

RESEARCH NOTE

Mycobiology 39(4) : 317-320 (2011)
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<http://dx.doi.org/10.5941/MYCO.2011.39.4.317>
pISSN 1229-8093
eISSN 2092-9323

Prevalence of *Nosema* and Virus in Honey Bee (*Apis mellifera* L.) Colonies on Flowering Period of Acacia in Korea

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(Received August 22, 2011. Revised September 26, 2011. Accepted October 4, 2011)

Honey production from approximately 1.6 million colonies owned by about 199,000 Korean beekeepers was almost 23,000 metric tons in 2009. *Nosema* causes significant losses in honey production and the virus decreases population size. We initiated a survey of honey bee colonies on the blooming period of Acacia to determine the prevalence of *Nosema* and virus in 2011. Most Korean beekeepers have moved from the south to north of Korea to get Acacia nectar for 2 mon. This provided a valuable opportunity to sample bees originating from diverse areas in one location. Twenty hives owned by 18 beekeepers were sampled in this year. *Nosema* spore counts ranged from zero to 1,710,000 spores per bee. The average number of *Nosema* spores per bee was 580,000. Approximately 95% of the colonies were infected with *Nosema*, based on the presence of spores in the flowering period of Acacia. This indicates that *Nosema* is the predominant species affecting honeybee colonies. Also, the seven most important honeybee viruses were investigated by reverse transcription-PCR. Among them, four different viruses were detected in samples. Black queen cell virus was present in all samples. Chronic bee paralysis virus was detected in 10% of samples. Deformed wing virus was present in only 5% of the samples. Prevalence of Sacbrood virus was 15%. However, Cloudy wing virus, Israel acute paralysis virus and Kashmir bee virus were not detected in any of samples.

KEYWORDS : Honeybee disease, *Nosema*, Virus

There are about 179,000 thousand beekeepers caring for approximately 1,600,000 colonies in Korea. Honey production totaled almost 23,000 metric tons in 2009. This production was estimated to be worth about 203 billion Korean won. *Nosema* and virus are a major cause of honey production decrease. *Nosema* disease (nosemosis) caused by the honey bee microsporidia is one of the most important diseases in honey bees and is worldwide in distribution [1]. Microsporidia are possibly the smallest single-cell organisms with a true nucleus. The genus *Nosema* is a parasitic fungus that infects insects such as honey bees, bumble bees and silkworms. *Nosema apis*, which infects the Western honey bee, *Apis mellifera*, was first described by Zander [2]. *Nosema ceranae*, which attacks the Asian honey bee, *Apis cerana*, was reported in 1996 by Fries *et al.* [3]. They invade the midgut epithelial cells of the worker bees, queens and drones. *Nosema* has negative effects on the bee colony. It can affect the productivity and survival of honeybee colonies including adult bee longevity, queen bees, brood rearing, bee biochemistry, pollen collection and other bee behavior [4].

The prevalence of *Nosema* has raised concerns especially with the recent declines in honey bee populations. Many of these losses have been attributed to Colony Collapse Disorder (CCD), although the specific causes of most losses are undetermined. It was reported that co-infection by virus and *Nosema* in honey bee might be associated with colony collapse [5]. However, *Nosema* disease is mostly overlooked by beekeepers, as there are no characteristic symptoms.

Viruses are one of the most major threats to the health of honey bees. Viruses were first identified as a new class of pathogens infecting honey bees at the beginning of the 20th century. Since then, at least 18 viruses have been reported to infect honey bees worldwide and about seven of these species have been isolated in Korea. Among them, six viruses cause severe disease in honeybees [6]. These include acute bee paralysis virus, black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), deformed wing virus (DWV), Kashmir bee virus (KBV) and Sacbrood virus (SBV). Viruses can attack different developmental stages and castes of the honey bees including

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eggs, larvae, pupae, adult worker bees, adult drones and queens. They are transferred both horizontally between bees and vertically from queen to offspring. Therefore, honey bee viruses may cause serious or even lethal disease in individual bees or the collapse of entire colonies [7]. In response to the increasing concern over honey bee disease, a survey of honey bee colonies was carried out to determine the prevalence of *Nosema* and virus on blooming period of Acacia in 2011.

