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Experimental honey bee studies using non-invasive imaging methods

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1. BACKGROUND OF THE RESEARCH AND OBJECTIVES

The dramatic global decline in both the diversity and abundance of pollinators represents one of the most pressing ecological challenges of our time. Among the affected taxa, the western honey bee (*Apis mellifera* Linnaeus, 1758) - also maintained as an economically important livestock species - is one of the most vulnerable pollinators from a human perspective. As a key component of ecological processes, honey bee populations are currently exposed to numerous adverse pressures. These include the decline in bee pasture diversity losses, poisoning resulting from improper pesticide use, and the transcontinental spread and increasing impact of bee-associated parasites, both microbial and arthropod (Rosenkranz et al., 2010; Martin et al., 2012; Mordecai et al., 2015; Morfin et al., 2020). Among these threats, the Varroa mite (*Varroa destructor* Anderson and Trueman, 2000), originating from the Far East, represents one of the most severe challenges due to damage it causes to *A. mellifera* colonies (Evans and Chen, 2021; Warner et al., 2024).

Following its global spread, *V. destructor* has become one of the most intensively studied parasites in apicultural research. Numerous studies have focused on its biology, morphology, and the potential transmission routes through which *A, mellifera* colonies become infected (Rosenkranz et al., 2010; Dietemann et al., 2012). The primary aim of these investigations has been to develop effective control strategies, particularly through the identification and application of acaricidal treatments capable of reducing colony losses. Although these applied and practice-oriented research efforts have significantly reduced the occurrence of high number of mass colony collapse caused by the parasite, but a comprehensive and sustainable solution has not yet been achieved (Rosenkranz et al., 2010; Warner et al., 2024). At the same time, the widespread use of conventional acaricides in beekeeping practice has begun to decline due to increasingly strict honey quality standards, growing societal expectations regarding food safety and sustainability, and the emergence of acaricide resistance in mite populations (Jack and Ellis, 2021; Mitton et al., 2022). Currently, neither conventional nor alternative

acaricides, nor biological control strategies, can fully eliminate the damage caused by the parasite. Likewise, breeding programs have not yet provided a universally applicable solution for commercial beekeeping operations (Le Conte et al., 2020; Jack and Ellis, 2021). The development of resistance further increases the demand for new control strategies and innovative approaches (Kolics et al., 2021; Mitton et al., 2022). As a practising beekeeper, I consider research aimed at addressing this major problem to be of particular importance; therefore, the *V. destuctor* was selected as the focal organism of my doctoral research.

The pathological effects caused by *V. destructor* manifest both at the level of individual and at the colony level, yet they are difficult to quantify due to the parasite's hidden lifestyle. The mite exhibits two main different life phases (Rosenkranz et al., 2010; Warner et al., 2024): a phoretic phase, during which female mites attach to adult bees and spread between colonies, and a reproductive phase within sealed brood cells. The primary damage caused by the parasite occurs at the individual level during the development of bees, sheltered by the sealed brood cells. However, as infestation levels increase, these effects may also have a significant impact on the social structure of the colony, which operates as a biological superorganism (Rosenkranz et al., 2010; Ramsey et al., 2019; Warner et al., 2024).

By feeding on the fat body and hemolymph of the developing pupae, the mite reduces lipid synthesis and disrupts the host's energy metabolism, which in turn negatively affects protein synthesis (Ramsey et al., 2019). The resulting reduction in lipid and protein synthesis impairs metabolic processes and may weaken the immune system. Another important consequence is the reduced tolerance of bees to pesticides (Bowen-Walker and Gunn, 2001; Van Dooremalen et al., 2013; Ramsey et al., 2019).

At the end of development, reduced body mass and abdominal deformities are clearly observable in imagoes. In worker bees, body mass is on average approximately 7% lower than in non-infested individuals (De Jong et al., 1982; Bowen-Walker and Gunn, 2001). In drones, this reduction can be even more pronounced, reaching 11-19%,

