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Original Research

In vitro assessment of Tropilaelaps mercedesae survival across different substrates

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ABSTRACT

Tropilaelaps spp. are parasitic mites that feed and reproduce within honey bee brood (*Apis spp.*), causing significant damage to *Apis mellifera* colonies. While traditionally believed to be incapable of surviving without brood, recent findings suggest *T. mercedesae* may persist during broodless periods. This study aimed to investigate the survival potential of T. mercedesae on various matrices in the absence of brood, with a focus on understanding possible mechanisms supporting its persistence and spread.

An in vitro survival experiment was conducted using *T. mercedesae* mites placed on three matrix types: live adult bees (*A. dorsata / A. mellifera*), decomposing pupae, and decomposing adults. Mite survival was monitored over time under controlled conditions. Survival duration was recorded and analysed using Log Rank tests and visualized with Kaplan Meier survival curves to identify differences in survival across matrices.

Mites survived for over 96 hours on live adult *A. mellifera*, over 144 hours on decomposing pupae, and up to 168 hours on decomposing adults. These findings demonstrate the mite's ability to survive for extended periods without access to live brood, challenging existing assumptions about its biology and survival limits.

This study highlights a potential survival mechanism of *T. mercedesae* outside brood environments, which may contribute to its spread through previously considered low-risk pathways, such as queen/package bee trade and used beekeeping equipment. These findings underscore the need for updated biosecurity protocols and further research into transmission dynamics and control strategies.

Keywords: *Apis mellifera, Tropilaelaps,* survival transmission, honey bee mites, broodless survival, parasitic mites, matrix-dependent viability.

INTRODUCTION

Western honey bee (*Apis mellifera*) queens, packages and colonies are frequently transported on a local, national, and international level as an agricultural pollinator and for the production of bee hive products such as honey, wax and propolis. This movement of honey bees has allowed for the global transmission and spread of multiple honey bee pests and pathogens such as *Varroa destructor*, small hive beetle (*Aethina tumida*) and American foulbrood (*Paenibacillus larvae*) (Neumann & Ellis, 2008; Roberts & Anderson, 2015; Papić et al. 2021). The ectoparasites *V. destructor* and *Tropilaelaps spp.* have jumped species from their natural honey bee hosts Apis cerana and Apis dorsata respectively to parasitise all Apis species of honey bee (Oldroyd, 1999; de Guzman et al. 2017). Both mites have spread outside of the ranges of their natural hosts, with *V. destructor* now being found on every continent and in almost every country where *A. mellifera* is present (Traynor et al. 2020; Owen et al. 2021). Currently the spread of *Tropilaelaps spp.* is not fully verified and until recently it was believed to be restricted to Asia (Chantawannakul et al. 2015). However, the presence of *T. mercedesae* has recently been confirmed in colonies of A. mellifera in the Krasnador and Rostov-on-don regions of Russia and the Samegrelo-Zemo Svaneti region of Georgia, thus confirming its presence in Europe for the first time (Brandorf et al. 2024; Janashia et al. 2024; Namin et al. 2024).

V. destructor causes physical damage to honey bee brood and vectors viruses during feeding, causing significant colony losses and is considered a major threat to global beekeeping (Rosenkranz et al. 2010). *Tropilaelaps spp.* are similar to *V. destructor* in that they primarily reproduce within sealed brood cells. Of the

