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Original article



CT-supported analysis of the destructive effects of *Varroa destructor* on the pre-imaginal development of honey bee, *Apis mellifera*

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Abstract – The ectoparasitic mite, *Varroa destructor*, is a most important health risk for European honey bee subspecies (*Apis mellifera*) globally. There is a scarcity in experimental approaches addressing this issue by using computed tomography. Our purpose was to determine the decrease in the volume, surface, density and weight alteration triggered by *V. destructor* in pre-imaginal stages of *A. mellifera* using computer-based 3-D reconstruction technology. Computer tomography used in the current study proved to be sufficiently sensitive for the detection of *V. destructor* in comb cells. This non-invasive experimental setup revealed that the developing worker pupae have retained their volume and surface values, whereas the weight loss triggered by *V. destructor* was statistically confirmed. In addition, the disappearance of lower radiodensity tissues is shown to be due to mite parasitism, which can be primarily explained by haemolymph losses.

computer tomography / pre-imaginal development / honey bee / impairment / *Varroa destructor*

1. INTRODUCTION

The honey bee mite *Varroa destructor* (Acari: Varroidae) is one of the most destructive natural enemy of the western honey bee, *Apis mellifera* (Anderson and Trueman 2000). Its original host is the eastern honey bee, *Apis cerana*; however, it attacks only its drone broods. It became a parasite for *Apis mellifera* subsequently, in areas in which both bee species coexist. Other host of this parasite mite is not known (Rosenkranz et al. 2010). It is native in Southeastern Asia, from where it started spreading worldwide. It has been present in Holarctic, Neotropical and

Indo-Australian regions for over 40 years (Sammataro et al. 2000).

There are several biological consequences in honey bee caused by *V. destructor*. Colony losses caused by *V. destructor* in the western honey bee are mainly due to worker bee injury caused by this parasite mite during pre-imaginal development (Duay et al. 2003). The most plausible side effect is the weight loss during the post-embryonic infestation. Body weight loss right after the onset of adult stage can reach 10% in infested populations (De Jong et al. 1982). The parasitised adult bee emerges injured with a reduced life expectancy and impaired orientation ability (De Jong and De Jong 1983).

Previous research results have been reported as to *V. destructor* being a typically haemolymph parasite of immature and adult honey bees (Shaw and Stobbart 1963). The latest results (Ramsey et al. 2019), however,

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showed that the parasite adheres to the body of the bee and weakens it by sucking its fat. It is a vector for at least 5 debilitating bee viruses including RNA viruses such as the virus inducing deformed wing development (Genersch and Aubert 2010; Anguiano-Baez et al. 2016). Through weakening the immune system, it facilitates the occurrence of other pathogens, which can cause the overall extinction of the infested bee family (Yang and Cox-Foster 2005).

Although a detailed, non-invasive study from Facchini et al.'s (2019) experiment on *V. destructor* infecting honey bees at different developmental stages has recently been published, experimental studies performed on honey bee hives mainly omitted the use of computed tomography hitherto, even though computed tomography offers a non-invasive approach.

The goal of this study was to analyse the effect brought about by *V. destructor* on the pre-imaginal stage of *A. mellifera* in a non-invasive manner. Our purpose was to assess the putative change in the volume, density and weight in post-embryonic stages of *A. mellifera* caused by this important mite parasite using computer-based three-dimensional reconstruction technology.

2. MATERIAL AND METHODS

2.1. Sampling

A “warm way”-built hive (with the frames perpendicular to the entrance) was placed next to Kaposi Somogy County Teaching Hospital, Dr. Baka Jozsef Diagnostic, Radiation Oncology, Research and Teaching Centre (GPS coordinates: WGS: X:46.381079 Y:17,826915), in which computer tomography (CT) was conducted at the commencement of August 2019. One colony of *Apis mellifera carnica* kept on 10 frames was the subject of our investigation. Copious honey and pollen were available to feed the colony, and in addition bees were fed with 50% sugar drops during the survey. The young honey bee queen providing the progeny

emerged on 15 May 2019. The colony infested with *V. destructor* was kept under natural conditions throughout the study. The average abundance calculated right before the implementation of the non-invasive analysis was 108.6 adult mites/frame. The last acaricide treatment of this colony was effectuated on 08 September 2018, after which other pesticides were not administered.