depending on the level of parasitism (Duay et al., 2003). Reduced body mass or abnormal wing development - often associated with infection by Deformed Wing Virus (DWV) - can significantly impair the functional performance of worker bees (Martin et al., 2012, Anguiano-Baez et al., 2016). In workers, this may result in shifts in their roles within the colony, including the premature start of foraging or, in some cases, the failure to initiate flight. These changes significantly shorten lifespan and impose unnecessary energetic costs of the colony and distrupt the balance of the superorganism (De Jong et al., 1982; Amdam et al., 2004). Furthermore, workers that were parasitized during development often exhibit impaired orientation abilities, which can further weaken the colony through increased drifting and loss of foragers (Martin et al., 2012; Mordecai et al., 2015). Overall, the severity of varroosis - the disease complex associated with *Varroa* infestation - is determined by the size of the mite population and the combination of viruses transmitted by the parasite. These effects may be further intensified by environmental stressors such as poor pollen quality, pesticide exposure, periodic nectar shortages leading to energy deficits, and negative impacts associated with suboptimal beekeeping practices (Van Dooremalen et al., 2013; Corby-Harris et al., 2019; Morfin et al., 2020; Taha and Al-Kahtani, 2020).

There is therefore an urgent need to develop new diagnostic methods that allow improved characterization of the hidden lifestyle and biology, and damage caused by *V. destructor*. Within entomological research - particularly in apicultural sciences - the application of digital imaging techniques remains limited (Facchini et al., 2019). Diagnostic technologies such as computed tomography (CT), micro-computed tomography (micro-CT), and infrared thermography represent innovative tools in this field. In certain cases, they allow deeper insight into the superorganism-level functioning, anatomy, and biology of *A. mellifera* colonies, as well as the biology and pathological effects associated with *V. destructor* infestation (Alba-Tercedor and Alba-Alejandre, 2017; Castejón et al., 2018; Facchini et al., 2019; De Paula et al., 2022).

The early application of X-ray radiation in entomological research was primarily aimed at the development of pest control strategies, such as the sterile insect technique (Hallman, 2013). Although the radiation doses applied during diagnostic CT and micro-CT imaging do not reach the gray (Gy) levels used in such control experiments, they may nevertheless influence individual vitality. Investigating the tolerance of *A. mellifera* and *V. destructor* to X-ray radiation is therefore essential for establishing the fundamental knowledge required for the application of ionizing-radiation-based imaging techniques in apicultural research (Wipfler et al., 2016; Hall and Martín-Vega, 2019; Facchini et al., 2019).

Based on these considerations, the primary objective of the present doctoral research was to address several previously unanswered research questions using a novel, non-invasive methodological approach. The long-term goal of these investigations is to develop research methodologies applicable to apicultural research that can support practical and theoretical efforts in the ongoing struggle against *V. destructor*, which has persisted for over 70 years.

1.1. OBJECTIVES

1. To evaluate the applicability and to establish objective parameters of imaging techniques (human diagnostic CT, micro-CT, and infrared imaging) for the detection of *V. destructor* and comb cell deformations.
2. To assess the presence of *V. destructor* within brood cells and to evaluate its effects on preimaginal developmental stages of honey bees, with particular emphasis on body deformities and changes in tissue density.
3. To further investigate and confirm the indirect relationship between the presence of *V. destructor* and social fever using infrared thermography under field conditions.
4. To evaluate the potential effects of ionizing radiation associated with CT diagnostic procedures on the host *A. mellifera* and parasite *V. destructor* viability, as well as to assess possible histological alterations in the host organism.

2. MATERIALS AND METHODS

2.1. EVALUATION OF THE APPLICABILITY OF HUMAN DIAGNOSTIC CT IN APICULTURAL RESEARCH WITH A FOCUS ON THE DETECTION OF *VARROA DESTRUCTOR*

2.1.1. SAMPLE PREPARATION

The honey bee colonies used in the experiment were maintained in Nagyboczonádi (NB) 18-frame horizontal hives at the Kaposvár Campus in 2019. In the beekeeping season preceding the study, no acaricidal treatments were applied in order to allow the natural increase of the parasite population. For the CT examinations, freshly capped brood comb samples (10x8 cm) were collected from the colonies in August 2019 and used as the basis for imaging analyses.

2.1.2. DIGITAL IMAGING PROCEDURES

Human diagnostic CT imaging was performed at the Dr. József Baka Diagnostic, Oncoradiology, Research and Education Centre of SVKMOK using a Siemens Somatom AS+ CT scanner. CT scans were carried out at four developmental time points, corresponding to days 14, 16, 18, and 20 of pupal development.

