Viral infections in queen bees (*Apis mellifera carnica*) from rearing apiaries

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Abstract

Viral infection could have an impact on the success of queen rearing and a potential effect on reduced queen quality. Newly mated honey bee (*Apis mellifera carnica*) queens were collected from mating nuclei in queen rearing operations in Slovenia. Altogether, 81 queens were sampled from 27 rearing apiaries in 2006 and 72 queens from 24 apiaries in 2008. Queens were analysed for the presence of four viruses: acute bee paralysis virus (ABPV), black queen cell virus (BQCV), sacbrood virus (SBV) and deformed wing virus (DWV) by using reverse transcription polymerase chain reaction (RT-PCR). In 2006, 12%, 9% and 1% prevalence was found for ABPV, DWV and SBV, respectively; BQCV was not detected. Two years later, DWV, BQCV, SBV and ABPV were detected in 58%, 24%, 11% and 10% bee queens, respectively. In 2006, fourteen out of twenty-seven apaiaries were virus free, whereas in 2008 only three out of twenty-four apiaries were virus free. This is the first evidence of virus infection occurring in newly mated queens from mating nuclei in rearing apiaries. The possible impacts of queen rearing technology and epidemiological influences on virus infection are discussed in this study.

Diagnosis, acute bee paralysis virus, black queen cell virus, sacbrood virus, deformed wing virus

Honey bee viruses have generally been found to be isometric particles containing a single positive strand RNA genome (Bailey and Ball 1991). The genome sequences of several honey bee viruses; acute bee paralysis virus (ABPV), black queen cell virus (BQCV), sacbrood virus (SBV), deformed wing virus (DWV), Kashmir bee virus (KBV), Israeli acute paralysis virus, chronic bee paralysis virus, and slow paralysis virus have been determined (Fujiyuki et al. 2004; de Miranda et al. 2004; Maori et al. 2007; Blanchard et al. 2009). Sacbrood virus is one of the few viral diseases of bees with striking well-defined clinical signs in honey bee colonies which can be identifiable by beekeepers. Other viruses usually cause inapparent infections in honey bees (Hung et al. 1996). Viruses are also prevalent in bees infested with Varroa mites. In severely infested colonies, ABPV has been found in adult bees and in mites (Bakonyi et al. 2002; Tentcheva et al. 2004a). The most wide-spread virus DWV appears in mites infested colonies throughout Europe (Tentcheva et al. 2004b).

Viruses may, however, cause the death of individual bees or even the collapse of entire colonies without clinical symptoms being observed (Allen and Ball 1996). Black queen cell virus and SBV are common in adult bees and affect queen prepupae or pupae, especially in spring and early summer, whilst DWV is mostly detected in Varroa-infested bees (Laidlaw 1979). Black queen cell virus found in dead honey bee queen larvae and pupae has been found to be a common cause of queen larval death in Australia (Anderson 1993). Acute bee paralysis virus can be detected in apparently healthy bees, but is also associated with the sudden collapse of honey bee colonies caused by heavy infestation with Varroa mites (Ball 1997).

Reverse transcription polymerase chain reaction (RT-PCR), a reliable, specific, and sensitive technique, has been used to detect a variety of RNA viruses including the picorna-

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like insect viruses (Hung and Shimanuki 1999). Although much research has been carried out on the characterization of bee viruses, little is known about the appearance and significance of bee viruses in honey bee queens and furthermore, on the influence of virus infected queens on the entire colony.

The aim of this study was to use highly sensitive detection methods based on the amplification of specific viral sequences by RT-PCR to detect four viruses (ABPV, BQCV, SBV and DWV) in recently mated queens collected from mating nuclei in queen mating apiaries. The survey was performed on queens reared in 2006 and 2008 to establish the incidence of these viruses and their influence on queen quality.

Materials and Methods

Sample collection

During the peak of the breeding season in June, queens were collected from queen rearing operations in Slovenia. In 2006 and 2008, three mated queens accompanied by seven workers from the same mating nucleus were collected from 27 and 24 registered queen rearing apiaries, respectively. Altogether 81 queens were sampled from 27 rearing apiaries in 2006 and 72 queens from 24 apiaries in 2008. Queens were collected directly from mating nuclei and together with attendant workers were transported in mailing cages to the laboratory and frozen at -20 °C for individual virus analysis.

RNA extraction and RT-PCR assay

The queen samples to be used for RNA isolation were separately crushed in a mortar and homogenized in PBS. The homogenized samples were centrifuged for 20 min at 3000 × *g* and 140 ml of the supernatants were used for the preparation of RNA. Viral RNA was isolated from the supernatants using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Reverse transcription and polymerase chain reaction was conducted in a reaction mixture, using an uninterrupted synthesizer programme: reverse transcription at 50 °C for 30 min, initial PCR activation at 95 °C for 15 min, 40 cycles with denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, extension at 72 °C for 1 min completed with a final extension step at 72 °C for 10 min. The reaction mixture using Qiagen OneStep RT-PCR Kit (Qiagen) was prepared according to the manufacturer's recommendations. Negative control was performed using the reaction mixture without RNA and verified positive samples were used as positive controls. The specific primer pairs for the four viruses were designed based on the partial or complete sequences of the virus genomes deposited in the GenBank database (Berényi et al. 2006).

The reaction products were analysed by electrophoresis on a 2% agarose gel stained with ethidium bromide. The bands were visualized by UV transillumination and documented. The bands obtained were compared to those of positive controls. To estimate the size of the amplicons we used the GeneRuler™ 100bp DNA ladder molecule size marker (MBI Fermentas; Vilnius, Lithuania).