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four know species of *Tropilaelaps* (*T. mercedesae, T. clareae, T. koenigerum and T. thaii*) *T. mercedesae* is the most widespread and is regarded as a more damaging parasite of *A. mellifera* than *V. destructor* (Anderson & Morgan, 2007). During feeding *Varroa* mites will open one or two large wounds on the brood to facilitate communal feeding of mites present within the sealed cell (Kanbar & Engels, 2005). In contrast *T. mercedesae* feed on both pre and post capped stages of brood, utilising multiple feeding sites, which can then go on to form wounds and deformities in the adult bee causing higher brood mortality than *V. destructor* infestations (Phokasem et al. 2019). Both *V. destructor* and *T. mercedesae* have been shown to vector viruses when they feed (Dainat et al. 2008; MinOo et al. 2018). When *T. mercedesae* vector deformed wing virus (DWV) there is a significant reduction in the longevity and emergence weight of parasitised hosts, plus an increase to the level of DWV load and associated clinical symptoms (Khongphinitbunjong et al. 2016). *T. mercedesae* are a major vector of honey bee pathogens and Turong et al. (2021) demonstrated that 100% of *T. mercedesae* mites they examined harboured DWV compared to only 81.8% of *V. destructor* mites examined in the same study. Israeli acute paralysis virus (IAPV) (47.4%), sac brood virus (SBV) and *Ascosphaera apis* (31.6%) were also prevalent honey bee pathogens detected in *T. mercedesae* (Turong et al. (2021). *T. mercedesae* are known to reproduce more quickly than *V. destructor* (Buawangpong et al. 2015) and unmated females have the ability to reproduce via deuterotokous parthenogenesis, producing both male and female offspring (de Guzman et al. 2018). The smaller size, increased mobility and shorter phoretic phase of *Tropilaelaps spp.*, coupled with the similarities between the visual symptoms of infestation, makes their detection and management more difficult than that of *V. destructor* (Pettis et al. 2013; Gill et al. 2024). To combat the high level of colony mortality caused by *Tropilaelaps spp. A. mellifera* colonies kept in infested areas require continual prophylactic treatment with miticides (Rinderer et al. 1994).

Understanding the mechanisms by which *Tropilaelaps spp.* may be transmitted is crucial to controlling their global spread. There is conflicting evidence as to whether *Tropilaelaps spp.* can survive and feed on adult bees. Rinderer et al. (1994) reported that *Tropilaelaps spp.* did not feed on adult *A. mellifera* and were therefore unable to survive for longer than 3 days without the presence of brood. However, possible feeding of *Tropilaelaps* mites at the soft membranes of the wing axillaries was reported by de Guzman et al. (2017). Equally under laboratory conditions Koeniger & Muzaffar (1988) observed *Tropilaelaps* survive for more than 5 days on *A. mellifera* pupae. Pettis & Chaimanee (2019) also observed *T. mercedesae* survive on *A. mellifera* and *A. cerana* larvae for 9-10 days and 5 days respectively under laboratory conditions. Less is known about the survival of *Tropilaelaps spp.* on their natural hosts *A. dorsata*. However, during migration *A. dorsata* colonies will 'bivouac' and not produce comb or brood for several weeks at a time (Robinson, 2012) with *Tropilaelaps* mites surviving by some unknown mechanism.

Beekeeping equipment and hive products pose an additional transmission route, and the World Organisation for Animal Health (OIE) recommends restricting the trade of honey bee products which are produced in colonies infested by *Tropilaelaps* mites (OIE, 2024). However, only limited research has been undertaken to assess the survival of *Tropilaelaps* mites on beekeeping equipment and hive products. Khongphinitbunjong et al. (2019) found that *T. mercedesae* were able to survive for up to three days in dry pollen and up to six days in honeycomb and Pettis & Chaimanee (2019) found that *Tropilaelaps* mites did not survive for longer than 3 days on honey/pollen, sugar candy and royal jelly.

This study set out to examine the survival of *T. mercedesae* on a range of matrices that represent transmission scenarios that might occur during the movement of honey bees (*A. mellifera* and *A. dorsata*), beekeeping equipment and hive products.

MATERIAL AND METHODS.

Study area and duration: The study was undertaken at Chaing Mai University, Thailand in July 2024.

Mite collection.

Adult mites were collected from colonies located at Chiang Mai University. Brood frames from colonies where phoretic mites were observed were selected from 10 colonies. Sealed brood was uncapped using forceps and mites were collected either with an entomological aspirator (pooter) or a slightly moist fine tipped paintbrush. Mites could be encouraged to leave the brood cells by blowing over the uncapped cells or by tapping the side of the brood frame on a solid surface. Mites were visually examined and confirmed as *T. mercedesae* and pooled in a sealable plastic container (350 ml) with freshly obtained 5th instar *A. mellifera* larvae prior to being individually transferred to treatment containers.