Samples for *Nosema* analysis were collected from beekeeper colonies during the blooming period of Acacia. Beekeepers agreed collecting samples of 30~40 bees from individual hives. Bee samples were defined as bees at the hive entrance or on frames away from the brood nest.

Bee samples collected for *Nosema* counts were stored in 70% ethanol at room temperature. From each sample, 10 worker bees were randomly selected and examined microscopically for the presence of *Nosema* spores using standard techniques. The abdomens removed from the bees were placed in mortar with 1 mL of distilled water for every bee and crushed with a pestle until the mixture was uniform. A droplet of the suspension was then placed on a hemacytometer (Superior, Marienfeld, Germany) for an estimation of spore numbers. Each sample extract was counted and the average number of spores per bee was calculated under a dissecting microscope.

Twenty hives owned by 18 beekeepers were sampled in 2011. Results of spore count per bee are shown in Table 1. The infection rate of colony for *Nosema* was 95% based on the presence of spores. *Nosema* spore counts ranged from zero to 1,710,000 spores per bee. The average number of *Nosema* spores per bee was 580,000.

Table 1. Spores of *Nosema* per bee

Province	City	Beekeeper code	Spores per bee
Gyeonggi-do	Dongducheon	A	0
		B	345,000
		C	125,000
	Uijeongbu	D	165,000
		E	200,000
	Pocheon	F	55,000
		G	245,000
		H	10,000
	Yeoncheon	I	540,000
		J	260,000
		K	510,000
	Paju	L	1,605,000
		M	705,000
		N	690,000
O		965,000	
P		845,000	
Gangwon-do	Cheorwon	Q	1,370,000
		R	1,710,000
		S	830,000
		T	425,000

Beekeeper code J sampled from Cheorwon city in Gangwon-do province had the highest number of spores per bee with 1,710,000 and beekeeper code A had no spores. This indicated a broad range of *Nosema* infection levels in hives. Approximately 95% of the colonies were infected with *Nosema* during the flowering period of Acacia, indicating the predominance of *Nosema* in the affected honeybee colonies. Few studies have been carried out to determine the prevalence of *Nosema* in Korea [8, 9]. *Nosema* disease has been long identified as a serious disease of honeybees. It increases the mortality of adult bees and causes significant loss of honey yields. Furthermore, *Nosema* drastically affects honey population because the bees are unable to produce enough royal jelly to feed the brood. Infections by *Nosema* can suppress the honey bee immune response and may increase susceptibility to other pathogens [10]. In addition, the horizontal transmission from workers to queens may increase the chances of queen failure and colony loss [11].

The presence of bee virus was investigated by reverse transcription-polymerase chain reaction (RT-PCR). Bee samples collected from the same colony by our research group were stored at -70°C until processed. Total RNA was extracted employing a total RNA Extraction Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Primers for real-time PCR were synthesized by Bioneer Corporation (Daejeon, Korea). The sequences of the primer pairs are shown in Table 2. Viral RNA was reverse transcribed and amplified by a continuous RT-PCR method with the One Step RT-PCR kit (Qiagen, Valencia, CA, USA) following the manufacturer's recommendations. RT-PCR amplification was performed in a PTC-200 apparatus

Table 2. Oligonucleotide primers employed in RT-PCR assays

Primer name	Primer sequence (5' → 3')
CWV F	ATC AGC GCT TAG TGG AGG AA
CWV R	TCG ACA ATT TTC GGA CAT CA
CWV F	GAT GAA CGT CGA CCT ATT GAA AAA GIT AAT C
CWV R	GTG GGT TGG CTA TGA GTC ATC ATG TAT ACT G
KBV F	GAT GAA CGT CGA CCT ATT GA
KBV R	TGT GGG TTG GCT ATG AGT CA
BQCV F	TGG TCA GCT CCC ACT ACC TTA AAC
BQCV R	GCA ACA AGA AGA AAC GTA AAC CAC
IAPV F	CTC CGC TCA ATT GCT TCA TTA ATA GTA TGG G
IAPV R	CCG CTC CTG AGC ATA ACC ACG ATA AGT G
SBV F	GGT GGA ACC CGA GTG TTT TGT AAT CCT CC
SBV R	GCT ATT TGA GCA TTA AAA CCT GAC CC
CBPV F	TCA GAC ACC GAA TCT GAT TAT TG
CBPV R	TCA GAC ACC GAA TCT GAT TAT TG

RT-PCR, reverse transcription-polymerase chain reaction; CWV, cloudy wing virus; F, forward; R, reverse; KBV, kashmir bee virus; BQCV, black queen cell virus; IAPV, Israel acute paralysis virus; SBV, Sacbrood virus; CBPV, chronic bee paralysis virus.

