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Original article

Interactions among *Varroa jacobsoni* mites, acute paralysis virus, and *Paenibacillus larvae larvae* and their influence on mortality of larval honeybees in vitro

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Abstract – The mortality of honey bee larvae and pupae reared in vitro caused by various combinations of oral inoculation with *Paenibacillus larvae larvae* spores, acute paralysis virus (APV), and infestation with *Varroa jacobsoni* was studied. The effect of the mite itself and the mite acting as a vector of APV on the mortality of larvae and pupae was investigated. Mortality caused by *P. l. larvae* ranged from 25 to 55% depending on spore dose. Oral inoculation with APV caused 9% mortality, which was not additive to the mortality caused by *P. l. larvae*. *P. l. larvae* did not induce the activation of APV infection. The mortality caused by *V. jacobsoni* itself was 25%, and by mites transmitting APV, 55%. Neither the mites themselves or the mites transmitting APV had an additive effect on mortality caused by *P. l. larvae*. The study suggests that APV transmitted by mites is the most significant cause of mortality of the treatment combinations tested. The results do not suggest that the mite itself or the mites transmitting APV act as a stress factor provoking clinical symptoms of American foulbrood (AFB) in individual larvae in the in vitro rearing system.

Varroa jacobsoni* / *Paenibacillus larvae larvae* / American foulbrood / acute paralysis virus / APV / honey bee / *Apis mellifera

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1. INTRODUCTION

American foulbrood (AFB) is a serious disease of honeybee brood caused by the spore forming bacterium *Paenibacillus larvae larvae* (White). The number of spores required to manifest clinical symptoms of AFB in a colony is variable [22, 29, 47] and related to larval resistance [42, 48] and the adult bees' ability to detect and remove infected larvae before the bacteria reach the infective sporulating stage [53]. Furthermore, environmental factors such as temperature, nectar flow [29] and pollen supplement [39] may influence the manifestation of clinical symptoms of the disease.

For the past 10 years, there has been an increase in the incidence of AFB in Europe. In former Czechoslovakia [16] and Austria [20] this was supposedly attributed to an increase in the number of colonies infested with the parasitic mite *Varroa jacobsoni*, Oudemans. From 1989 to 1991, a doubling of the number of cases of AFB was reported in Western Germany [32], and Otten [33] reported a correlation between symptomatic AFB infections and varroa infestations. It has been suggested that susceptibility to viral and fungal infections is altered if the bees are infested with external parasites that damage their integument [19] and that *V. jacobsoni* mites act as inducers of viral [9] and bacterial infections [19, 28], and carriers of fungal infections [30] of honey bees. However, it has been suggested that the *V. jacobsoni* mite is not a vector of *P. l. larvae* [1, 34]. Although *P. l. larvae* spores have been found on the body surface of mites [1, 49] they have not been detected in the gut lumen of mites from infected bee colonies [49]. On the other hand, a clear correlation between infestation with *V. jacobsoni* and certain virus infections is reported [13]. The mite has been implicated as a virus vector in several cases [12, 13] but in other cases it is not yet established whether mite infestations merely stress honey bee colonies to such an extent that the colonies become more susceptible to latent virus infections [6]. The latter relationship may also exist between *V. jacobsoni* infestations and the manifestation of clinical symptoms of infections with *P. l. larvae*. Latent infections with *P. l. larvae* (spores detected in honey without apparent clinical symptoms in the colony) have been reported from several countries [20, 23–25, 40] and sublethal infection with *P. l. larvae* may make the larvae more susceptible to acute paralysis virus (APV) as has been suggested with European foulbrood and Kashmir bee virus [3].

The aims of the present study were to elucidate if the *V. jacobsoni* alone, APV (one of the viruses the mite is a vector for [10]) alone, or the combination of the mite and APV has an influence on the larval susceptibility to *P. l. larvae* infection. The relationship was studied in vitro as this method enabled us to exclude the influence of nursing or cleaning bees.