Survival study.

Sealable plastic containers (350 ml) were prepared with matrices and three replicates were used in each treatment group. The treatment groups comprised of

- An empty container (control),
- Five *A. dorsata* adults collected from a wild colony at Chiang Mai University,
- Five *A. mellifera* pupae at the pink-eyed pupal stage,
- Five newly emerged *A. mellifera* adults,

- Five newly emerged freshly euthanised *A. mellifera* adults.

A sugar cube was glued to the side of containers containing live bees to act as a feeder and a ventilation hole covered with 0.025 mm nylon mesh was also added. *A. mellifera* pupae at the pink eyed development stage and adults were collected from frames and colonies that were randomly selected from ten colonies at Chiang Mai University. Pupae and adult bees were pooled in sealed containers before being assigned to replicate containers. Thirty *T. mercedesae* mites (n = 90 mites per treatment) were carefully individually introduced to treatment containers with a fine-tipped paintbrush. Containers were then sealed with parafilm and randomly placed in an incubator at 34 ^{O}C and 60% R.H. to limit possible spatial effects. *T. mercedesae* mortality was assessed every 24 hours until all the mites were dead. Mites were deemed dead if they were immobile, could not be encouraged to move when gently stimulated with a fine tipped paintbrush and were observed to have their legs curled up beneath them. It should be noted that the pink-eyed pupae and dead A. mellifera adults were not replaced and decomposed throughout the trials.

STATISTICAL ANALYSIS.

All statistical analysis was performed using R Studio (Antoch, 2008). Since the data were not normally distributed non-parametric tests were used to assess *T. mercedesae* survival between different treatments. Treatments were compared using Log Rank tests and visualized with Kaplan Meier survival curves (Fig 1.). The level of significance was p < 0.0001.

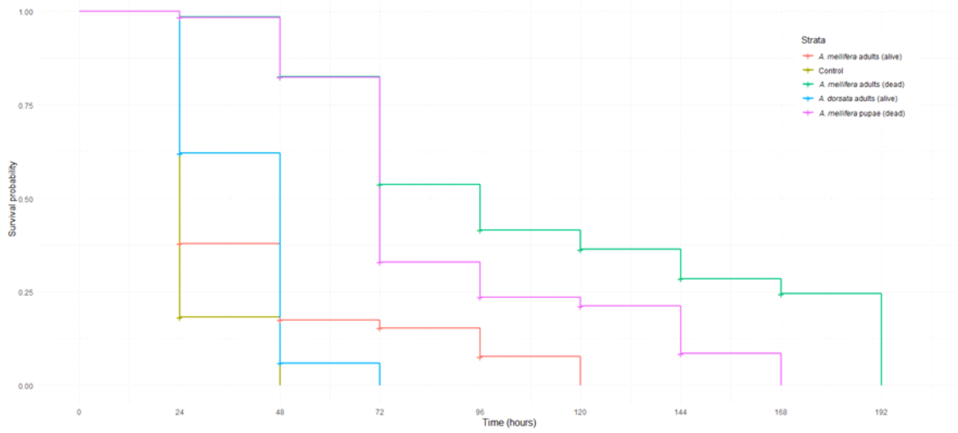

Figure 1. Kaplan-Meier survival curve of *T. mercedesae* mites across five treatments: live *A. mellifera* adults, dead *A. mellifera* adults, dead *A. mellifera* pupae, live *A. dorsata* adults, and empty control (p < 0.0001; n = 30 mites per treatment).

RESULTS.

