Control of Deformed Wing Virus-B in a Commercial Apiary Following Queen Vaccination with an Experimental *Paenibacillus larvae* Bacterin

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Deformed Wing Virus (DWV) is a viral disease present in the majority of honey bee colonies worldwide. In addition to vertical transmission, DWV is vectored by *Varroa destructor* mites, which facilitate virulence through suppression of the bees' immune system. DWV and Varroa are both strongly linked to colony losses and are of great concern to beekeepers. There is no available treatment or preventative for DWV, therefore management of the disease is limited to indirect mite control. In a 400-hive placebo-controlled field study, we demonstrated that colonies with queens vaccinated using an experimental *Paenibacillus larvae* bacterin have significantly reduced DWV-B loads compared to colonies with unvaccinated queens, and that this reduction is independent of Varroa load.

Deformed Wing Virus (DWV) is one of the most widespread and destructive diseases affecting honey bee colonies (1–3), with the APHIS honey bee survey finding DWV present in over 85% of US hives since 2013 (4). DWV is split into 3 'Master' variants: the common DWV-A (5) and DWV-B (6), and the less common, DWV-C (7). While DWV-A has historically been most prevalent, DWV-B is rapidly overtaking it worldwide (8). Both DWV-A and DWV-B have been directly linked to overwintering failure (3,9), and are easily identified in the hive due to their most striking clinical symptom - undeveloped and non-functional wings on newly emerged adult bees. Bees displaying clinical DWV symptoms are typically never able to forage and die in less than a week after emerging (2).

All DWV variants propagate principally within developing honey bee pupae and are highly associated with the obligate honey bee ectoparasitic mite Varroa destructor (10). V. destructor mites act as vectors to facilitate indirect horizontal transmission of DWV between pupae (11), and increase the severity of infection by weakening their hosts immune system (12). V. destructor are themselves highly associated with colony loss (13) and are often listed as the factor of greatest concern among beekeepers (14). DWV remains an issue even in the absence of *V. destructor*, as it can be vertically transmitted in from queen to brood, or by direct horizontal transmission to larvae through food (2).

Despite its prevalence and virulence, there is no specific treatment or preventative for DWV (15). Currently, the primary method of reducing DWV levels in a colony is to reduce *V. destructor* levels (16). This is generally done using acaricides and insecticides (17) which stresses the bees (13) and contaminates honey (18). Additionally, reduction of mites does not eliminate DWV, as mites have no impact on vertical or direct horizontal transmission. It has been hypothesized that Trans-Generational Immune Priming (TGIP) using live or inactivated DWV virus could bolster honey bee immunity against DWV, though results to date are mixed (19,20).

DVW-B Levels Are Significantly Reduced in Honey Bee Colonies With Queens Vaccinated Using Experimental *Paenibacillus larvae* Bacterin, Independent of *V. destructor* Levels

We placed queens vaccinated with an

experimental *Paenibacillus larvae* bacterin into 200 colonies across 8 sites, alongside an equal number of unvaccinated control colonies, in a large commercial beekeeping operation in Georgia, USA. Vaccinate and control colonies were managed identically for an entire season. All colonies were treated for mites once per month with Amitraz and supplemented with sugar water as necessary according to local nectar availability. Mite counts were taken one week prior to vaccination (N=35 control, N=38 vaccinated) and 6 months after vaccination (N=44 control, N=44 vaccinated) by alcohol washing 300 bees taken from above brood comb. Additionally, pooled samples of adult bees from 10 hives per treatment at each of the 8 yards were taken one week before vaccination (N=8 control, 8 vaccinated), and four months after vaccination (N=8 control, 8 vaccinated). Bee samples were sent to the National Agricultural Genotyping center (21) to have DWV-B viral loads quantified by qPCR.

We found no difference in mite counts between treatments either before (Welch two sample t-test, T=0.38, df=61 P= 0.71) or after vaccination (Welch two sample t-test, T=-1.30, df=48 P= 0.20) (Figure 1). Mite counts were under 2% (6/300 bees) in >85% of sampled colonies in each treatment at both timepoints.

We found that DWV-B levels were identical between groups prior to vaccination (Wilcoxon rank sum test, W=31, P=0.96), and were significantly reduced in vaccinated hives compared to control hives 4 months after vaccination (Wilcoxon rank sum test, W=54.5, P=0.021). DWV-B quantities were reduced in all 8 yards, with an average reduction of 83% (Figure 2). To our knowledge, these data represent the first use of TGIP with a bacterial vaccine to provide protection against a virus in an invertebrate, and the first field example of direct control of DWV over an entire season, independent of mite control.*

This study demonstrates vaccinating queen honey bees with *P. larvae* bacterin provides significant cross-protection benefits against DWV-B to honey bee colonies in a commercially-relevant field setting, and that this protection occurs independent of *V. destructor* levels.
(Figures continue page 3)

**The current data do not constitute an efficacy claim for DWV-B for Dalan's existing P. larvae product. Further studies and regulatory reviews will be necessary to establish any official claims.*

About Dalan Animal Health, Inc.