2. MATERIALS AND METHODS

2.1. Larval and pupal material

The test larvae and pupae originated from 11 *Apis mellifera* ligustica Spinola colonies. The queens of the colonies were sisters of a Danish strain, mated on an island with a known line of drones. The colonies were naturally non-infested with *V. jacobsoni* mites and had never been treated with acaricides. The larvae were obtained by caging queens for 4-hour periods on broodless combs in the colonies. When the larvae reached the age of 24–28 hours, they were thereafter reared in vitro. Prior to the experiment the colonies were checked for clinical symptoms of *P. l. larvae* according to White [51] and honey samples were analysed according to Hansen [21]. As the symptoms of APV are similar to European foulbrood, brood in the test colonies was examined for visible signs of European foulbrood [52], and brood samples were analysed for the presence of *Melisococcus pluton* according

to Bailey and Ball [6] and Ritter [41], and for three viruses by immunodiffusion (see Sect. 2.6). No pathogens were detected in any of the colonies.

2.2. In vitro rearing of larvae

The larvae were reared in vitro using the method described by Rembold and Lackner [38] with the modifications described by Brødsgaard et al. [14]. An incubator with precise regulation of humidity and temperature (Biomed CO_2 incubator, ASSAB Classic T305 GF) was used for the rearing. The larvae were reared until the moment when healthy adults normally emerge (day 21). The number of dead larvae was recorded twice a day during larval feeding and the total number dead was calculated at the end of each day. Larvae were recorded dead when they stopped respiration, lost their body elasticity, and, for the group inoculated with *P. l. larvae* spores, when they also displayed the colour changes described by Jaeckel [26]. In the prepupal stages (day 8 to 10) the larvae were not checked as the they are very sensitive to vibrations during these stages (Reiche, pers. comm.).

2.3. Experimental groups

The following combinations of inoculation with *P. l. larvae* spores (see Sect. 2.4), or APV (see Sect. 2.5), or infestation with 'cleaned' *V. jacobsoni* mites (see Sect. 2.7), or APV fed mites (see Sect. 2.7) were established: inoculated with 3, 8, or 24 spores ('spore' group), fed with APV ('APV' group), infested with a 'cleaned mite' ('cleaned mite' group), infested with an 'APV mite' ('APV mite' group), inoculated with 3, 8, or 24 spores plus fed with APV, inoculated with 3 or 8 spores plus infested with a 'cleaned mite', inoculated with 3 or 8 spores plus infested with an 'APV mite', and a non-infested/infected control group. Samples of pupae of the 'APV' group, 'cleaned mite' group, 'APV mite' group, and control group were tested for the presence of APV (see Sect. 2.6). The experiments were conducted from late May to mid-July. All the experiments were repeated three times. The results were analysed statistically by means of Kruskal-Wallis Multiple Comparisons (K-W) [45].

2.4. Inoculation with *P. l. larvae* spores

Larvae were inoculated with different doses of *P. l. larvae* spores by feeding 2 µl spore solution directly to the mouth parts at a larval age of 24–28 hours. The spores were extracted from foulbrood scales of a Danish *P. l. larvae* strain, JT-79, and were prepared and counted as described in Brødsgaard et al. [14].

2.5. Oral inoculation with APV

Larvae were inoculated with 2.5 µl of a solution (1:9) of purified APV and sterile physiological saline. The inoculated amount contained approximately 2.5×10^2 ng particles [9]. The AVP solution was applied directly to the mouth parts of the larvae at a larval age of 48–52 hours. The APV was extracted from bees from heavily mite infested colonies and was purified as described by Bailey and Woods [7]. Virus concentration was measured photometrically as described by Ball [9]. At a larval age of 5–6 days (sealing time in the colony), a proportion of the larvae/prepupae of the APV-inoculated group and the control group was injected with a 0.2 µl 0.01 M potassium phosphate buffer (KPB) by means of a Hamilton syringe (33-gauge, 701-RN modified to fit repeater adapter PB 600–1) through the intersegmental membrane of the abdomen and incubated for 96 h at 35 °C, in order to stimulate the replication of inapparent virus [4]. These individuals did not participate further in the experiment and were tested for the presence of APV as described in Section 2.6.

