

First Detection of Honey Bee Viruses in Jordan by RT-PCR

Nizar Haddad*[✉], Mohammad Brake**, Hussein Migdadi* and Joachim R. de Miranda***

ABSTRACT

Mortality of honeybees is a serious problem that beekeepers have to face periodically in Jordan and worldwide. The presence of honeybee viruses, in addition to other pathogens and parasites may be one of its possible causes. In this work, individual bee samples from Ajlun area of Jordan were screened for six honey bee viruses using RT-PCR and specific primers. Acute Bee Paralysis Virus (ABPV), Sac Brood Virus (SBV) and Deformed Wing Virus (DWV) were detected in the samples, while Black Queen Cell Virus (BQCV), Chronic Bee Paralysis Virus (CBPV) and Kashmir Bee Virus (KBV) were not detected. This is the first report of the detection of bee viruses in Jordan utilizing molecular techniques.

Keywords: Honeybee viruses, RT-PCR, Molecular markers.

INTRODUCTION

The beekeeping industry plays a key role in agricultural production in Jordan. A recent study estimated that, in Jordan, the total production value of 12 bee-pollinated crops was \$117.4M in 2005, of which \$ 50.7M could be directly attributed to pollination by honey bees (Haddad et al., 2007), which is over 16 times the value of the locally produced honey (\$3.1M). Recently, a large proportion of beekeepers complained of considerable bee mortality in their hives, especially in Ajlun province. Mass mortality of honeybees (*Apis mellifera*) is one of the most serious problems that beekeepers and dependent industries face worldwide. A

graphic example is the widely publicised economic, social and political upheaval caused by the extensive colony collapse among migratory beekeepers in the USA (Cox-Foster et al., 2007; Stokstad, 2007a; 2007b) and similar colony collapses and agricultural disruption during the previous century (Beuhne, 1910; Wilson and Menapace, 1979; Oertel, 1965; Foote, 1966; Rennie, 1921; Bailey, 1964; Goodacre, 1943).

There are many factors that may contribute to bee mortality, including bee parasites and diseases (Ball and Bailey, 1997; Cox-Foster et al., 2007), the effects of etiological agents used to treat such diseases, climatic factors (Bailey et al., 2001; Wilson and Menapace, 1979) and exposure to insecticides or pesticides used in agriculture (Antunez et al., 2006; Suchail et al., 2004).

The association of viruses with honeybee mortality is a particular cause for concern world-wide (Bailey et al., 1981; 1983; Hornitzky, 1987; Allen and Ball, 1996; Nordstrom et al., 1999; Tentcheva et al., 2004a,b; Todd et al., 2007). The honeybee is host to at least 18 viruses (Bailey and Ball, 1991). All are normally asymptomatic and persist in the

* Bee Research Unit and Biotechnology Unit, National Center for Agricultural Research and Technology Transfer (NCARTT), Baq'a 19381, Jordan.

**Department of Biological Sciences, Jerash Private University, Jerash, Jordan.

***Department of Biological Sciences, Queen's University, Belfast, BT9 7BL, Belfast, Northern Ireland.

[✉]dmizarh@yahoo.com

Received on 21/11/2007 and Accepted for Publication on 29/5/2008.

colonies as covert infections (Bailey and Ball, 1991; Ball and Bailey, 1997). Some, such as Chronic Bee Paralysis Virus (CBPV), Black Queen Cell Virus (BQCV), Sac Brood Virus (SBV), Deformed Wing Virus (DWV) and Cloudy Wing Virus (CWV), will also produce recognizable clinical symptoms at certain life stages (Bailey and Ball, 1991; Allen and Ball, 1996). Nucleotide sequences are now available for many bee viruses, enabling the development of nucleic acid based diagnostic assays (e.g., Tentcheva *et al.*, 2004b; Chen *et al.*, 2005; Topley *et al.*, 2005; Chantawannakul *et al.*, 2006; Berenyi *et al.*, 2006; Grabesteiner *et al.*, 2007). In this study we use several such assays to assess the presence of honeybee viruses in some Ajlon province apiaries.

MATERIALS AND METHODS

Sample Collection

Adult worker bee samples were collected from 13 collapsing colonies originating from 7 widely separated apiaries from Ajloun province. The samples were collected randomly from inside the colonies, transported on ice and transferred to -25 °C until use.

RNA Extraction

Individual bees were homogenized in by using pestle and mortar with 1 ml Trizol reagent (Biobasic) and transferred to 1.5 ml microfuge tubes. The protein fraction was separated out in the organic (lower) phase using chloroform and the RNA was precipitated from the aqueous (upper) phase with 1 ml of iso-propanol and centrifugation (14000 rpm). The resultant nucleic acid pellets were washed twice with 70% ethanol and resuspended in nuclease-free water.