Dalan Animal Health is dedicated to bringing the world transformative animal health solutions to support a more sustainable future. This platform vaccine technology uses transgenerational immune priming, allowing the maternal animal to pass immune modulators (e.g., antigens, anti-microbial molecules) to the next generation larvae before they hatch. Dalan plans to develop vaccines for other honeybee diseases and underserved industries, such as shrimp, mealworms, and insects used in agriculture. The company is headquartered in Athens, Georgia, at the University of Georgia's Innovation Hub.

Figures:

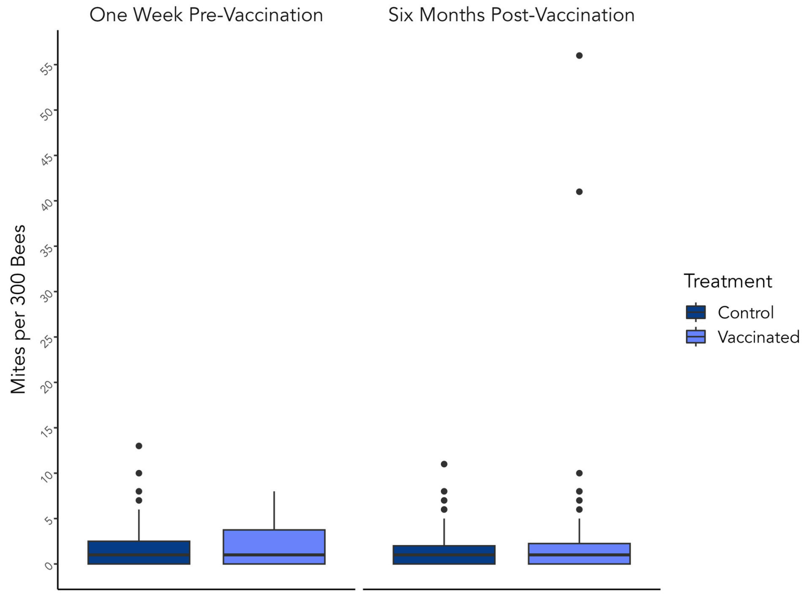

Figure 1: Mite count per 300 bees in unvaccinated (navy blue) and vaccinated (light blue) colonies. Bees were collected from above the brood comb and washed for mites one week prior to vaccination (N=35 control, 38 vaccinated colonies), and 6 months post-vaccination (N=44 control, N=44 vaccinated colonies). There was no difference in mite counts between treatments prior to (Welch two sample t-test, T=0.38, df=61 P= 0.71), or after vaccination (Welch two sample t-test, T=-1.30, df=48 P= 0.20).

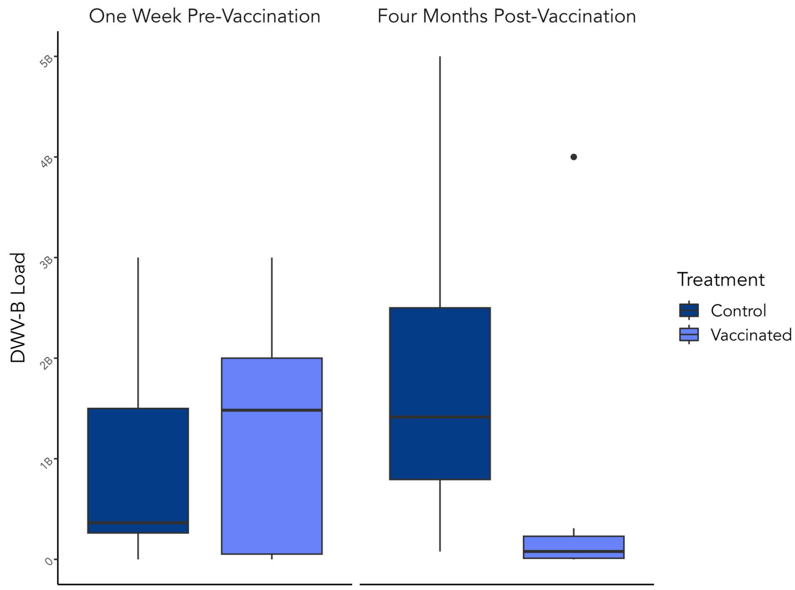

Figure 2: Box and whisker plot showing DWV-B viral load in pooled bee samples from colonies with either unvaccinated (navy blue) or vaccinated (light blue) queens both before and four months after vaccination. For each treatment, samples were pooled equally from 10 hives in 8 yards (N=8 samples/treatment). Prior to vaccination there was no difference in DWV-B viral load (Wilcoxon rank sum test, W=31, P=0.96). Four months post vaccination viral load was significantly decreased in vaccinated colonies (Wilcoxon rank sum test, W=54.5, P=0.021).

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