2.6. Serology

Prior to the experiment the colonies were examined for inapparent infection (using the method described above) with the following honey bee viruses: APV, Deformed Wing Virus (DWV) and Sacbrood Virus (SBV). From each colony 30 pupae and 30 adults were tested by immunodiffusion [31] for inapparent virus infections after injection of 0.2 µl KPB and subsequent incubation for four days. All were found to be negative.

Antisera for the immunodiffusion test were prepared as described by Cooper [15]. The specificity of the antisera was tested as described by Koch [27].

After the experiments samples of the larvae, pupae and bees from all 11 colonies were stored in micro tubes at –20 °C until they were tested for the presence of APV by immunodiffusion. The detection level was approximately 10^9 particles/ml [2] which corresponds to about 10^2 ng/ml (Ball, personal communication). Furthermore, Koch's postulates were fullfilled.

2.7. Infestation with *V. jacobsoni*

The mites were obtained from capped brood from infested Danish colonies by

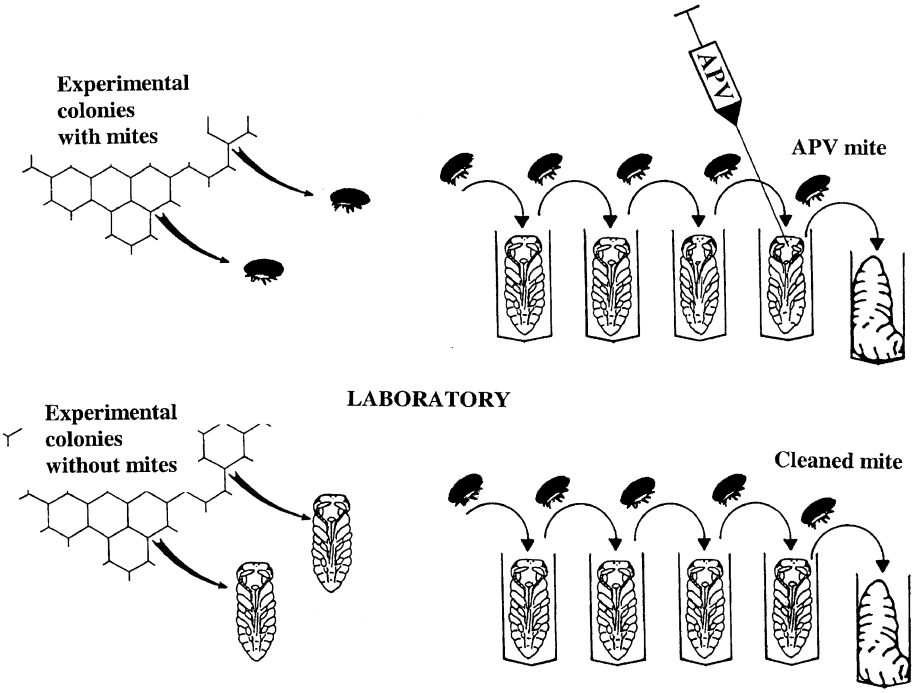

Figure 1. Infestation with *Varroa jacobsoni* in the laboratory. Test larvae and pupae originated from colonies non-infested with *Varroa* mites. The mites were obtained from capped brood from infested colonies. The mites used in the 'cleaned mite' groups were cleaned of pathogens by transferring the mites to four batches of pupae (Pw stage) at three-day intervals. The pupae were placed in micro-tubes and kept at 35 °C in an incubator. The mites in the infection group ('APV mite') were infected with APV by transferring the mites to pupae already injected with APV after the general cleaning for pathogens. When the larvae reared in vitro were 6 days old and had just reached the end of L5 stage, each of the living larvae was infested with one mite. Thereafter, the edges of the artificial cells were sealed with nylon mesh.

