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Scientific note

A scientific note on the detection of Kashmir bee virus in individual honeybees and *Varroa jacobsoni* mites

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Kashmir bee virus / *Varroa jacobsoni*/ detection / direct RT-PCR

The immunodiffusion test has been routinely used to detect bee viruses because it is rapid, inexpensive and specific [1]. This technique, however, is relatively insensitive and the samples must contain sufficient virus to be readily detected. Stoltz et al. [4] used reverse transcription-polymerase chain reaction (RT-PCR) to detect Kashmir bee virus (KBV). This technique requires the time-consuming virus purification and RNA extraction steps. Direct RT-PCR can circumvent these steps [2, 3]. Here we report a protocol that further simplifies the use of RT-PCR in bee virus detection.

Each pupa or adult bee (*Apis mellifera* L.) was homogenized in 0.5 mL of 0.01 M potassium phosphate buffer (pH 6.7). Individual *Varroa jacobsoni* mites were homogenized in 15 µL of buffer. The homogenate was centrifuged at 4 000 *g*. Two microliters of supernatant were mixed with 10 mL of sterile H_2O, heated at 95 °C for 2 min, centrifuged briefly and cooled to 50 °C. The mixture was added to master mix 1 of the Titan™ One Tube RT-PCR System (Boehringer Mannheim) which contains the KBV-specific primers (GATGAACGTCGACCTATTGA and TGTGGGTTGGCTATGAGTCA) [4] at the final concentration of 0.4 µM each. After adding master mix 2, the final mix was spun briefly, overlaid with 50 µL of mineral oil and incubated at 50 °C for 30 min for the RT step. Cycling temperature settings were 94 °C for denaturation, 57 °C for annealing and 68 °C for elongation. The template was first denatured for 2 min. For the first ten cycles, cycling times were 30 s for denaturation, 30 s for annealing and 45 s for elongation. During the subsequent 25 cycles denaturation and annealing times were set at 30 s; however, the elongation time was set at 45 s plus 5 s for each cycle (e.g. cycle no. 11 has an additional 5 s, cycle no. 12 has an additional 10 s). The final elongation time was set at 7 min. This 'time step' protocol took about 3 h and 10 min from the RT step and first denaturation to the last elongation. As in regular RT-PCR, a specific primer pair must be used in this protocol in order to generate specific RT-PCR products.

We used this 'time step' protocol to detect KBV in individual bees and *V. jacobsoni* mites in a transmission study. We not only circumvented the virus isolation and RNA extraction steps, but could also detect KBV in individual *V. jacobsoni* mites (*figure 1*) and sequenced the PCR products. The immunodiffusion test failed to detect KBV in any individual mites and most individual bees in our study. It would be very difficult (if not impossible) to isolate KBV and

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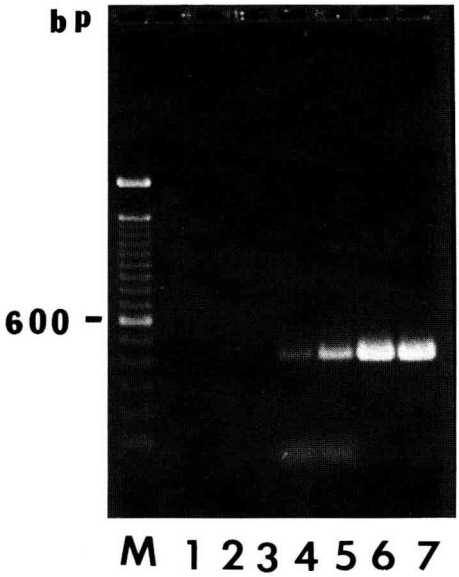

Figure 1. Amplification of a KBV-specific PCR product by direct RT-PCR.
M: 100-bp DNA ladder. Lanes 1–7: individual *Varroa* mites. Note the expected 410-bp KBV amplicon in lanes 4–7, but not in lanes 1–3.

extract RNA from a single mite for RT-PCR. Furthermore, this protocol requires only 2 µL of supernatant; therefore, sufficient homogenate remains that can be used in other tests. As a result, we can not only compare data from RT-PCR/sequencing and immunodiffusion tests from the same individual bee or mite, but also re-evaluate the results of transmission studies carried out by other workers. This improvement in methodology will greatly facilitate our on-going study on the role of *Varroa* in bee virology.

Similar results were obtained with the Calypso™ RT PCR System (DNAmp Ltd.). We, therefore, believe that other commercial RT-PCR systems will work as well. Some of the 410-bp fragments of the KBV RNA polymerase gene in our transmission study have been sequenced and registered with GenBank (accession nos AF085479, AF093457 and AF117953). The size of the 417-bp product reported for Canadian KBV by Stoltz et al. [4] was based on their estimation using the molecular weight DNA markers. Although we also used their two primers in PCR, the 410-bp length of our sequenced amplicon represents the actual count of bases.

Note scientifique sur la détection du virus Kashmir de l'abeille chez des abeilles (*Apis mellifera* L.) et des acariens *Varroa jacobsoni* Oud.

Eine wissenschaftliche Notiz zur Entdeckung des Kashmir Bienenvirus in einzelnen Honigbienen und Varroamilben

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