Monitoring the season-prevalence relationship of *Vairimorpha ceranae* in honey bees (*Apis mellifera*) over one year and primary assessment of probiotic treatment

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Supplementary Materials

Supplementary Methods

DNA extraction

Total 100 μL of the homogenized suspension was used was for DNA extraction by EDNA HISPEX (Easy DNA High-Speed Extraction) Tissue Kit (Fisher Biotec, Australia). Briefly, 64 μL of solution 1A and 16 μL of solution 1B was mixed with 100 μL homogenized suspension, incubated at 95 °C for 20 minutes, then mixed with

20 μL solution 2 for total 200 μL. The DNA sample was then 100-fold diluted with sterilized ddH_2O and store at −20 °C until used.

Absolutely quantitation of *V. ceranae* genome copy number

For the absolutely quantitation, a standard curve based on the serial-dilution of Nc-MSP was established as followed; Centrifuged at 16000 ×*g* for 3 min to removed midgut tissue of honey bees and the 1×10^7 spores per mL were harvest from the homogenized suspension as described above. The DNA was extracted as described above and then 10-fold serial dilution was performed for seven times to prepare the DNA sample with the spore concentrations from 1×10^6 spore / mL to 1×10^0 per mL. These DNA samples were subjected to real-time quantitative PCR (qPCR) to generate the standard curve of *V. ceranae* genome (y= -3.3043x + 37.909 R^2 = 0.9894). For qPCR, the ribosomal RNA gene specific primer set was design: SSU-F: 5'- CCCTGACTGGACGAACAGAAG-3'; SSU-R: 5'- CCAGCTTACGTCCTTGTTCAA A-3' was used to quantify the genome copy number of *V. ceranae* in both DNA of collected samples and DNA for standard curve. The linear standard equation for *V. ceranae* quantification was generated by plotting the crossing point (Cp) versus the $\log_{10}$ of the initial plasmid copy number as follows: y =-3.3043x+ 37.909, R^2 = 0.99. Real-time qPCR was performed by using iQ™

SYBR® Green Supermix (Bio-Rad) in a CFX Connect Real-Time PCR Detection System (Bio-Rad). Total reaction volume of 20 μL was consisted of 10 μL 2× iQ™ SYBR® Green Supermix (Bio-Rad), 1 μL per forward (10 mM) and reverse primers (10 mM) and 1 μL DNA template. The qRT-PCR program was performed as follows: 95 °C for 3 min, 40 cycle at 95 °C for 10 s, 59 °C for 30 s All samples were performed in five repeats. Convert the qPCR result to the spore number by formula: copy number $\times 2 \times 100 \times 100$. 2 was back calculation for the dilution of DNA extraction, one of 100 was for the diluted after DNA extraction and other was used 1 μL 100× DNA for qPCR.

Supplementary Figure 1

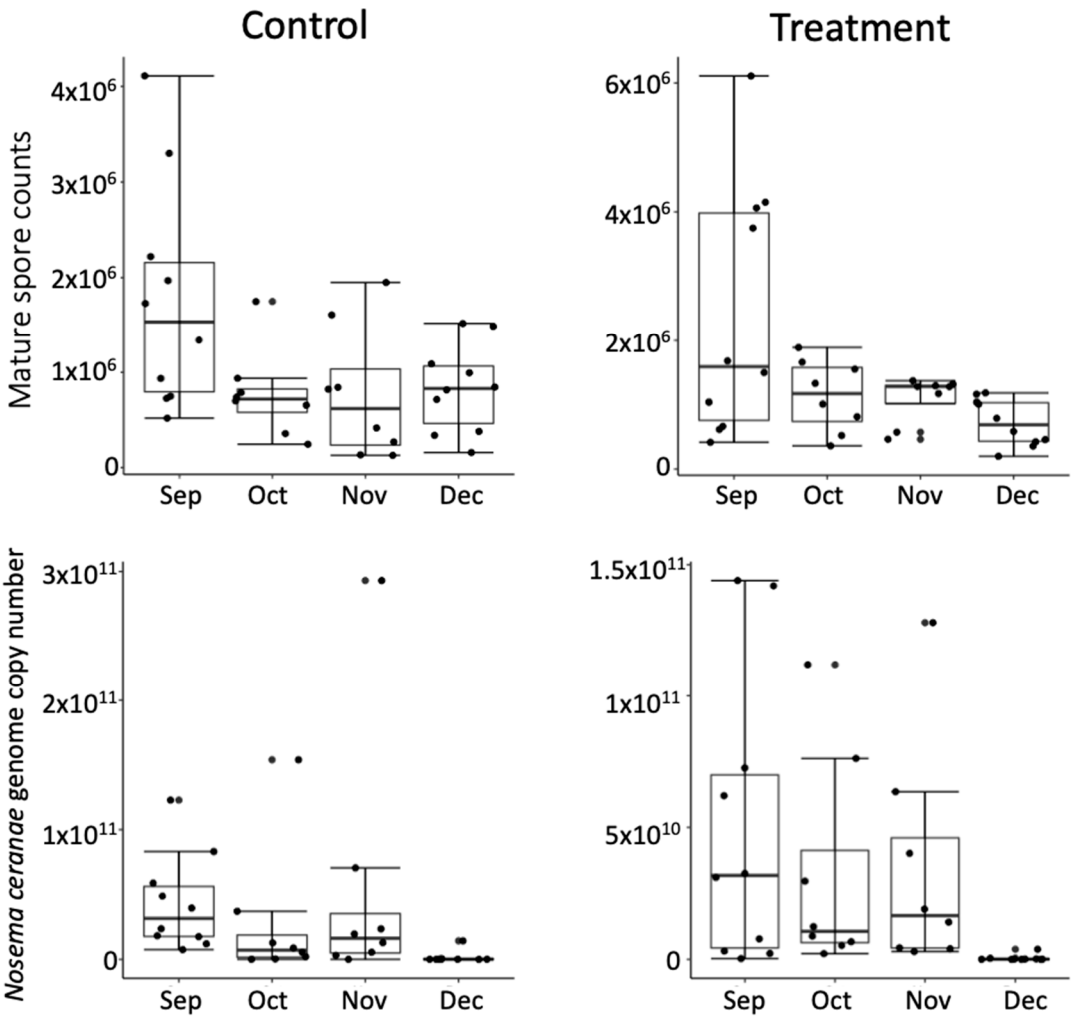

Figure S1. Total spore counts and genome copy numbers of the control group (A and C) and treatment group (B and D) from September to December.