RT-PCR Amplification

Each RNA sample was analysed for the presence of bee virus sequences using the Access RT-PCR system (Promega, USA) according to the company protocol. Amplification occurred in a 25- μ l reaction mixture containing 1x reaction buffer, 0.2mM dNTP, 1 μ M each of the two primers for each virus, 2 mM MgSO₄, 0.1 U of avian myeloblastosis virus

reverse transcriptase, 0.1 U of Tfl DNA polymerase and 250 to 500 ng of total RNA. The primer sequences and expected length of the RT-PCR products are shown in Table 1. Reverse transcription at 48°C for 48 min. was followed by 40 cycles of 95°C for 30 s, 55°C for 1 min. and 68°C for 2 min. and a final extension at 68°C for 7 min. Negative controls were included in each RT-PCR experiment. Ten microliters of amplified product was electrophoresed in a 2% agarose gel and stained with ethidium bromide. Bands were photographed under UV light. Fragment sizes were determined with reference to 100-bp ladder (Promega).

Table (1): The sequences of the six primer pairs.

No.	The Virus	5'-3' Primer*	Weight (pb)
1	BQCV	F: tggtcagctcccactaccttaaac R: gcaacaagaagaacgtaaaccac	700
2	DWV	F: ctactctgccgtegecca R: ccgtaggaactcattatcgcg	194
3	KBV	F: gatgaacgtcgacctattga R: tgtgggttgctatgagtca	415
4	SBV	F: gctgaggtaggatctttgctg R: tcacatcttcaccatcega	824
5	ABPV	F: ttatgtgtccagagactgtat R: gctcctattgctcggttttc	900
6	CBPV	F: agttgtcatggttaacaggatacag R: tctaactcttagcacgaaagccgag	455

* Antunez *et al.*, 2006; Berenyi *et al.*, 2006; Tentcheva *et al.*, 2004b.

RESULTS AND DISCUSSION

Ajloun province is the second most important region in Jordan for beekeeping, in terms of the number of beekeepers and managed colonies. Adult worker bees collected from thirteen colonies in seven widely dispersed apiaries from Ajloun province were assayed for the presence of six honeybee viruses (ABPV, BQCV, CBPV, DWV, KBV and SBV) using RT-PCR. The results are shown in Figure 1. Out of the 13 colonies examined, 92% were infected with DWV, 8% with SBV and 16% with ABPV. None were infected with CBPV, BQCV or KBV. Several colonies were infected

with more than one virus. This indicated that DWV is the most common virus in collapsing bee colonies in Jordan. These data are similar to comparable surveys conducted elsewhere (Tentcheva et al., 2004a). Nearly all the samples from colonies that presented bee mortality were infected with at least one virus or co-infected with more than one virus.

This is the first instance of nucleic acid-based bee virus detection in Jordan. Robinson (1981) previously observed sacbrood virus symptoms in Jordan and several bee viruses have been previously detected serologically in other countries of the Arabian peninsula (Allen and Ball, 1996). DWV has been detected by RT-PCR in the United Arab Emirates (Berenyi et al., 2007) although no other viruses were surveyed in these studies.

These preliminary results show the presence of several bee viruses, in particular DWV, in dying Jordanian bee

colonies. This is similar to surveys conducted in other countries (Nordström et al., 1999; Antunez et al., 2006; Tentcheva et al., 2004b; Berenyi et al., 2006; Todd et al., 2007; Cox-Foster et al., 2007). Further research is needed to determine which other factors are also differentially associated with colony mortality, such as infestation with parasitic mites (*Varroa destructor*, *Acarapis woodi*), *Nosema apis* and *N. cerana* (PSU, 2007), bacterial diseases and any possible effects of chemical treatment of colonies or foraging resources. These results are also important to increase the awareness among beekeepers, bee researchers and bee-dependent agricultural industries in Jordan and the Arab world that the diseases and parasites that threaten the bee industry world-wide are similarly present in Arab countries and are similarly associated with bee colony mortality.