2.2. Description of the examination setup

In the course of the experiment, the colony was observed daily and the batches of the eggs laid simultaneously were analysed by CT (18 August 2019.). The broods were uniformly closed on the 9th day following egg-laying. After the brood coverage, a brood comb of 8×10 cm of the workers was cut from the centre of the frame, which was subsequently assigned to CT examination at equal intervals, four times during the post-embryonic development: on the 14th, 16th, 18th, and 20th day from the onset of egg-laying. One hundred fifty pupae were assayed, from which 10 intact and 10 infested pre-imaginal specimens were randomly selected for comparison. The sensitivity of the CT-method was visually examined by detecting *V. destructor* in the brood's cells, which was tested by extracting unexamined brood's cells in 10 replications. This comparison method was always carried out by two persons. Volume and tissue density data of pupae were gathered by means of computed tomography, from which the change in physiological characteristics of the honey bee pupae could be concluded. Between each recording, the comb sample was placed back to its original position in the frame. At the end of the survey, these examined worker pupae were removed from their brood cells and the number of adult female mites per cell was determined by their distinct, darker cuticle and signs of wear; subsequently the host bees were weighed on a micro-scale (Sartorius A120S) with the accuracy of 1 mg.

2.3. Computed tomography-assisted imaging analysis

The CT measurements were performed using a Siemens Somatom AS + CT scanner (Siemens Ltd., Erlangen, Germany). The following scanning parameters were used for data collection: 140 kV, 200 mAs and spiral data collection with pitch 0.7, UHR (Ultra High Resolution) mode. Axial scans were reconstructed at every 0.1 mm increment with 0.6-mm slice thickness and 60-mm field of view using V80 u convolution kernel. The images were archived in DICOM (Digital Imaging and Communications in Medicine) format and each of the series was subsequently converted to NIFTI (Neuroimaging Informatics Technology Initiative) metafiles. The resolution of the meta-images was nearly isotropic: $0.117 \times 0.117 \times 0.1 \text{ mm}^3$.

The first step during the implementation of the analysis was the separation and identification of the individuals. The pupae were segmented manually using the segment editor module of the 3D Slicer program (Fedorov et al. 2012). The average radiodensities and volumes of the pupae were calculated using the voxels belonging to the pupae. Subsequently, the coordinates of those pupae voxels were considered to be the coordinates of the surface points of the pupae. Based on these 3-D point clouds, the surfaces of individual seeds were reconstructed through triangularisation. The 3-D models of seeds were created for measuring the surface size and visualisation of the pupae, in order to test the volume, surface and tissue density [(Hounsfield Unit (HU) is a quantitative value for describing radiodensity. It is frequently used in CT scans, where its value is also termed CT number].

2.4. Statistical analysis

For Hounsfield Unit and the weight values of the examined honey bee pupae, the Shapiro–Wilk test was used. For the survey of the normal distribution of data ($P < 0.05$), the Ghasemi- and Zahediasl-type methods

were employed. The effects of *V. destructor* (post-embryonic development and health condition were the independent variables) on the volume (mm^3), the surface (mm^2) and the tissue density (HU) of infested pupae (as dependent variables) and its interaction with pre-imaginal development were statistically analysed by two-way ANOVA. The impact of mite parasitism on the weight of honey bee pupae measured at the end of the survey was evaluated by one-way ANOVA via using the SPSS for Windows 11.5 software package. Mean values were separated by using the Tukey test, at $P \leq 0.05$.

3. RESULTS

3.1. Sensitivity of CT for mite detection and infestation rate

The detection rate of *V. destructor* present in the analysed comb by CT has continuously increased during the progress of post-embryonic development of honey bee pupae. The early developing stage of parasite can be seen in the lateral side of pre-imaginal honey bee pupae in Figure 1. The perception rates of the parasite in the four non-invasive recordings were the following: 1st recording, 43.33%; 2nd, 76.66%; 3rd, 90%; 4th, 100%.

The infestation in the experimental comb was 21.31% (Figure 2). Additionally, the average number of mites per one infested cell was 4.68, which has been determined after the opening of cells at the end of the experiment.

3.2. Volume, surface and tissue density data of pupae

The Shapiro–Wilk normality test showed that our CT-measured data are of normal distribution, $P > 0.05$. Uniform significant differences in the tissue density of pupae between intact and infested samples were revealed by statistical analysis.

