduce birth weight, influence water content and protein level in hemolymph of newly emerged worker bees, decrease flight performance of drones, change gene expression patterns related to immune system of honeybees, and result in honeybee colony losses (Duay et al. 2002; Bowen-Walker and Gunn 2010; Zhang et al. 2010; Annoscia et al. 2012; Dooremalen et al. 2013; Locke 2016). The *Varroa* mite, as an ectoparasite of the honeybee, prefers living in the sealed brood cells (Bogdanov 2006). Thus, bees are more likely to become hosts of mites in the pupal stage. In this study, we systematically investigated the current impacts of *V. destructor* infestations on the development from capped larvae to emerged bees. It is hypothesized that *Varroa* infestations in the pupal stage of honeybees will exert further influences on adult bees even if they successfully get rid of the *Varroa* mite later in life, and there is still data gap for understanding the potential effects of *Varroa* infestations on survival and foraging performance of adult bees throughout their life cycle. The radio frequency identification (RFID) technology could be favorable for documenting the life cycle of honeybees (Shi et al. 2020). Here, effects of *V. destructor* mite infestation on lifespan, age at onset of foraging, the number of foraging flights, and homing rates of worker bees without deformed wings were examined using RFID system. Detail methods are described in Supplementary Materials. Two mites were selected to deposit in one capped cell in this study as the treatment group.

To understand the effects of *Varroa* mite infestation on the development of worker bees, we first counted the birth weight and emergence rates of bees from the control group and *Varroa* mite from the treatment group. Results showed that the birth weight of treatment group was only 111.96 ± 17.19 mg (mean ± standard deviation), which was 19.40 mg on averagely lower than the control group (Mann–Whitney *U*-test, $W = 566.5$, $P < 0.001$; Figure 1A), suggesting that *Varroa* infestation could suppress the growth of bees, which may be due to the long-term nutritional utilization of worker larvae by mites, similar results were reported by Bowen-Walker and Gunn (2010). Mean emergence rates of treatment group also decreased by 5.83% compared with control group, although there was no statistical significance between 2 groups (Mann–Whitney *U*-test, $W = 9$, $P = 0.136$; Figure 1B), indicating that *Varroa* infestation may reduce the survival of pupae. In this study, length of wings in the treatment group decreased to 14.78 μm on averagely than that in the control group (Mann–Whitney *U*-test, $W = 186$, $P < 0.001$); the thorax of bees in treatment group also significantly reduced than that in the control group, which on averagely decreased by 21.60% (Unpaired *t*-test with Welch's correction, $t = 7.023$, $P < 0.001$), which may directly influence the flying ability of worker bees. As for the length of