The acquired images were analysed using 3D Slicer software (version 4.11). Several modules were applied during the analysis (Fiducial, Segment Editor, and Markups) to evaluate 10 intact and 10 parasitized individuals. The occurrence of mites was recorded on the CT images at each time point for the parasitized specimens. The presence of mites was subsequently validated retrospectively through dissection of the brood cells.

2.1.3. MEASURED PARAMETERS

Following diagnostic imaging, the brood cells were carefully opened using microforceps and dissecting needles. During dissection, the number of mites present in each cell was recorded. In addition, the developmental stage and body mass of the bees were determined. Body mass was measured with a precision of 1 mg using a Sartorius A120S analytical balance.

2.1.4. STAISTICAL ANALYSES

Statistical analyses were performed using SPSS software (version 11.5). The normality of the data distribution was assessed using the Shapiro–Wilk test. Differences in pupal volume (mm^3), surface area (mm^2), and tissue density (HU) between parasitized and non-parasitized individuals were analysed using two-way ANOVA, considering both parasitism and developmental stage as explanatory variables. The relationship between body mass and parasitism was analysed using one-way ANOVA ($p<0.05$).

2.2. Investigation of the combined effects of Deformed Wing Virus (DWV) and *Varroa destructor* on developing honey bee pupae

2.2.1. Sample preparation

The honey bee colonies used in the experiment were maintained in Nagyboczonádi (NB) 18-frame horizontal hives at the Kaposvár Campus. No acaricidal treatments were applied during the beekeeping season preceding the experiment in order to allow the natural increase of the mite population. For the CT examinations, brood comb samples measuring 10x8 cm were collected from the colonies in September 2019 and used as the basis for the imaging analyses.

2.2.2. Diagnostic imaging protocoles

Human diagnostic CT imaging was performed at the Dr. József Baka Diagnostic, Oncoradiology, Research and Education Centre of SVKMOK using a Siemens Somatom AS+ CT scanner. CT scans were conducted at four developmental time points, corresponding to days 14, 16, 18, and 20 of individual development.

The acquired images were analysed using 3D Slicer software (version 4.11) with several modules (Fiducial, Segment Editor, and Markups). Distances between marker points in three-dimensional space were calculated using Python software (version 3.6).

2.2.3. Measured parameters

Following diagnostic imaging, the brood cells were opened and the number of mites present in each cell was recorded. The developmental stage of the bees was also documented. The body mass of the pupae was measured with 1 mg precision using an

Ohaus Explorer Pro EP214CE analytical balance. The samples were subsequently stored at -80 °C until molecular biological analyses were performed.

2.2.4. Molecular biological analyses, detection of Deformed Wing Virus

For the detection of Deformed Wing Virus (DWV), five intact and five parasitized pupae were randomly selected. RNA extraction was performed using the RNeasy Fibrous Tissue Mini Kit, and cDNA synthesis was carried out using the QuantiTech Reverse Transcription Kit. PCR amplification was conducted according to the protocol described by Berényi et al. (2006). PCR products were analysed using 2% Tris-acetate-EDTA agarose gel electrophoresis, stained with SYBR™ Gold Nucleic Acid Gel Stain, and visualized using a GeneRuler™ 1 kb Plus DNA Ladder.

2.2.5. Statistical analyses

Data normality was assessed using the Kolmogorov–Smirnov test (n=50). The effect of parasitism on the measured morphological parameters of the pupae was analysed using one-way analysis of variance (ANOVA) ($p<0.05$).

Changes in the size of the head, thorax, and abdomen during development were analysed using regression analysis and Pearson correlation coefficients.

2.3. Analysis of the structure and size of worn comb cells and the morphology of developing honey bee pupae using micro-CT imaging

2.3.1. Sample preparation

Comb samples required for the study were collected from Nagyboczonádi ("nb") 18-frame horizontal hives located at the Kaposvár Campus. Brood comb fragments measuring 10x8 cm were collected from the colonies in April 2022 and used for CT imaging. Following a preliminary screening of the CT images, 10 intact and 10 worn comb cells were selected for subsequent micro-CT analysis. The selection was based on the degree of comb wear, as the brood comb fragments originated from frames that had been used in the colonies for three beekeeping seasons.