using a pair of sharp-pointed forceps to open the cell, forceps in a light-alloy metal to pull out the pupae, and a brush to catch the mite. Thereafter, the mites that were used in the non-infected groups ('cleaned mite') were cleaned of pathogens by transferring the mites to four subsequent batches of disease-free pupae (cf. 2.1 and 2.6) (Pw stage) at three days intervals (after defecation) (Fig. 1). The pupae were placed in micro-tubes and kept at 35 °C in the incubator mentioned above. Only mites that defecated in each of the micro tubes were used further in the experiment. The group of 'cleaned mites' used later in the infection experiments ('APV mite') was fed with APV in the following way: prior to transferring the mite to the last of the four pupae, the pupae were injected with 0.2 µl of a purified APV: 0.01M KPB solution (1:9) [35] containing approximately 2.0×10^{-7} ng APV particles (approximating 2.5×10^{9} particles) [9] by means of a syringe (described above) between the 5th and 6th abdominal segment at the ventro-lateral side [46]. When the larvae reared in vitro (in the 'cleaned' and 'APV mite' groups, cf. 2.3) were 6 days old and had just reached the end of L5 stage, each of the living larvae was infested with one mite. Thereafter, the edge of the artificial cells were lubricated and covered with nylon mesh (Streno®, mesh size 100 µm) in order to confine the mites to the cells. The handling was done at 35 °C by means of an infrared lamp with a thermoregulator. 16 of the injected pupae used to feed mites with APV, were subsequently tested for the presence of APV as described in Section 2.6.

3. RESULTS

We tested our APV infection methodology by immunodiffusion tests of samples of our experimental groups (Tab. I). No APV was detected by immunodiffusion in larvae in the 'no-mite' groups. The APV inoculation groups showed 15% positive reactions when not injected with KPB and 79% positive when injected. Of the tested pupae infested with an 'APV mite' 80% were positive. Furthermore, of the APV injected pupae used to feed mites with APV, 94% were positive by immunodiffusion.

The control mortality (larvae not inoculated/infested) was approximately 25% (Figs. 2 and 3). A significant difference was seen between the control group and the group only inoculated with virus, which had a mortality of approximately 34% (K-W, $p < 0.05$) (Fig. 2). When the larvae were only inoculated with spores the mortality ranged from about 50 to 82% and from 58 to 86% in the group also inoculated with APV. No significant difference between these results was found. However, the tendency seen in Figure 2 suggests a slightly higher death-rate in the APV inoculated larvae. Furthermore, a significant difference was found between the control group and the

Table I. Methodology test of APV handling. Number of larvae, pupae, or adults tested positive or negative, respectively, for acute paralysis virus (APV) by immunodiffusion.

Treatment	APV negative	APV positive	% APV positive
APV inoculation	23	4	15
APV inoculation + KPB injection	14	51	79
control (0 spores, 0 APV, 0 mites)	35	0	0
control + KPB injection	67	0	0
'cleaned mite'	18	0	0
'APV mite'	4	16	80