(MJ Research, Waltham, MA, USA). Amplifications were carried out in 25 μ L of reactions containing 200 ng of RNA template and 2.5 pmole of each primer. Reverse transcription at 42°C for 60 min was followed by denaturation and polymerase activation steps at 94°C for

5 min and by 35 cycles of PCR, each consisting of 30 sec at 94°C, 45 sec at 48°C and 1 min at 72°C. Reactions were completed by a final elongation step for 10 min at 72°C. The PCR products were electrophoresed in a 1.2% Tris-acetate-EDTA-agarose gel and stained with ethidium bromide. Bands were photographed under ultraviolet illumination.

A total of 60 samples from 20 hives were investigated by RT-PCR for the presence of the seven important honeybee viruses including BQCV, CBPV, cloudy wing virus (CWV), DWV, Israel acute paralysis virus (IAPV), KBV and SBV. Four different bee viruses were detected in samples (Table 3). BQCV was detected in all samples. However, CWV, IAPV and KBV were not detected in any of samples. CBPV was detected in Beekeeper code I sampled from Yeoncheon city and Beekeeper code S sampled from Cheoron city (Fig. 1). DWV was present in Beekeeper code J sampled from Yeonchen city. SBV was detected in Beekeeper code M, N and O sampled from Paju city in Gyeonggi-do province (Fig. 2). BQCV was the most widespread bee virus in the samples. It mainly affects developing queen larvae and pupae in the capped-cell stage. Infected queen pupae die and darken, and the cell walls turn black. The second most prevalent virus was SBV. It primarily affects the brood of the honeybee and results in larval death. SBV affects adult bees without causing obvious signs of disease, but the infected adult bees may have a decreased life span. CBPV disease is referred to as the "hairless black syndrome" because of being not easily detected. However, its disease rarely causes

Table 3. Virus infections in honeybee samples

Province	City	Beekeeper code	Virus type
Gyeonggi-do	Dongducheon	A	B
		B	B
		C	B
	Uijeongbu	D	B
		E	B
	Pocheon	F	B
		G	B
		H	B
	Yeoncheon	I	B, CB
		J	B, D
		K	B
	Paju	L	B
		M	B, S
		N	B, S
		O	B, S
P		B	
Gangwon-do		Cheorwon	Q
	R		B
	S		B, CB
	T		B

B, black queen cell virus; CB, chronic bee paralysis virus; D, deformed wing virus; S, Sacbrood virus.