2.3.2. Human diagnostic imaging

Human diagnostic CT imaging was performed at the Dr. József Baka Diagnostic, Oncoradiology, Research and Education Centre of SVKMOK using a Siemens Somatom AS+ CT scanner. The spatial resolution of the images was 0.0977 mm × 0.0977 mm × 0.1 mm. The human diagnostic CT images were used to calibrate the Hounsfield unit (HU) values of the micro-CT images using the following equation:

HU = (micro-CT voxel value x 20.78)-1024. Image datasets obtained from the two devices were registered using the Elastix module of the 3D Slicer software.

Micro-CT examinations were conducted at the János Szentágothai Research Centre, University of Pécs, using a Bruker Biospin SkyScan 1176 micro-CT scanner. The resulting images had a voxel size of 8.75 μm.

Image analysis was performed using 3D Slicer software, applying the Fiducial, Segment Editor, and Markups modules.

2.3.3. MEASURED PARAMETERS

Different structural elements of the comb cells were distinguished on the images based on Hounsfield unit values, using five sampling points for each structural component.

On the micro-CT images, the diameter and length of the head, thorax, and abdomen were measured in 10 pupae developing in intact cells and 10 pupae developing in worn cells. Additionally, the following parameters were determined: total body length of the pupae, surface area, body volume, volume of the comb cells

2.3.4. STATISTICAL ANALYSES

Data normality was assessed using the Kolmogorov–Smirnov test.

The effect of comb cell size on the morphological parameters of the pupae was analysed using one-way analysis of variance (ANOVA) (p<0.05). Post hoc comparisons were performed using the Tukey test.

2.4. EFFECTS OF X-RAY IRRADIATION ON THE VIABILITY AND HEMOCYTE COMPOSITION OF *VARROA DESTRUCTOR* AND *APIS MELLIFERA*

2.4.1. SAMPLE PREPARATION

Honey bee samples used in the experiment originated from artificial swarms established in June 2022, each consisting of 1.5 kg of worker bees and provided with a queen reared in 2022. To achieve approximately similar levels of mite infestation among the colonies, the artificial swarms were treated with oxalic acid sublimation two weeks after colony establishment. For laboratory experiments, 20 samples were prepared, each consisting of 60 g of bees (approximately 500 in each cage). The samples were placed in special polypropylene containers during irradiation and subsequent laboratory analyses.

The experimental bees were supplied ad libitum with 40% sucrose solution. Laboratory conditions were maintained at 25 °C and 70% relative humidity.

2.4.2. HIGH-ENERGY X-RAY IRRADIATION PROCEDURE

CT scans required for irradiation planning were performed at the Dr. József Baka Diagnostic, Oncoradiology, Research and Education Centre of SVKMOK using a Siemens Somatom AS+ CT scanner.

The irradiation protocol was designed using the Varian Eclipse treatment planning system. High-energy X-ray irradiation was delivered using a Varian Clinac IX linear accelerator. The irradiation field size was 40x40 cm, the dose rate was 600 MU min^{-1}, and the nominal photon beam energy was 6 MV. To ensure a homogeneous dose distribution, solid water phantom plates were placed above and below the samples.

To evaluate the effects of X-ray irradiation on both the parasite and the host organism, the samples were exposed to average radiation doses of 15, 50, 100, and 150 Gy.

2.4.3. MEASURED PARAMETERS

During the laboratory experiments, mortality of honey bees and *V. destructor* was recorded at the following time points: 12 h, 24 h, 2 d, 3 d, 6 d, 12 d, 18 d, and 24 d.

For cytological analyses, hemolymph samples were collected on days 6 and 12 following irradiation and analysed using microscopic examination.

The analyses focused on changes in the relative proportions of hemocyte types.

2.4.4. STATISTICAL ANAYSES

Mortality values of bees and mites were corrected using Abbott's formula. Data distribution was assessed using the Kolmogorov–Smirnov normality test.

The effects of different X-ray dose levels on mortality and hemocyte parameters were analysed using two-way analysis of variance (ANOVA) ($p<0.05$). Post hoc comparisons were performed using Duncan's multiple range test.

2.5. RELATIONSHIP BETWEEN SOCIAL FEVER AND *VARROA DESTRUCTOR*

2.5.1. PREPARATION OF THE EXPERIMENTAL HONEY BEE COLONIES

The experiments were conducted in 2022 and 2023, involving five honey bee colonies per year. Colonies were maintained on eight Nagyboczonádi (NB) frames in identical NB-18 type hives.