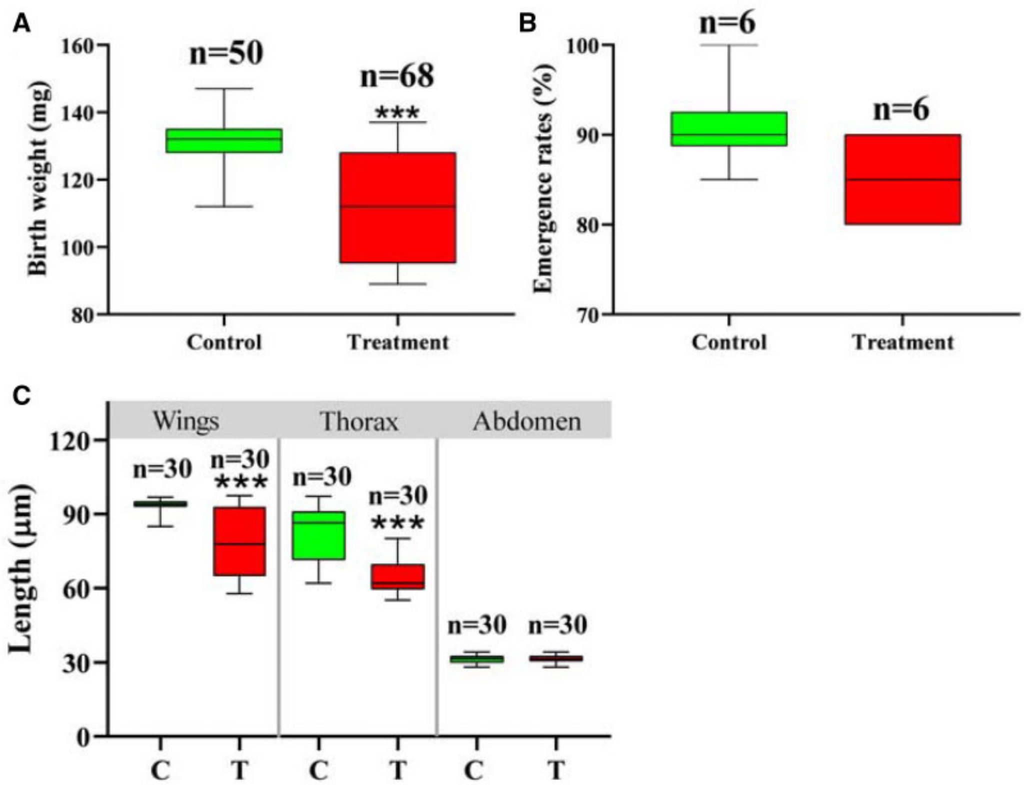

Figure 1. Effects of *Varroa* infestations on birth weight, emergence rates, the length of wings, thorax, and abdomen of worker bees. (**A**) Body weight of each newly emerged worker bees from the control group ($n = 50$) and treatment group ($n = 68$) were counted, respectively. Tested by Mann–Whitney *U*-test. (**B**) Emergence rates of worker bees from 2 groups. A total of 6 biological replicates from each group from 3 different colonies were included. Data were uniformed by conducting arcsine substitution to perform Mann–Whitney *U*-test. (**C**) Length of wings between 2 groups was analyzed by Mann–Whitney *U*-test, Length of thorax was analyzed by unpaired *t*-test with Welch's correction and length of abdomen was analyzed by unpaired *t*-test ($n = 30$). Error bars represent SD. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

abdomen, there was no significance between 2 groups (Unpaired *t*-test, $t = 0.263$, $P = 0.794$), which maybe explained that *Varroa* mites feed mainly on the bee's thorax, rather than its abdomen. Due to this fact, we could basically infer that the parasitic mites affect the development of bees by feeding on nutrients from the bees rather than by influencing the food intake of bees. Above results showed that *Varroa* infestation would bring adverse impacts on the morphological development of honeybees.

After we established that *Varroa* mite infestation induced overt symptoms of emerged bees during the period of infection, we further investigated the differences in foraging performance of adult worker bees between 2 groups after the *Varroa* mite-treated bees got rid of the *Varroa* mites after emergence. *Varroa* infestation affects foraging behaviors of honeybees throughout their life has not yet been fully elucidated. Adult worker bees were continuously monitored throughout their life using RFID technology; in this study, the age at onset of foraging, number of foraging flights, and lifespan of worker bees were analyzed. There was no significance on age at onset of foraging of worker bees between the 2 groups (Mann–Whitney *U*-test, $W = 1{,}774$, $P = 0.891$; Figure 2A), bees were about 6–7 days old at onset of foraging. Similar phenomenon was recently reported in other literature (Shi et al. 2020), but there is no evidence in our results that mite parasitism induces precocious foraging activity of worker bees. The mite-infected group presented higher mortality rate in the first 20 days, while there was no statistical difference in average lifespans of worker bees between the control and treatment groups ($\chi^2 = 0.214$, $P = 0.644$; mean 17.05 and 15.50 days, respectively; Figure 2B). The reason for above results could be that we excluded the fresh worker bees with deformed wings before conducting monitor. Another reason could be that honeybees can improve some part of physiological functions when they are fed with nutrients after emerging. In addition, we found that the number of foraging flights per worker bee from the treatment group was significantly lower than that from the control group, which was decreased by 21.24% (Mann–Whitney *U*-test, $W = 1{,}271$, $P < 0.01$; Figure 2C). This may be parasitic mites that disturbed the development of bees' wings and thorax, thus decreasing the flying efficiency. Our results highlighted the adverse effects of *Varroa* infestation on the development and foraging ability of honeybee colonies.