Results

In 2006, the highest percentage of infection was detected for ABPV, the second for DWV, the third for SBV, while BQCV was not detected (Table 1). Queens without detected viruses were found in 14 queen rearing apiaries, 10 apiaries had one virus and three apiaries had two viruses (Table 2). In 2008, all viruses tested were found in the samples from 24 queen rearing apiaries, the most common being DWV (Table 1). Three apiaries were without virus infected queens, six apiaries had one virus detected, ten had two, four had three and one had all four, and simultaneous infections of different viruses also occurred (Table 2).

Table 1. The incidence of four bee viruses (acute bee paralysis virus, black queen cell virus, sacbrood virus and deformed wing virus) diagnosed in 81 bee queens sampled from 27 rearing apiaries in 2006 and in 72 bee queens from 24 apiaries in 2008.

Year	n	Positive (%) ABPV	BQCV	DWV	SBV
2006	81	10 (12%)	0 (0%)	7 (9%)	1 (1%)
2008	72	7 (10%)	17 (24%)	42 (58%)	8 (11%)

ABPV - acute bee paralysis virus; BQCV - black queen cell virus; DWV - deformed wing; SBV - sacbrood virus

Table 2. Viruses detected in bee queens in 2006 and 2008 shown for each queen rearing apiary.

Apiary	Rearing year 2006	2008
1	ABPV	DWV
2	neg.	BQCV, DWV
3	neg.	BQCV, DWV, SBV
4	ABPV, SBV	BQCV, DWV
5	neg.	DWV
6	neg.	BQCV, DWV
7	DWV	BQCV, DWV
8	neg.	BQCV, DWV, SBV
9	ABPV, DWV	ABPV, DWV
10	neg.	BQCV, DWV, SBV
11	DWV	-*
12	neg.	BQCV, DWV
13	neg.	ABPV, DWV
14	DWV	-
15	neg.	neg.
16	neg.	DWV
17	neg.	neg.
18	ABPV	-
19	neg.	BQCV, DWV, SBV
20	neg.	DWV
21	ABPV	neg.
22	neg.	ABPV, BQCV, DWV, SBV
23	ABPV, DWV	BQCV, DWV
24	ABPV	DWV
25	ABPV	DWV, SBV
26	DWV	BQCV, DWV
27	DWV	DWV

ABPV - acute bee paralysis virus; BQCV - black queen cell virus; SBV - sacbrood virus; DWV - deformed wing virus; neg. - negative result; *no queen analysed

Discussion

Queen quality is an important factor influencing colony productivity, and is characterized by different morphological and functional characteristics (Kaftanoglu et al. 2000). Queen age has influence on high colony productivity (Tarpy et al. 2000), so beekeepers need to replace them, especially after the second year in nomadic beekeeping conditions (Akyol et al. 2007). Queen rearing is therefore a very important beekeeping activity in order to ensure the availability of quality queens. Keeping young queens in productive colonies is a priority, but continuing queen replacement is also associated with some risk of virus introduction. Deformed wing virus has been extensively studied in recent years, and has often been found in newly mated queens, as in our experiment. It can be transmitted both horizontally (Bowen-Walker et al. 1999), and vertically through infected eggs or semen (Chen et al. 2006). Varroa mites are also thought to activate virus replication and suppress immunity in the host bee. The symptoms of DWV infection do not necessarily result from Varroa mite co-infestation (Yang and Cox-Foster 2005), and the virus can produce wing deformity and other visible morphological abnormalities in workers and drones (e.g. Bowen-Walker et al. 1999). Symptomatic individual workers live <67 h after emergence (Yang and Cox-Foster 2007) but there are no data on DWV infected queen longevity. Deformed wing virus has been found in workers and drones regardless of wing morphology, but only in newly emerged queens with deformed wings (Williams et al. 2009). In our study, DWV was found in normally developed laying mated queens mating nuclei. Virus appeared in queens reared and examined in both 2006 and 2008, at incidences of 9% and 58%, respectively. This was despite the regular treatment for Varroa mites, necessary in queen rearing apiaries. Acute bee paralysis virus spreads via salivary gland secretions of adult bees and in food stores to which these secretions are added (Ball 1985) and the virus is widespread as a latent and inapparent infection (Allen and Ball 1996). In our study, ABPV appeared in queens in both years and is thus potentially present in newly reared queens in all rearing apiaries. In 2006, BQCV was not detected in any samples tested, but in 2008, one quarter of the samples contained it. This virus may damage queen pupae and

thus have a direct impact on the success of queen rearing. Sacbrood virus was also detected in both years, but with lower frequencies.

Some queens were found to be infected with two viruses simultaneously. This is in agreement with observations at the colony level, showing that multiple infections frequently occur in apiaries (Evans 2001; Tentcheva et al. 2004a) and are associated with Varroa mites as an activator of virus replication (Dandeu et al. 1991). All viruses detected in queens can affect queen performance. In 2008, only two queen rearing apiaries were found without virus infected queens, but the reasons for this remain unknown. It has been established that mites act as an inducer of viral infection (Ball 1985), but in queen rearing operations there are other potentially non pathogenic inducers of viral activation which should be further studied, such as queen rearing manipulations, type of mating nuclei, and the feeding system. Further epidemiological studies in queen rearing apiaries are needed to clarify the transmission, spread and prevalence of viruses and their effects on queen fertility and on entire colonies.

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