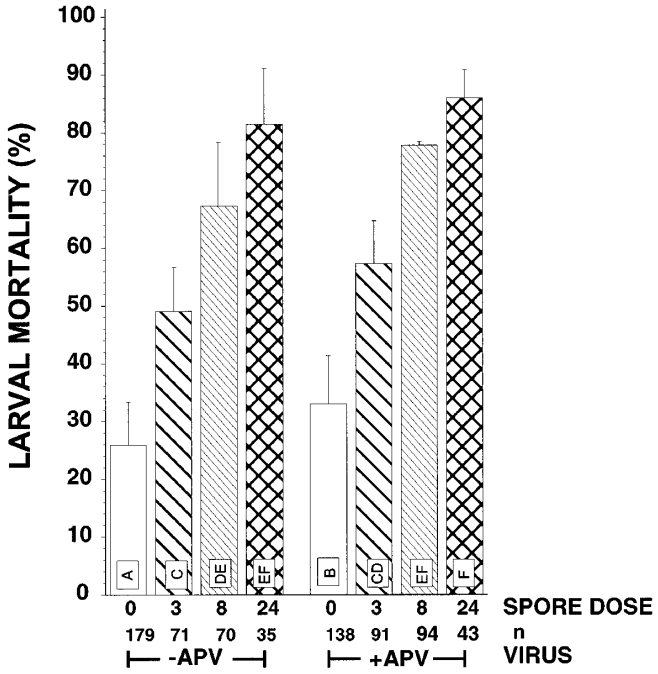

Figure 2. Mean percent (+ s.e.) mortality of honeybee larvae/pupae inoculated with *Paenibacillus larvae larvae,* APV and uninoculated controls. The larvae were inoculated with 3 different doses of *P. l. larvae* at a larval age of 24–28 h. The larvae were inoculated with a single APV dose (2.5×10^9 particles) at a larval age of 48–52 h. n = number of individuals per group. Means with the same letter are not significantly different ($p < 0.05$, Kruskal-Wallis Multiple Comparisons).

groups inoculated with spores (K-W, $p < 0.05$). No significant difference was seen between the larvae inoculated with 8 and 24 spores in either of the virus groups. However, significance was seen in both the APV inoculated and the group not inoculated with APV between larvae inoculated with 3 spores and 8 or 24 spores (K-W, $p < 0.05$).

The results of the mite part of the experiment are shown in Figure 3, where the control group (no spores, no mites) is a proper subset of the control group shown in Figure 2. The control group differed significantly from all other groups (K-W, $p < 0.05$). In the 'no mite' group inoculated with 3 and 8 spores, the mortality was 49 and 67%, respectively. In the 'clean mite' group, the corresponding mortalities were 56 and 80%, respectively. The tendency of a higher mortality in the 'spore' groups of 'clean mite' compared to 'no mite' was not significant. In the 'APV mite' group the mortality ranged from 76 to 90%. There were no differences between the group infested with only mites and the groups infested with both mites and spores, but there were significant differences from the corresponding groups of the 'no mite' group (K-W, $p < 0.05$). Furthermore, a significant difference was seen between the groups infested with a 'clean mite' and 0 or 3 spores and the corresponding groups of the 'APV mite' group (K-W, $p < 0.05$).

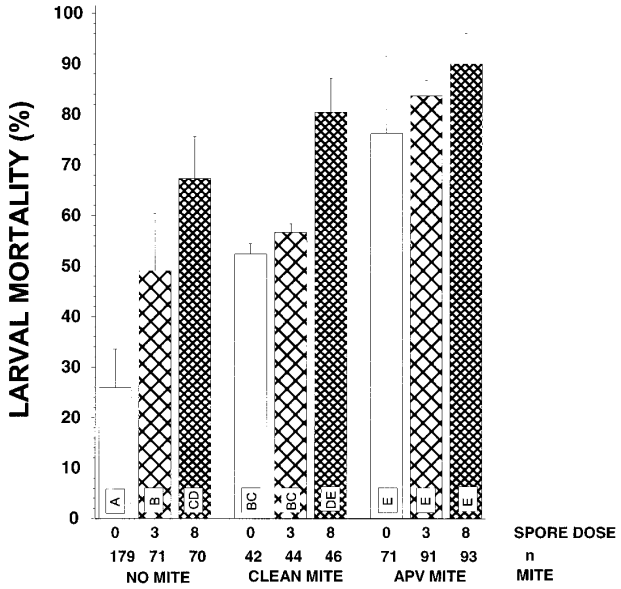

Figure 3. Mean percent (+ s.e.) mortality of honeybee larvae/pupae inoculated with *Paenibacillus larvae larvae*, infested with APV transferring *V. jacobsoni* mites or 'clean mites' and uninoculated controls. The larvae were inoculated with 2 different doses of *P. l. larvae* at a larval age of 24–28 h. The larvae were infested with mites at a larval age of 6 days. n = number of individuals per group. Means with the same letter are not significantly different ($p < 0.05$, Kruskal-Wallis Multiple Comparisons).