Each colony was established in June from 1.5 kg artificial swarms. Within two weeks after colony establishment, the swarms were treated with oxalic acid sublimation in order to reduce parasite load and thereby simulate natural infestation dynamics.

All colonies were equally insulated to minimize heat loss and ensure comparable thermal conditions.

2.5.2. INFRARED THERMOGRAPHIC MEASUREMENTS

Infrared thermographic measurements were conducted over five consecutive days, between 12:00 and 16:00, each year between 10 and 15 October.

Thermal images were captured using a FLIR E5-XT WiFi handheld infrared camera, with a resolution of 160x120 pixels and a thermal sensitivity of 0.1 °C. Images were taken from capped brood frames at a distance of 50 and 16-18 cm. Prior to imaging, adult bees were carefully removed from the frames. The frames were photographed in

a closed, shaded environment with stable ambient temperature. To minimize heat loss, frames were imaged within 30 seconds after removal from the hive.

Thermal images were analysed using Teledyne FLIR Thermal Studio software, which allowed the temperature of 1005 intact and 1005 parasitized brood cells to be determined.

2.5.3. COMB INSPECTION

Following thermographic measurements, the brood frames were placed in a freezer and stored at -20 °C until dissection. For each dissected individual, the developmental stage was determined according to Rembold (1980). In addition, the number of mites present within each brood cell was recorded.

2.5.4. STATISTICAL ANALYSES

Data normality was assessed using the Shapiro–Wilk test.

To compare temperature values recorded on different days, Pearson correlation analysis was applied. The relationship between elevated cell temperature and parasitism was analysed using one-way analysis of variance (ANOVA) ($p<0.05$). The same method was also used to examine the relationship between mite number and brood cell temperature.

3. RESULTS AND DISCUSSION

Based on the human diagnostic CT images, the number of mites detectable on the surface of developing honey bee pupae increased progressively as individual development advanced. The proportion of detectable mites showed a clear increasing trend over time. On images obtained on day 14 of development, mites were detected in 43.33% of the infested brood cells. By day 16, the presence of the parasite could already be identified in 76.66% of the cells. On day 18, mites were visible in 90% of the examined brood cells, and by day 20, during the final stage of preimaginal development, the presence of *V. destructor* was detectable in all infested brood cells.

The timing of image acquisition, corresponding to the progression of individual development, had a significant effect on pupal volume ($p<0.0001$), surface area ($p<0.0001$), and tissue radiodensity ($p<0.0001$). However, the reductions in pupal volume ($p=0.271$) and surface area ($p=0.842$) associated with *V. destructor* parasitism were not statistically significant at every developmental stage based on CT imaging. In contrast, the mean difference in radiodensity expressed in Hounsfield units between parasitized and intact individuals was statistically significant ($p<0.0001$), with the most pronounced difference observed on 20th day of pupal development.

Body mass reduction was also clearly visible in parasitized individuals. The mean body mass of parasitized bees was 105.2±1.84 mg, whereas healthy individuals had an average body mass of 121.9±1.32 mg ($p<0.0001$).

Measurements of pupal body segment lengths based on CT imaging revealed that the combined effect of parasitism and Deformed Wing Virus (DWV) infection resulted in statistically significant morphological alterations ($p<0.001$). The average total body length of infected individuals was 11.025±0.065 mm, while the mean abdominal length was 5.21±0.062 mm. In contrast, intact individuals exhibited an average pupal body length of 11.37±0.062 mm and an abdominal length of 5.57±0.056 mm.

Based on ratios calculated from body segment measurements obtained from CT images, parasitism clearly influenced body proportions (n=35, $p<0.001$). In parasitized

individuals, the ratio between total body length and abdomen length was 3.77% lower compared with intact individuals. Similarly, the head-to-abdomen ratio was 6.86% lower in virus-infected individuals, whereas the ratio between total body size and thorax length showed a 3.33% increase in intact individuals. While the relative sizes of the abdomen and head decreased in parasitized individuals, the relative size of the thorax increased in healthy individuals when expressed as a proportion of total body length.

Micro-CT-based investigations of comb cells demonstrated that the method is suitable for distinguishing different structural components of comb cells and for analysing the morphology of developing honey bee pupae. Micro-CT imaging clearly revealed that worn comb cells, resulting from intensive use, consist of two distinct structural layers: a lighter inner layer with higher X-ray absorption and a darker outer layer with lower density on the CT images.