We further observed whether there were differences in homing ability of worker bees between the 2 groups, 20-day-old bees from each group were released at 1 and 2 km away from their hives. Results suggested that either at 1 or 2 km, there were no significant differences between the control group and treatment group (1 km: unpaired *t*-test, $t = 1.706$, $P = 0.127$; 2 km: unpaired *t*-test, $t = 1.706$, $P = 0.127$; Supplementary Figure S1). It indicates that *Varroa* infestation on honeybee pupae may not affect the cognitive ability of >20-day-old bees. The reason may be the same as above. The freshly worker bees with deformed wings were excluded and the honeybee can improve some part of physiological functions when they are fed with nutrients after emerging. Another reason is that seriously affected bees have died in the first 20 days after emerging. The real reasons need to be

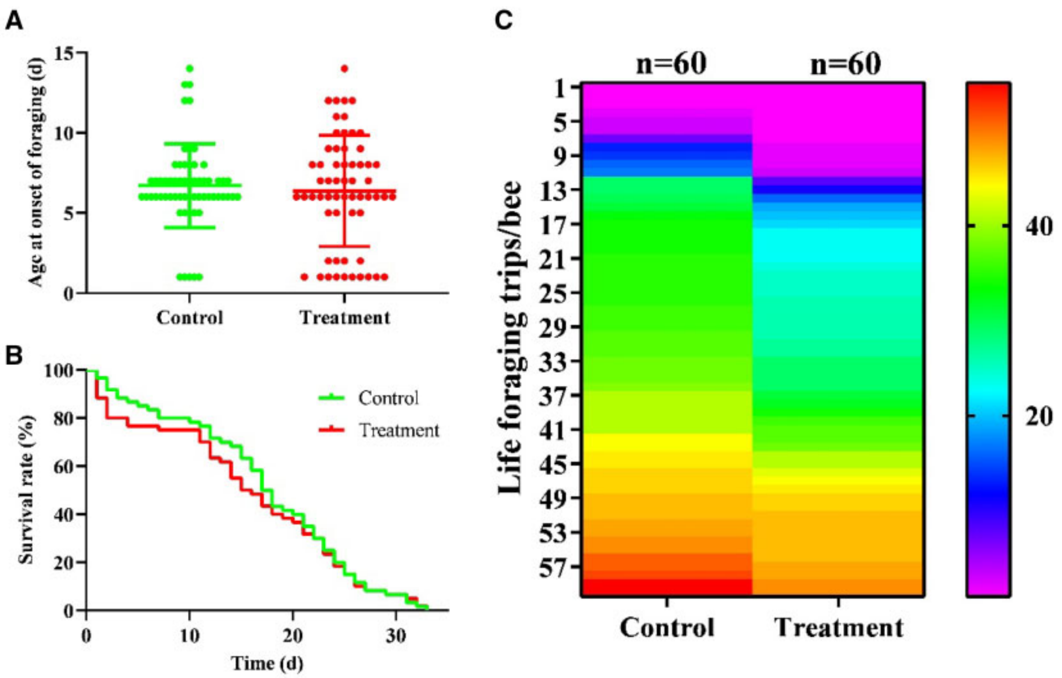

Figure 2. Effects of *Varroa* infestations on age at onset of foraging (A), lifespan (B), and the number of foraging flights (C) of worker bees. A total of 60 bees from each group were monitored ($n = 60$).

investigated further. Additionally, homing rates of 2 km were regularly lower than that of 1 km, which reflects that >2 km away will reduce the regress of bees.

In all, *Varroa* infestation in the pupa stage of honeybees not only affected the development, but also negatively influenced the foraging activity of adult worker bees. This study highlights the detriment of *Varroa* infestation in capped brood cells of worker bees and inspires us to attach the importance to control mites in the pupal stage in beekeeping.

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Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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