The effect of post-embryonic development on the change in volume ($P = 2.47 \times 10^{-19}$), surface ($P = 4.91 \times 10^{-18}$) and tissue density ($P = 1.73 \times$

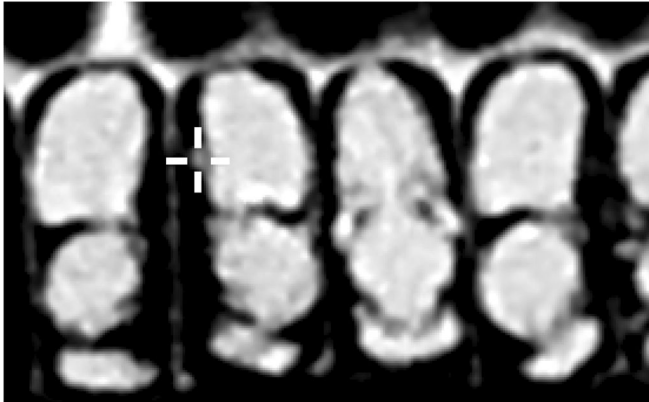


Figure 1. Axial CT recordings of honey bee pupae with feeding *V. destructor* (marked by white cross).

10^{-8}) data of pupae was confirmed by two-way ANOVA.

Volume reduction in pupae triggered by *V. destructor* could be detected in this

experiment (Figure 3a, b, c, d, e, f, g, and h). The volumes of the parasite-infested pupae and intact pupae as well decreased in each observation record with the exception of the 2nd recording. We showed a remarkable difference on the 1st recording as well (2.898 mm³), but the most spectacular difference (3.435 mm³) between the volumes of the infested samples and those of the intact ones was measured on the 3rd recording. Nonetheless, the effect of mite parasitism on volume decrease in bee pupae could not be proven statistically ($P = 0.271$) by two-way ANOVA. The combined effect of pre-imaginal development and parasitism on the volume change in the bee pupae showed a not statistically proven relationship ($P = 0.692$).

The surface of intact and infested samples within the same recording was examined in conjunction with the volume. The surface of the examined intact samples was 136.711 ± 1.276 mm² at the end of the examination. The surface loss in the infested pupae was 0.667 mm² on average on the 4th recording, but this decrease ($P = 0.842$) and the interaction with pre-imaginal development ($P = 0.085$) were not statistically confirmed.

The pupae tissue densities expressed by Hounsfield Unit (HU) of intact and infested honey bees are shown in Figure 4a, b, c, d, e, f, g, h, and i. The samples infested by *V. destructor* have lower radiodensity values than those of the intact samples, which has

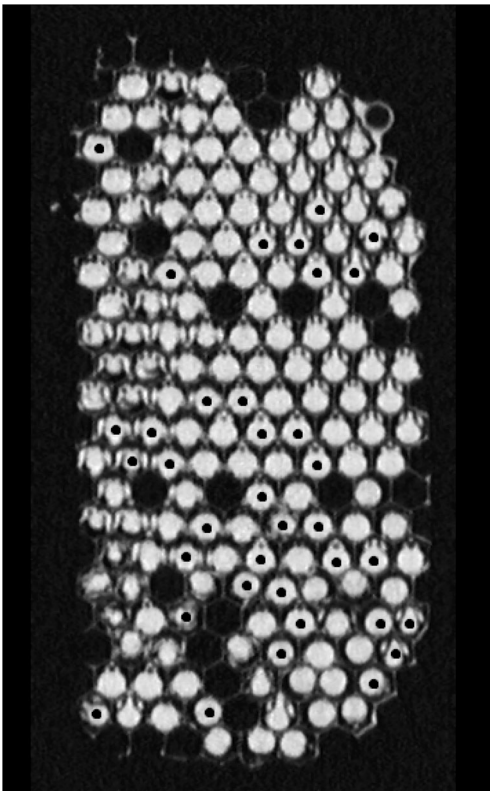


Figure 2. Experimental sample of honey bee comb with black spot marking of infested cells by *V. destructor*.

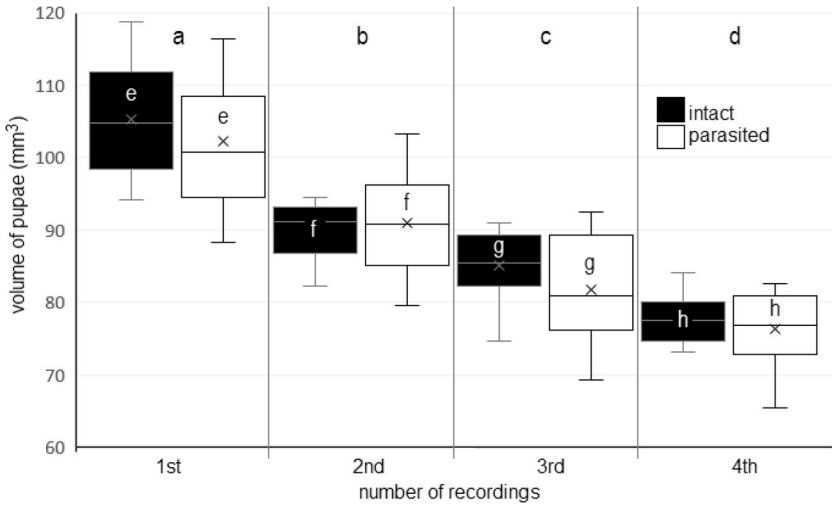


Figure 3. The volume (mm^3) changing of honey bee pupae during the examination as a function of *V. destructor* parasitism ($n = 20$). (a, b, c, d) Small letters indicate significant difference ($p \leq 0.05$) between means of different recording times; (e, f, g, h) small letters indicate significant difference ($p \leq 0.05$) between intact and parasite samples.