4. DISCUSSION

The mortality of in vitro reared bees does not exceed 10% if the rearing is stopped at a larval age of 14 days (Brødsgaard, unpublished data). The relatively high control mortality (25%) seen in the present experiment is normally observed when the rearing is continued until emergence of adults (Reiche, pers. comm.).

In the present experiment each larva was fed 2.5 µl APV solution containing approximately 2.5×10^9 APV particles [9], and the mortality was only 8% greater than the control (Fig. 2). This low mortality corresponds with our result that only 15% of the larvae gave a positive reaction to APV antiserum in immunodiffusion tests and the findings of Bailey et al. [8] who reported that when fed to adult bees the LD_{50} of APV is 10^{11} particles. APV can be transferred to larvae as an inapparent infection by adult bees secreting virus in the larval food [5]. The results of the immunodiffusion test in the KPB injected 'APV' group show that an inapparent infection with APV was present in 64% of these inoculated larvae (Tab. I). The findings correspond with the results of Anderson and Gibbs [4] showing that the injection of KPB can stimulate the multiplication of the virus to such an extent that it is recognizable by means of immune diffusion. It therefore seems that feeding larvae large amounts of APV (in the present study: 2.5×10^9 APV particles per larva) will mainly cause non-lethal inapparent infection. Furthermore, the results of the immune diffusion test suggest that the larvae of the control group were (naturally) non-infected (not expressing dormant APV) as no

inapparent infection was observed after injection with KPB.

A significant difference in mortality between the group inoculated only with spores and the group also inoculated with APV could not be found (Fig. 2). The result suggests that the AFB infection itself is severe and that APV inoculation, although it causes a significant higher mortality compared with the control group, does not have an additive effect on death due to *P. l. larvae* infection, nor is *P. l. larvae* able to induce activation of an inapparent infection with APV.

In the 'clean mite' group (Fig. 3) there was a significantly higher mortality in the group not inoculated with spores compared to the control group (no mite, no spores) suggesting that the mite itself has an influence on the larval mortality at least in the in vitro rearing system. However, in the 'clean mite' groups inoculated with 3 or 8 spores there was no difference in mortality compared to the corresponding 'no mite' groups suggesting that mites have no additive effect on the mortality caused by *P. l. larvae*.

In the 'APV mite' group there were no significant differences in mortality between the group only infested with mites and the groups also inoculated with spores, but there were significant differences from the corresponding groups of the 'no mite' group suggesting that the APV infected mite is the most severe death factor in this experiment. This corresponds to the results of Bailey et al. [8] showing that as few as 100 particles of APV can cause infection when injected into the blood. The results of the 'clean mite' group showed that the mite itself was also an important death factor in vitro though not as important as *P. l. larvae*. When the larvae are subjected to both the mite with the APV it transfers (as shown by Ball [11] and our own immunodiffusion tests), and *P. l. larvae* mite-transferred APV is the primary cause of death.

In each of the three groups inoculated with 8 spores 10–30% of the larvae survived (Fig. 3). These surviving larvae may simply be stronger in terms of general fitness, and thus, survive both pathogens. Reduced fitness may be a result of simultaneous infection with other pathogens or alterations in the immune defense of individuals. Colony fitness has been suggested as an important factor in the manifestations of clinical symptoms of disease by Otteni [35] who has shown that colonies with low fitness seem to be more susceptible to APV infection. Individuals from these colonies had a much lower LD_{50} (APV injection) compared to colonies with higher fitness [35]. Gliński and Jarosz [18] reported a remarkable reduction in total proteins in haemolymph of drone brood infested with *V. jacobsoni* and related this reduction to the infestation level of the colony. This was later supported by experiments on worker honey bees [43, 50]. From these experiments it was assumed that the reduction was a result of protein depletion caused by the sucking of the mites. Furthermore, the immune defense of the mite infested honeybees has been found to be altered in terms of reduced lysosome activity and lowered phagocytic index [18].

