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

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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 Ajlon 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 Ajlon 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

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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-µl reaction mixture containing 1x reaction buffer, 0.2mM dNTP, 1 µM each of the two primers for each virus, 2 mM $MgSO_4$, 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:gcaacaagaagaaacgtaaaccac	700
2	DWV	F: cttactctgccgtcgccca R: ccgttaggaactcattatcgcg	194
3	KBV	F: gatgaacgtcgacctattga R: tgtgggttggctatgagtca	415
4	SBV	F: gctgaggtaggatctttgcgt R: tcatcatcttcaccatccga	824
5	ABPV	F: ttatgtgtccagagactgtat R: gctcctattgctcggtttttc	900
6	CBPV	F: agttgtcatggttaacaggatacgag R: tctaatcttagcacgaaagccgag	455

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

RESULTS AND DISCUSSION

Ajlon 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 Ajlon 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 penninsula (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.

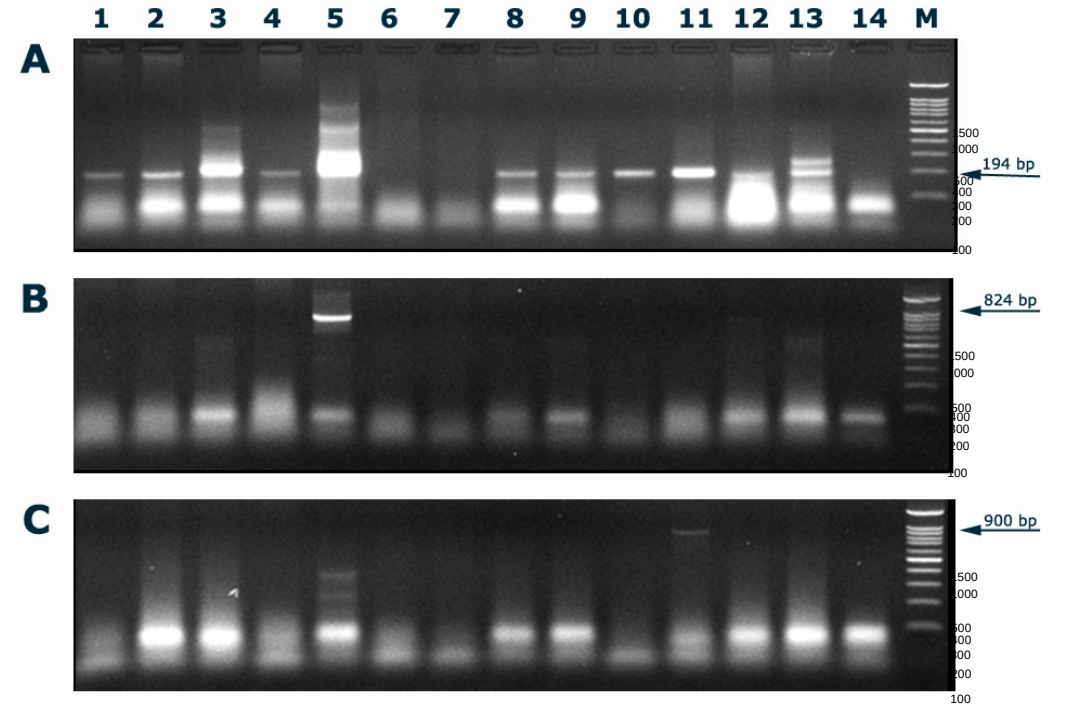

Figure (1): Detection of different viruses in the Jordanian affected hives. RT-PCR was performed with differential primers amplifying virus-specific sequences. Panel A: Deformed Wing Virus (DWV), Panel B: Sac Brood Virus (SBV), Panel C: Acute Bee Paralysis Virus (ABPV). Lanes 1-13: Different bee samples.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

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التسجيل الأول في الأردن للأمراض الفيروسية لنحل العسل باستخدام التقانات الحيوية (RT-PCR)

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ملخـص

إن ظاهرة موت النحل هي واحدة من أهم المشاكل التي تواجه النحالين في الأردن والعالم عامة. إن وجود الأمراض الفيروسية بالإضافة إلى مسببات مرضية أخرى ومتطفلات يمكن أن يكون السبب لهذا الموت. في هذا البحث تم جمع عينات نحل من منطقة عجلون لفحص ما إذا كانت مصابة بالأمراض الفيروسية باستخدام البادئات الوراثية المتخصصة لستة فيروسات تصيب النحل. تم تسجيل إصابة العينات بفيروس شلل النحل الحاد، وفيروس تكيس الحضنة، وفيروس تشوه الأجنحة بينما تبين عدم إصابة العينات المفحوصة بفيروس البيت الملكي الأسود أو فيروس الشلل المزمن أو فيروس النحل الكشميري. إن هذا التقرير هو الأول أردنيا الذي يقوم بتشخيص الأمراض الفيروسية في الأردن باستخدام التقانات الحيوية.

الكلمات الدالة: فيروسات نحل العسل، (RT-PCR)، المعلمات الوراثية.

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