The accumulation of organic residues (FRP component) on the bottom and inner surfaces of the cell walls was responsible for the abnormal thickening of the walls, while the outer wax layer (FRPW) primarily represented freshly deposited wax covering of the brood cell opening.

Our experiments demonstrated that the internal volume of worn comb cells decreases significantly. The average internal volume of normal cells was 234.103±4.105 mm^3, whereas the value measured in worn and constricted cells was only 151.237±4.957 mm^3. Consequently, the internal volume of heavily used cells was 35.4% smaller, a difference that proved to be statistically significant ($p<0.001$).

Worker pupae developing in these constricted cells exhibited reduced body size compared with those developing in normal cells. The average body surface area of pupae developing in worn cells was 218.97±1.94 mm^2, compared with the values 266.86±2.23 mm^2 in normal cells, representing a reduction of approximately 17.9% ($p<0.001$).

Similarly, the body volume of 18-day-old pupae was significantly reduced. Pupae developing in normal cells had an average body volume of 120.61±1.43 mm^3, whereas

those developing in worn cells had an average volume of 105.87±1.29 mm^3, corresponding to an approximately 12.2% reduction ($p<0.001$).

The total body length of pupae developing in worn cells (8.87±0.11 mm) was also significantly shorter than that measured in normal cells (11.06±0.05 mm). Moreover, the lengths of all three body regions - head, thorax, and abdomen - were significantly smaller in pupae originating from worn cells ($p<0.001$).

Our experiments further demonstrated that short-wavelength X-ray irradiation at a dose of 15 Gy resulted in only a 7% higher mortality rate compared with the control group after 24 days, corresponding to a total mortality of 27%. Two-way ANOVA confirmed statistically significant differences between radiation doses, honey bee mortality, and mite mortality ($p<0.001$).

Furthermore, it was determined that radiation doses exceeding 50 Gy were poorly tolerated by honey bees, causing irreversible cellular damage under in vitro conditions by day 12, resulting in nearly 100% mortality. In our experiment, all groups exposed to 50, 100, and 150 Gy irradiation died by day 18.

The experiments conducted in 2023 demonstrated that *V. destructor* and honey bees exhibit different levels of tolerance to X-ray radiation. Based on these results, it can be concluded that the electromagnetic radiation doses generated during diagnostic CT and micro-CT imaging are unlikely to compromise honey bee viability.

Due to the obligate parasitic nature of the mite, a severe deterioration in host condition ultimately leads to parasite mortality as well. This phenomenon was observed in the 50, 100, and 150 Gy irradiation treatments. In contrast, mites exposed to 15 Gy irradiation showed a gradual increase in mortality throughout the experiment, following a polynomial trend, reaching its maximum by day 24. In the experimental population, 48% of mites dropped onto the hygienic bottom board, compared with 21% in the control group, representing a 27% difference at the 15 Gy dose level.

Mortality of irradiated mites was already detectable on days 6 and 12, whereas no parasite mortality was observed in the control group until day 18. These results suggest that 15 Gy irradiation affects mite viability more strongly than that of the host organism.
Analysis of hemocyte composition revealed that X-ray doses above 50 Gy significantly affected the differentiated hemocyte profile of honey bees ($p<0.001$). The most pronounced changes were observed in plasmatocytes and prohemocytes, while oenocytes and granulocytes also showed temporal variation, although these changes were not dose-dependent.
Statistical analyses confirmed that different X-ray doses significantly influenced plasmatocyte and prohemocyte counts ($p<0.001$). The mortality trends observed in the 50, 100, and 150 Gy groups corresponded with shifts in hemocyte composition, characterized by a decrease in prohemocytes and an increase in plasmatocytes.
In contrast, bees exposed to 15 Gy showed a hemocyte composition similar to that of the control group according to post hoc Duncan test results.