Ball and Allen [13] examined unsealed larvae from *V. jacobsoni* infested German colonies and found a high proportion of these to be infected with APV. They, therefore, concluded that it is unlikely that larvae become infected by mites feeding on them, and they suggested that adult bees containing multiplying APV infect larvae via the food. Later, it was shown in the laboratory that the mite acts as a vector and can transmit virus to pupae [11]. The latter is supported by the results of the present study suggesting that APV fed mites are capable of transmitting the disease to larvae in vitro causing high mortality and that the larvae though fed huge amounts of APV only seldom become lethally infected by this way of transmission. The results of the feeding experiment with APV followed by KPB injection suggest that feeding APV to the larvae by adult bees only becomes fatal

in nature if mites subsequently infest the larvae/pupae.

The results of the present experiment do not suggest that the mite itself or the APV transmitting mite acts as a stress factor provoking clinical symptoms of AFB in the individual larvae in the in vitro rearing system. However, that does not exclude *V. jacobsoni* as a stress factor in the bee colony. It has been shown that the weight of larvae from colonies infested with mites is much lower than the weight of larvae from non-infested colonies [44]. This lower weight could be due to insufficient brood care by nurse bees infested with *V. jacobsoni* [17] and/or infected with APV during the pupal stage [37] which results in poorly developed hypopharyngeal glands [36, 44]. Hence, it could be imagined that substantially fewer spores are needed to infect smaller larvae compared to larger healthy larvae. This could explain why the colonies infested with *V. jacobsoni* could be more susceptible to *P. l. larvae*.

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Résumé – Interactions entre l'acarien *Varroa jacobsoni*, le virus de la paralysie aigüe et *Paenibacillus larvae larvae* et leur influence sur la mortalité des larves d'abeilles in vitro. La mortalité des larves et des nymphes d'abeilles (*Apis mellifera* L.) élevées in vitro a été étudiée après plusieurs combinaisons d'inoculation par voie orale de spores de *P. larvae larvae*, d'inoculation par voie orale de virus de la paralysie aigüe (APV) et d'infestation par *V. jacobsoni*. Les acariens ont été soit débarrassés des pathogènes, soit infectés par l'APV. La procédure de nettoyage consistait à transférer les acariens à quatre lots successifs de nymphes d'abeilles saines à trois jours d'intervalle (Fig. 1). L'infection des acariens avec l'APV a été faite de la façon suivante : on a injecté de l'APV purifié aux nymphes d'abeilles du 4^{e} lot avant de transférer les acariens. La relation a été étudiée in vitro afin d'exclure toute influence de la part des abeilles nourrices ou nettoyeuses.

Les résultats suggèrent que l'infection par la loque américaine (AFB) est sévère avec des mortalités allant de 25 à 55 % selon la dose d'infection. Les effets de l'inoculation par voie orale de l'APV, qui a provoqué une mortalité de 9 %, ne s'additionnent pas à ceux de l'infection par *P. larvae larvae* et *P. larvae larvae* ne peut activer une infection par l'APV passée inaperçue.

La mortalité provoquée par *V. jacobsoni* seul a été de 25 %, ce qui laisse penser que l'acarien lui-même a une influence sur la mortalité larvaire au moins en élevage in vitro. Pourtant, il n'y a pas eu de différence de mortalité dans les groupes inoculés avec trois ou huit spores de *P. larvae larvae* et infestés par un acarien par rapport aux groupes correspondants non infestés. La mortalité provoquée par *V. jacobsoni* infecté par l'APV a été de 55 %. Il n'y a pas eu de différence significative de mortalité entre le groupe infesté uniquement avec des acariens et l'APV et les groupes également inoculés avec des spores de *P. larvae larvae*, mais les différences étaient significatives avec les groupes inoculés correspondants mais non infestés. Les résultats suggèrent que l'infestation par les acariens n'a pas eu d'effet s'additionnant à la mortalité causée par *P. larvae larvae*.