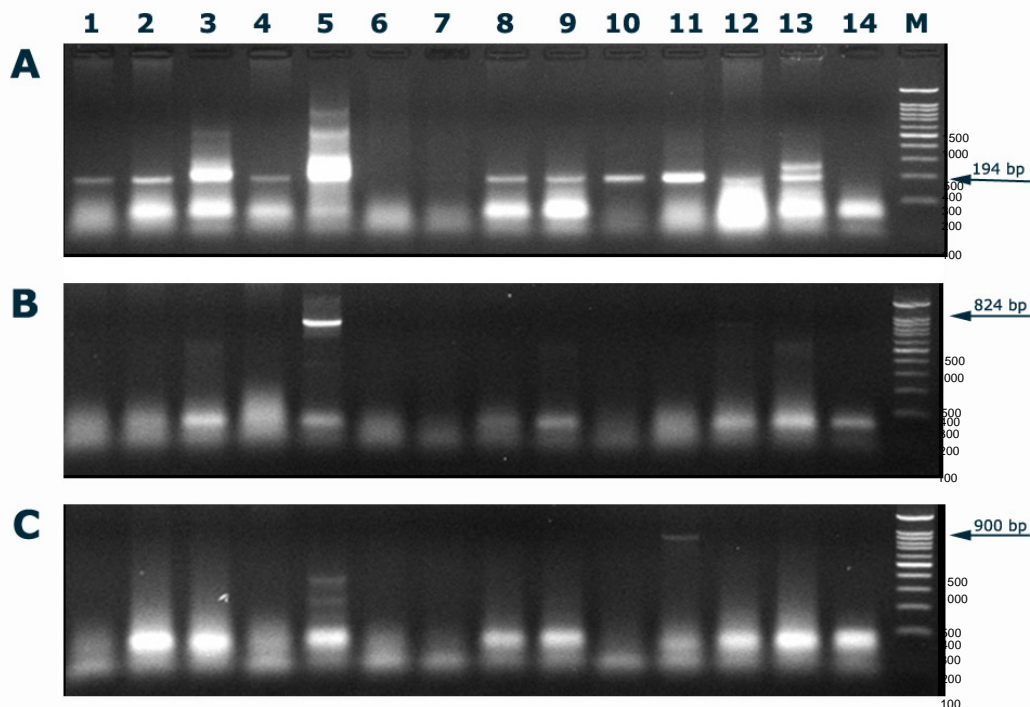


Figure (1): Detection of different viruses in the Jordanian affected hives. RT-PCR was performed with differential primers amplifying virus-specific sequences. Panel A: Deformed Wing Virus (DWV), Panel B: Sac Brood Virus (SBV), Panel C: Acute Bee Paralysis Virus (ABPV). Lanes 1-13: Different bee samples. Lane 14: Negative control, RT-PCR without a template. Lane M: DNA size marker.

ACKNOWLEDGMENTS

The research group would like to thank the Vita-Europe and the National Center for Agricultural Research and Extension for financial support and Ahmad Bataeneh from the Bee Research Unit for help

REFERENCES

- Allen, M.F. and Ball, B.V. 1996. The incidence and world distribution of honey bee viruses. *Bee World* 77, 141–162.
- Antunez, K.A., D'Alessandro, B., Corbella, E., Ramallo, G. and Zunino, P. 2006. Honeybee viruses in Uruguay. *Journal of Invertebrate Pathology* 93, 67–70.
- Bailey, L. 1964. The 'Isle of Wight Disease': the origins and significance of the myth. *Bee World* 45, 32–37.
- Bailey, L. and Ball, B.V. 1991. Honey Bee Pathology, second ed. Academic Press, London.
- Bailey, L., Ball, B.V. and Perry, J.N. 1981. The prevalence of viruses of honey bees in Britain. *Ann. Appl. Biol.* 97, 109–118.
- Bailey, L., Ball, B.V. and Perry, J.N. 1983. Association of viruses with two protozoal pathogens of the honey bee. *Ann. Appl. Biol.* 103, 13–20.
- Bailey, L. and Perry, J.N. 2001. The natural control of the tracheal mite of honey bees. *Exp. Appl. Acarol.* 25, 745–749.
- Ball, B.V. and Bailey, L. 1997. Viruses. In: Morse, R.A., Flottum, K. (Eds.), Honey Bee Pests Predators and Diseases. AI Root Co., Medina, 11–31.
- Berenyi, O., Bakonyi, T., Derakhshifar, I., Köglberger, H. and Nowotny, N. 2006. Occurrence of six honeybee viruses in diseased Austrian apiaries. *Appl. Environ. Microbiol.* 72, 2414–2420.
- Berenyi, O., Bakonyi, T., Derakhshifar, I., Köglberger, H., Topolska, G., Ritter, W., Pechhacker, H. and Nowotny, N. 2007. Phylogenetic analysis of deformed wing virus genotypes from diverse geographic origins indicates recent global distribution of the virus. *Appl. Environ. Microbiol.* 73, 3605–3611.
- Beuhne, R. 1910. Bee mortality. *J. Dept. Agric. Victoria* 8, 149–151.
- Chantawannakul, P., Ward, L., Boonham, N. and Brown, M. 2006. A scientific note on the detection of honeybee viruses using real-time PCR (TaqMan) in Varroa mites collected from a Thai honeybee (*Apis mellifera*) apiary. *J. Invertebr. Pathol.* 91, 69–73.
- Chen, Y.P., Higgins, J.A. and Feldlaufer, M.F. 2005. Quantitative analysis of deformed wing virus infection in the honey bee *Apis mellifera* L. by real-time RT-PCR. *Appl. Environ. Microbiol.* 71, 436–441.
- Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A., Quan, P.-L., Briese, T., Hornig, M., Geiser, D.M., Martinson, V., van Engelsdorp, D., Kalkstein, A.L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchinson, S.K., Simons, J.F., Egholm, M., Pettis, J.S. and Lipkin, W.I. 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* 318, 283–287.
- Foote, H.L. 1966. The mystery of the disappearing bees. *Am. Bee J.* 106, 126–127.
- Goodacre, W.A. 1943. Dwindling troubles may cause heavy mortality. *Australasian Beekeeper* 45, 57–59.
- Grabensteiner, E., Bakonyi, E., Ritter, W., Pechhacker, H. and Nowotny, N. 2007. Development of a multiplex RT-PCR for the simultaneous detection of three viruses of the honeybee (*Apis mellifera* L.): Acute bee paralysis virus, Black queen cell