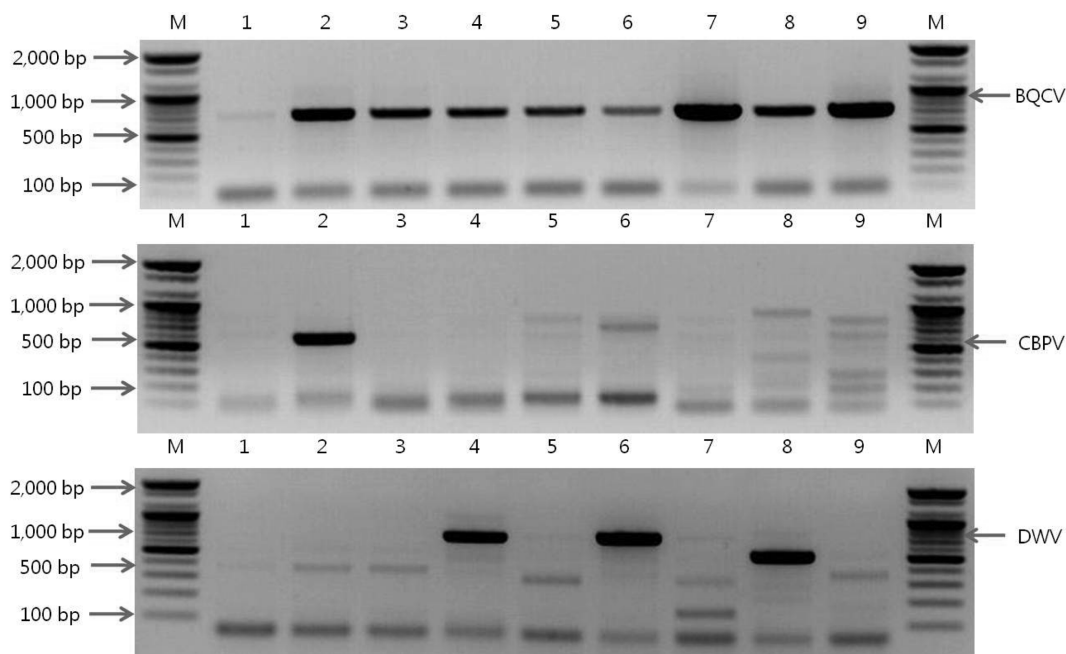


Fig. 1. Detection of viruses in samples collected from Yeonchen apiaries. Lane M, DNA size markers; 1~3, Beekeeper code I; 4~6, Beekeeper code J; 7~9, Beekeeper code K. BQCV, black queen cell virus; CBPV, chronic bee paralysis virus; DWV, deformed wing virus.

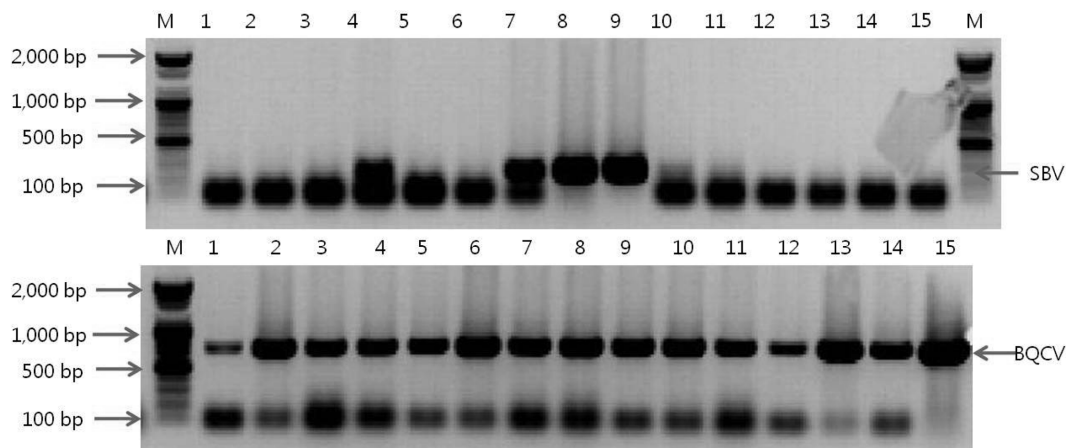


Fig. 2. Detection of viruses in samples collected from Paju apiaries. Lane M, DNA size markers; 1~3, Beekeeper code I; 4~6, Beekeeper code J; 7~9, Beekeeper code K. SBV, Sacbrood virus; BQCV, black queen cell virus.

economic damage because the colonies usually recover spontaneously from the disease [12]. DWV has been shown to be one of the most prevalent infections in honey bees in recent years, and is known to be transferred by the varroa mites. It is one of a few bee viruses that cause well-defined disease symptoms in infected bees. Typical disease symptoms of DWV infection include shrunken, crumpled wings, decreased body size and discoloration in adult bees.

Even the present limited comparison of honeybee diseases indicated that beekeepers can deal with decreased production and increased maintenance costs if the infested colonies are left untreated.

Acknowledgements

This study was carried out with the support of “Research Program for Agricultural Science & Technology Development (Agenda Program, PJ 006994 and PJ 006755),” National Academy of Agricultural Science, Rural Development Administration, Republic of Korea.

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