Ils suggèrent que les acariens vecteurs de l'APV ne sont pas le facteur de mortalité le plus élevé parmi les combinaisons testées. Mais les acariens eux-mêmes ou les acariens vecteurs de l'APV agissent comme facteur de stress, provoquant les symptômes cliniques de la loque américaine chez les larves élevées in vitro. Cela n'exclut pas néanmoins que l'acarien *V. jacobsoni* est un facteur de stress pour la colonie d'abeilles.

Varroa jacobsoni* / *Paenibacillus larvae larvae* / loque américaine / APV / virus paralysise aigüe / *Apis mellifera

Zusammenfassung – Wechselwirkungen zwischen *Varroa jacobsoni,* Akute Paralyse Viren und *Paenibacillus larvae larvae* auf die in vitro Sterblichkeit von Honigbienenlarven. Wir untersuchten die Sterblichkeit von in vitro aufgezogenen Honigbienenlarven und Puppen nach unterschiedlicher oraler Beimpfung mit Sporen von *Paenibacillus larvae larvae*, mit dem Akute Paralyse Virus (APV), und nach Infektion mit *Varroa jacobsoni*. Die Varroamilben wurden entweder von den Pathogenen gereinigt oder mit APV infiziert. Die Reinigung wurde dadurch erreicht, dass die Milben über jeweils drei Tage auf vier aufeinanderfolgende Gruppen von infektionsfreien Puppen übertragen wurden (Abb. 1). Für die Infektion mit APV wurden den Milben vor der Übertragung auf die letzte der vier Puppen gereinigte APV injiziert. Die Untersuchungen wurden in vitro ausführt, um den Einfluss der Ammenbienen oder der die Zellen reinigenden Bienenarbeiterinnen auszuschlieβen.

Die Untersuchungen legen nahe, dass bereits AFB allein ernste Folgen hat und abhängig von der Infektionsdosis eine Sterblichkeit zwischen 25 % und 55 % hervorruft. Orale Beimpfung mit APV verursachte eine Sterblichkeit von 9 %, sie bewirkte aber keinen zusätzlichen additiven Effekt auf die Sterblichkeit durch *P. l. larvae*. Weiterhin bewirkte *P. l. larvae* auch keine Aktivierung einer noch nicht in Erscheinung getretenen Infektion durch APV.

Die durch *Varroa* allein bewirkte Sterblichkeit von 25 % zeigte zumindest in dem in vitro Aufzuchtsystem einen Einfluss der Milbe auf die Larvensterblichkeit. Allerdings bestand zwischen den mit 3 oder 8 *P. l. larvae* Sporen und einer Milbe infizierten Gruppen kein Unterschied zu den jeweiligen uninfizierten Gruppen. Die durch AVPinfizierte Varroamilben hervorgerufene Sterblichkeit betrug 55 %. Es bestanden aber keine signifikanten Unterschiede der Sterblichkeit der allein mit AVP-infizierten Milben infizierten Gruppen zu den zusätzlich mit *P. l. larvae* Sporen beimpften, allerdings bestanden signifikante Unterschiede zu den entsprechenden beimpften aber nichtinfizierten Gruppen. Nach diesen Ergebnissen hat keine der Milbengruppen einen additiven Effekt auf die Sterblichkeit durch *P. l. larvae*.

Die Ergebnisse der Studie belegen, dass in den Kombinationstests die APVübertragenden Milben den stärksten Mortalitätsfaktor darstellten. Hiernach sind innerhalb des in vitro Aufzuchtsystems weder die Milben selbst oder die APVübertragenden Milben ein Stressfaktor, der die klinischen Symptome von AFB zu Tage treten lässt. Dies schlieβt allerdings nicht zwingend aus, dass die Varroamilben innerhalb der Bienenvölker einen solchen Stressfaktor darstellen könnten.

Varroa jacobsoni* / *Paenibacillus larvae larvae* / Amerikanische Falbrut / Akute Paralyse Virus / APV / Honigbienen / *Apis mellifera

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