Infrared thermographic analyses demonstrated that different developmental stages (larval, prepupal, and pupal stages) could be clearly distinguished based on temperature differences ($p=0.001$).
Infrared thermography of capped worker brood cells revealed that higher temperatures were detected on the wax capping surface of cells containing mite-infested pupae. One of the major findings of the study was the identification of two distinct heating patterns responsible for the elevated temperature of parasitized cells.
The most common pattern was an open heating cell, located adjacent to a heating cell and exhibiting higher temperatures than neighbouring cells. The second pattern observed during the experiments was a “hotspot” pattern, in which the mite-infested cell was located at the centre of a localized temperature increase. In these cases, the temperature of parasitized cells was on average 0.65 °C higher than that of surrounding cells.

The results obtained in the two-year experiment showed consistent trends in both infected and intact cells. In 2022, the average temperature of parasitized cells was 30.78±0.09 °C, approximately 0.82 °C higher than that of healthy brood cells (29.96±0.08 °C).

Similarly, the 2023 experiments confirmed that parasitized cells exhibited higher temperatures (30.62±0.14 °C) than neighbouring healthy cells (29.86±0.08 °C), corresponding to an average increase of 0.76 °C. In both experimental years, the temperature of parasitized cells was significantly higher than that of intact cells (p<0.001).

4. CONCLUSIONS AND RECOMMENDATIONS

Based on our investigations, it can be concluded that non-invasive CT imaging, which represents a novel approach in apicultural research, is suitable for detecting the presence of *V. destructor* within brood cells. However, the sensitivity of the method strongly depends on the developmental stage of the mites present in the cell. This can be explained by the biological characteristics and reproductive cycle of female mites (Rosenkranz et al., 2010; Ramsey et al., 2019; Warner et al., 2024).

As host development progresses, the degree of parasitism can be determined with increasing accuracy. This phenomenon can be explained by the concealed behaviour of female mites at the early stages of brood development, when they remain hidden at the bottom of the brood cell and only the foundress mites are present (Rosenkranz et al., 2010; Warner et al., 2024). During feeding, mite nymphs typically exploit the wound created by the mother mite on the fifth abdominal segment of the host, which is located near the so-called fecal accumulation zone at the bottom of the brood cell. This observation was also confirmed in our experiments (Kanbar and Engels, 2003; Rosenkranz et al., 2010).

Our investigations confirmed that parasitism significantly affects several parameters of developing honey bees. Although differences in body volume and surface area between healthy and parasitized individuals were not detectable, body mass, radiodensity, body length, and body composition differed significantly between the two groups. These findings are consistent with the conclusions of Duay et al. (2003), who reported reduced body mass in parasitized drone brood at the red-eyed developmental stage. The decrease in radiodensity observed in the tissues of developing honey bee pupae affected by varroosis can be explained by the reduction of both organic compounds with higher radiodensity (e.g. proteins and carbohydrates) and inorganic components (e.g. water and minerals) (Annoscia et al., 2012). These observations are in agreement with previous studies showing that the parasite not only consumes the host fat body but also reduces the content of the host hemolymph (Ramsey et al., 2019).

Furthermore, varroosis induces degenerative development in honey bee pupae, reduced lipid and protein synthesis, dehydration, and metabolic disturbances, which ultimately contribute to increased winter mortality, shortened lifespan, and decreased pesticide tolerance (Van Dooremalen et al., 2013).

In this study, we were the first to characterize morphological changes occurring during the preimaginal developmental period after brood cell capping, including changes in average body volume, radiodensity, surface area, and body proportions. In addition, we provide the first evidence that the combined effects of parasitism and DWV infection primarily induce abdominal deformities during honey bee development.

Micro-CT analyses clearly demonstrated that structural deformation of comb cells resulting from long-term use negatively affects the development of honey bee larvae and pupae (Berry and Delaplane, 2001). By applying micro-CT technology, these structural alterations and their consequences were visualized non-invasively in three-dimensional images for the first time. The results are consistent with previous observations indicating that aged comb negatively affects colony performance (Berry and Delaplane, 2001; Al-Kahtani, 2018).

The present study also demonstrated that modern imaging techniques can be applied effectively in apicultural research in a complementary manner. Human diagnostic CT allows rapid evaluation of larger comb sections, while micro-CT enables the detection of fine structural details. Our findings provide a scientifically sound basis for the importance of replacing old and heavily used combs, which may contribute to maintaining colony vitality and productivity in the long term (Berry and Delaplane, 2001; Al-Kahtani, 2018; Taha and Al-Kahtani, 2020).