- virus and Sacbrood virus. *Journal of Invertebrate Pathology*. 94: 222-225.
- Haddad, N., Shammout, A. and Al-Nsour, A. 2007. The economic value of honeybees for crop pollination in Jordan. Documents of the 40th Apimondia International Apicultural Congress, Melbourne, Australia, 115.
- Hornitzky, M.A.Z. 1987. Prevalence of virus infections of honey bees in Eastern Australia. *J. Apic. Res.* 26, 181-187.
- Nordstrom, S., I. Fries, A. Aarhus, H. Hansen and S. Korpela. 1999. Virus infection in Nordic honey bee colonies with no, low or severe *Varroa jacobsoni* infestations. *Apidologie* 30:475-484.
- Oertel, E. 1965. Many bee colonies dead of an unknown cause. *Am. Bee J.* 105, 48-49.
- PSU. 2007. Bee Researchers Close in on Colony Collapse Disorder. *Am. Bee J.* 147, 930-931.
- Rennie, J., White, P.B. and Harvey, E.J. 1921. Isle of wight disease in hive bees. *Trans. Roy. Soc. Edinb.* 52, 737-779.
- Robinson, W.S. 1981. Beekeeping in Jordan. *Bee World* 62, 91-97.
- Stokstad, E. 2007a. The case of empty hives. *Science* 316, 970-972.
- Stokstad, E. 2007b. Puzzling decline of U.S. bees linked to virus from Australia. *Science* 317, 1304-1305.
- Suchail, S., Debrauwer, L. and Belzunces, L.P. 2004. Metabolism of imidacloprid in *Apis mellifera*. *Pest Manag. Sci.* 60, 291-296.
- Tentcheva, D., Gauthier, L., Jouve, S., Canabady-Rochelle, L., Dainat, B., Cousserans, F., Colin, M.E., Ball, B.V. and Bergoin, M. 2004a. Polymerase chain reaction detection of Deformed Wing Virus (DWV) in *Apis mellifera* L. and *Varroa destructor*. *Apidologie* 35, 431-439.
- Tentcheva, D., Gauthier, L., Zappulla, N., Dainat, B., Cousserans, F., Colin, M. E. and Bergoin, M. 2004b. Prevalence and seasonal variations of six bee virus in *Apis mellifera* L. and *Varroa destructor* mite populations in France. *Appl. Environ. Microbiol.* 70, 7185-7191.
- Todd, J.H., de Miranda, J.R. and Ball, B.V. 2007. Incidence and molecular characterization of viruses found in dying New Zealand honey bee (*Apis mellifera*) colonies infested with *Varroa destructor*. *Apidologie* 38, 354-367.
- Topley, E., Davison, S., Leat, N. and Benjeddou, M. 2005. Detection of three honeybee viruses simultaneously by a single multiplex reverse transcriptase PCR. *Afric. J. Biotech.* 4, 763-767.
- Wilson, W.T. and Menapace, D.M. 1979. Disappearing disease of honey bees: A survey of the United States. *American Bee Journal* 118-119; 184-186; 217.

(RT-PCR)

*** * ** ☒*

(RT-PCR) :

.dnizarh@yahoo.com .

☒*

**

.2008/ / / /