been confirmed by two-way analysis of variance ($P = 8.64 \times 10^{-6}$). The mean changing density of the infested pupae was 1.657 HU (1.481%). Eventually, the dense tissue formation has disappeared from the pupae of honey

bee, which may be due to sucking by *V. destructor*. Nonetheless, the interaction of the parasitism and the development could not be statistically confirmed ($P = 0.084$).

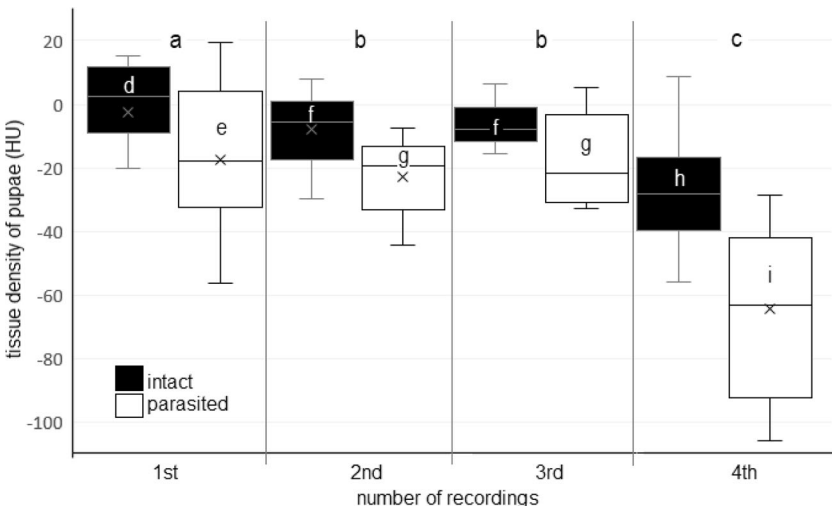


Figure 4. The tissue density (HU) changing of honey bee pupae during the examination as a function of *V. destructor* parasitism ($n = 20$). (a, b, c) Small letters indicate significant difference ($p \leq 0.05$) between means of different recording times; (d, e, f, g, h, i) small letters indicate significant difference ($p \leq 0.05$) between intact and parasite samples.

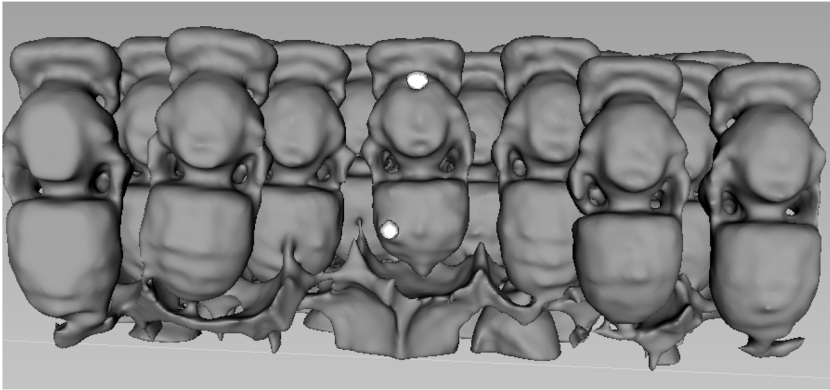


Figure 5. Computer-based three-dimensional reconstructions of honey bee pupae infested by *V. destructor* in the comb. *V. destructor* are marked by white spots.

3.3. Weight of pupae

The weight loss of honey bee pupae attacked by *V. destructor* (Figure 5) was evinced. The mean of calculated weight of the infested pupae was 105.2 ± 0.001 mg, while the same developing stage (healthy stage) was 121.9 ± 0.001 mg, which represents 16.6 mg (13.665%) weight loss in the case of the damaged honey bee pupae. The effect of parasitism on the weight of this pre-imaginal stadium of honey bee was statistically demonstrable ($P = 8.41 \times 10^{-7}$) by one-way ANOVA.