The mean survival time of *T. mercedesae* mites was 13.3 hours in the control treatment, 15 hours in the treatment group containing live *A. mellifera* adults, 21.8 hours in the treatment group containing live *A. dorsata* adults, 54.6 hours in the treatment group containing dead *A. mellifera* pupae and 55.6 hours in the treatment group containing dead *A. mellifera* adults. The final *T. mercedesae* died after 168 hours in the treatment group containing dead *A.*

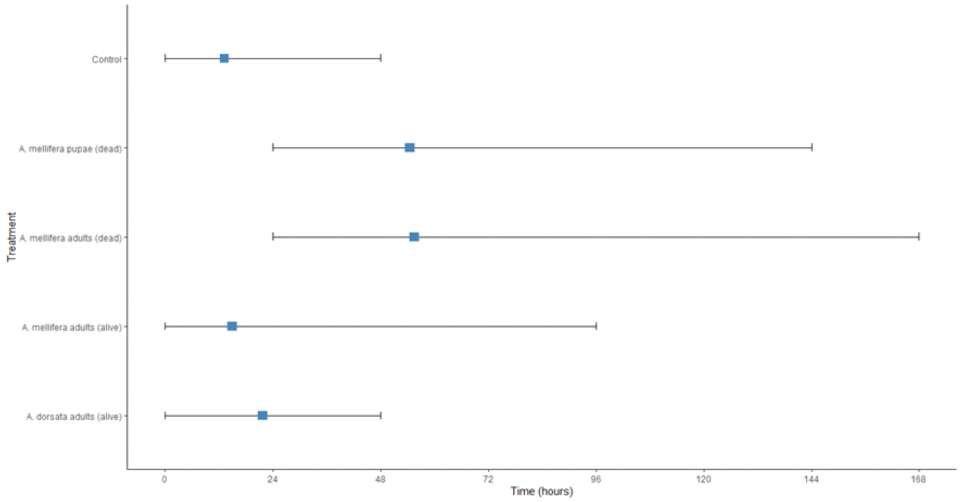

Figure 2. Diagram showing the survival times of *T. mercedesae* with blue squares showing mean survival time.

mellifera adults and survived for significantly longer than mites in the other treatment groups (Fig 2.).

A log rank test (Wilcoxon - Breslow) determined that the survival distributions between the differing treatments were statistically significantly ($\chi2(4) = 478.71$, $p < 0.0001$). Pairwise comparison using the Log Rank test showed that there was a statistically significant difference in survival distributions between all treatment groups apart from live *A. dorsata adults* vs live *A. mellifera* adults and p values are shown in Table 1.

	Live *A. mellifera* adults	Control	Dead *A. mellifera* adults	Live *A. dorsata* adults
Control	$p<0.001$	-	-	-
Dead *A. mellifera* adults	$p<0.0001$	$p<0.0001$	-	-
Live *A. dorsata* adults	$p = 0.0776$	$p<0.0001$	$p<0.0001$	-
Dead *A. mellifera* pupae	$p<0.0001$	$p<0.0001$	$p<0.01$	$p<0.0001$

Table 1. *p-values* from pairwise comparison using the Log Rank test.

DISCUSSION.

T. mercedesae mites experienced high mortality in the control group, with 90% of mites not surviving for more than 24 hours in the treatment which consisted of an empty container with no food source, and these results corroborate those of Koeniger & Muzaffar (1988) and Pettis & Chaimanee (2019). However, *T. mercedesae* survived for longer than previously observed in other studies in the treatment groups containing dead *A. mellifera* adults, dead *A. mellifera* pupae and live *A. mellifera* adults (Koeniger & Muzaffar, 1988; Pettis & Chaimanee, 2019). De Guzman et al. (2017) observed the apparent feeding of *T. mercedesae* on adult *A. mellifera* where the mites mouthparts appeared to pierce the soft membrane of the wing axillaries and this was accompanied by a pumping or pulsating motion of the opisthosoma. Feeding on live adult bees was not observed during this study, however a small proportion of mites (~10%) were able to survive for significantly longer on live adult *A. mellifera* than mites in the control group, which could suggest that these mites were able to feed on the adult bees. Another possibility is that *Tropilaelaps*, like Braula coeca, may receive food during trophallaxis between bees. Koeniger & Muzaffar (1988) observed the “conspicuous reactions of mites” during trophallaxis between *A. dorsata* and suggested that mite feeding might be taking place. This theory might also explain why the survival distribution between the live *A. mellifera* and live *A. dorsata* treatments was not statistically significantly different, although both groups did survive for significantly longer than the control group. Mites did not survive for longer than 48 hours in the treatment group containing live *A. dorsata* adults, which is again in line with the findings of Koeniger & Muzaffar (1988). Koeniger & Muzaffar (1988) observed injuries and lost appendages on *T. mercedesae* in their A. dorsata treatment groups, whereas the mites in other treatment groups were uninjured. The dead mites in this study were not examined for injuries, however *T. mercedesae* survived for up to 48 hours longer on live *A. mellifera* when compared to live *A. dorsata* and this may be due in part to the parasite host relationship and grooming behaviours of *A. dorsata*.