Experiments designed to assess the harmful effects of X-ray irradiation demonstrated that high-energy X-ray exposure increases mortality in both the host and the parasite in a dose-dependent manner and induces significant immunological changes in honey bees at doses above 15 Gy. However, these dose levels are several orders of magnitude higher than those applied during diagnostic imaging procedures (Easton,

2012). Therefore, our results confirm that X-ray-based diagnostic imaging techniques can be safely applied in apicultural research, particularly in studies focusing on *V. destructor*. With respect to pest control applications, however, X-ray irradiation did not achieve the desired level of effectiveness. Although the 15 Gy dose produced more promising results than higher exposures, the practical applicability of this method remains limited, as both honey bee lifespan and immune function may be adversely affected.

Measurements performed with a handheld infrared camera demonstrated that parasitism induces significant temperature differences in capped brood. The approximately 0.8 °C temperature increase recorded in infested cells reliably distinguished parasitized pupae from intact ones. This phenomenon can be explained by the social fever response of honey bee colonies, which likely functions to impair the survival conditions of the parasite (Seeley, 2009; Bauer et al., 2018; Goblirsch et al., 2020).

To our knowledge, this phenomenon has been demonstrated for the first time *in situ* specifically in relation to *V. destructor*. Based on these findings, infrared thermography appears to be a promising non-invasive diagnostic tool for the field detection of varroosis and may also have potential applications in selective breeding programs. Nevertheless, wider implementation of the method will require further technological development, particularly through the integration of AI-based image analysis systems.

Overall, the present research revealed the potential of two novel diagnostic approaches in apicultural research: infrared thermographic analysis and human diagnostic CT imaging. The practical implementation and wider use of these methods may open new opportunities for both scientific and applied research that have previously been difficult to achieve.

5. NEW SCIENTIFIC RESULTS

1. We were the first to demonstrate the applicability of human diagnostic computed tomography (CT) for the *in-situ* detection of *Varroa destructor* within *Apis mellifera* sealed brood cells.
2. Using non-invasive imaging techniques, we were the first to document developmental abnormalities and tissue composition alterations in the preimaginal stages of honey bees, with the mean density change of infested pupae reaching 1.657 HU (1.481%) (p=0.001).
3. The dissertation presents the first quantitative micro-CT analysis of intact *Apis mellifera* brood cells and pupae, providing direct structural evidence that comb aging, wall thickening, and reduced cell volume for larval and pupal development (-12.22% in volume **(**p<0.05)).
4. Dose- and time-dependent high energy X-ray irradiation experiments define biological response thresholds in both *Apis mellifera* and *Varroa destructor* (effect of X-ray on mortality of examined organisms $p < 0.001$). The results provide the first evidence in *Apis mellifera* that ionizing radiation induces dose-dependent shifts in plasmatocyte (p<0.001) and prohemocyte counts ($p < 0.001$).
5. This work delivers the first *in situ*, field-based demonstration that *A. mellifera* colonies exhibit localised temperature elevation of Varroa-infested brood cells (+0.79 °C), confirming social fever as an active, collective immune defense mechanism (p=0.001).

PUBLICATIONS AND PRESENTATIONS

Peer-reviewed papers relevant to the dissertation:

Keszthelyi, S., **Sipos, T.**, Csóka, Á., & Donkó, T. (2021), CT-supported analysis of the destructive effects of *Varroa destructor* on the pre-imaginal development of honey bee, *Apis mellifera*. Apidologie, 52(1), 155-162.

Sipos, T., Donkó, T., Jócsák, I., & Keszthelyi, S. (2021), Study of Morphological Features in Pre-Imaginal Honey Bee Impaired by *Varroa destructor* by Means of Computer Tomography. Insects, 12(8), 717.

Sipos, T., Donko, T., Csoka, A., Kiss, T., & Keszthelyi, S. (2023), Comparative micro-computed tomographic analysis of the structure of brood cells and its effect on the development of the pupae of honey bee (*Apis mellifera*). European Journal of Entomology, 120.

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Oral presentations and conference abstracts:

Sipos T., Donkó T., Jócsák I., Keszthelyi S., (2021), A varroatózis méhegészségügyi vonatkozásainak feltárása CT képalkotásra alapozott non-invazív technikával, LXIII. Georgikon Napok Nemzetközi Tudományos Konferencia Méhészeti Workshop, 2021. 10. 7-8. Keszthely

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