4. DISCUSSION

It is claimed that computer tomography as a new, non-invasive method in apiary researches is suitable for the detection of *V. destructor* in comb cells. Naturally, the detection rate depends on the operator's aptitude, but generally, the later the scan, the easier it is to spot a mite in a cell. However, it proved most suitable in observations at more advanced developing stages of mite, at which the deutochrysalis has already exhibited the final body shape. At the deutochrysalis stage, the coloration starts on the periphery of the opisthosoma and shifts to a reddish-brown colour after the moult of the female (Rosenkranz et al. 2010). Further, the better detection of parasites by CT at late developmental stages can be partly explained by the hidden lifestyle of both the

founder mite and the larvae at earlier developing stages. The nymphs are generally located on the 5th segment on the bee pupa and near the so-called faecal accumulation site at the bottom of the cell (Kanbar and Engels 2003).

The infestation rate of *V. destructor* reported in this study can be considered to be the average infestation rate on the basis of the relevant data, which substantially vary between 7% (De Guzman et al. 2007) and 30% (Rosenkranz et al. 2006). Besides, it cannot be excluded that the infestation rate can reach higher value in a comb as *V. destructor* might not infest cells in a uniform way within a comb (Facchini et al. 2019). The recorded number of parasite mites in one cell coincided with the normal progeny rate of *V. destructor* reported in previously published works. It is normal for a fertile mite to lay five eggs (one male and four females) in worker cells, which can be located close to each other (Martin 1995; Rosenkranz et al. 2010).

The anatomical features measured by CT in developing honey bee pupae were unequivocally influenced by parasitism. According to our CT-assisted observation, the developing honey bee pupae have retained their volume and surface values (which can be partially explained by the normal post-embryonic development too), while the weight loss triggered by *V. destructor* was observable. These results are in line with the data presented previously in the relevant literature (Colin et al. 1999; van Engelsdorp et al. 2007;

Facchini et al. 2019). Duay et al. (2003) have presented the first reliable data on individual drone weight loss, especially from the red-eyed pupal stage onwards. The resulting reduction in the weight of adult drones was related to the number of female mites (up to 20) that had invaded a brood cell.

The diminished radiodensity in animal tissues (e.g. fat) in infested pupae can be explained by the disappearance of high-density organic (e.g. proteins, carbohydrates) and inorganic (e.g. water) materials (Romvari et al. 1996; McEvoy et al. 2008). In this context, it is worth mentioning that water (as an essential component) can be found in the largest quantities in insect haemolymph (Shaw and Stobbert 1963).

Albeit direct tissue analysis was not carried out in this study, based on our results, it can be inferred that the content of haemolymph could be decreased primarily by *V. destructor* parasitism due to water being the densest and the highest proportion component of this insect tissue. The results of former experiments are confirmed by our findings. Ramsey et al. (2019) showed that this parasite does not only consume body fat but also damages host bees by haemolymph sucking, a phenomenon which could not be verified by our study. *Varroa* parasitism is associated with impaired development of immature bees, decreased lipid synthesis, reduced protein titers, desiccation, impaired metabolic function, inability to replace lost protein, precocious foraging, heightened winter mortality, impaired immune function, decreased longevity and reduced pesticide tolerance (Bowen-Walker and Gunn 2001; van Dooremalen et al. 2013).

In summary, our results can shed light on the consequences of the damage brought about by *V. destructor* during the pre-imaginal stages of honey bee development in a non-invasive way. Our reconnaissance study in the detailed biology of this important ectoparasite can play an important role in the understanding of some viral disorders transferred by mite (e.g. CCD) as well as the elaboration of strategies aimed at successful protection method against it. These novel data originating from CT imaging can contribute to the understanding of the complex phenomenon of colony collapse disorder.

AUTHORS' CONTRIBUTIONS

Sandor Keszthelyi and Tamas Donko conceived and designed the research project. Tamas Sipos provided the experimental bee colony. Tamas Donko and Tamas Sipos conducted the experiments. Adam Csoka and Tamas Sipos did the CT image post-processing and connected data analysis. All authors analysed the data. Sandor Keszthelyi wrote the manuscript. All authors read and approved the manuscript.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest The authors declare that they have no potential conflict of interest in relation to the study presented in this paper.

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Analyse par tomographie assistée par ordinateur des effets destructeurs de *Varroa destructor* sur le développement préimaginal de l'abeille, *Apis mellifera*.

tomographie par ordinateur / développement préimaginal / abeille domestique

Eine CT-Analyse der negativen Auswirkungen von *Varroa destructor* auf die on the preimaginal development of honey bee, *Apis mellifera*.

Computertomograph / präimaginale Entwicklung / Honigbiene

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