T. mercedesae mites survived for up to 168 hours in the treatment group containing dead *A. mellifera* pupae and up to 192 hours in the treatment group containing dead *A. mellifera* adults and it should be noted that dead pupae and adults were not replaced throughout the trial but instead decomposed. It is unclear if Pettis & Chaimanee (2019) replenished the *A. mellifera* larvae used in their study to achieve mite survival of 9-10 days (216 – 240 hours) or allowed them to decompose, however Koeniger & Muzaffar (1988) replaced *A. mellifera* pupae every 3 days and observed mite survival of up to 120 hours. Mites were observed spending the majority of their time on the dead pupae and dead bees and appeared to feed on the exudate created during decomposition. This finding suggests that *T. mercedesae* could be transported in scenarios where live *A. mellifera* brood and adults are not present, such as in used beekeeping equipment containing decaying brood and bees or in queen shipments and packages where bees have died during the caging / packaging and transportation process. Feral colonies have been transported to countries outside of the current distribution of *T. mercedesae* on shipping containers, boats, and airplanes (Heersink et al. 2016) and it has been assumed that broodless colonies and colonies that have died at sea do not pose a transmission risk for *Tropilaelaps spp.* (EFSA, 2013). Our findings suggest that aside from the obvious transmission root of brood within feral *Apis spp.* colonies *T. mercedesae* could survive in a broodless scenario on live adult bees or on decaying brood or adult bees that have died during transportation.

T. mercedesae can survive during broodless periods in *A. mellifera* colonies (Brandorf et al. 2024) and during broodless periods on their natural hosts *A. dorsata* during bivouacking (Robinson, 2012), but the mechanisms by which they are able to do so are little researched and not understood. This study has demonstrated *T. mercedesae* ability to survive on decaying *A. mellifera* brood and bees and live newly emerged *A. mellifera* bees, and these findings coupled with that of Robinson (2012) and Brandorf et al. (2024) demonstrate that the transmissibility of *T. mercedesae* is higher than previously evaluated. Given the increasing global spread of *T. mercedesae* and its recent incursion into regions bordering areas which produce significant amounts of honey each year such as Ukraine and Türkiye (Popescu et al. 2024) and given that political and socioeconomic factors in these regions could impact successful monitoring for *Tropilaelaps spp*. it is important that beekeepers and authorities globally are vigilant to the threat that the transmission of *Tropilaelaps* poses.

CONCLUSION.

In conclusion, this study demonstrates that *T. mercedesae* can survive significantly longer on decaying *A. mellifera* brood and adults than previously documented, with survival of up to 192 hours. A small proportion of mites also persisted on live adult bees, suggesting possible feeding or trophallactic interactions. These findings highlight that *T. mercedesae* may be transmitted not only through brood but also via dead bees or brood in hive products and equipment. Such survival potential in broodless or decomposing environments broadens known transmission pathways and underscores the need for heightened biosecurity measures. This expanded understanding of *T. mercedesae* survivability should inform global management and trade policies to curb its spread.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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