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An investigation of honey bee virus prevalence in managed honey bees (Apis mellifera and Apis cerana) undergone colony losses: a case study in China

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Research note

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Abstract

Objective: In the absence of known clinical symptoms, viruses were considered to be the most probable key pathogens of honey bee. Therefore, the aim of this study was to investigate the prevalence and distribution of honey bee viruses in managed *Apis mellifera* and *Apis cerana* in China.

Results: We conducted a screening of 9 honey bee viruses on *A. mellifera* and *A. cerana* samples collected from 54 apiaries from 13 provinces in China. We found that the types and numbers of viruses significantly differed between *A. mellifera* and *A. cerana.* Black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), *Apis mellifera* filamentous virus (AmFV) and Kakugo virus (DWV-C/KV) were the primary viruses found in *A. mellifera* colonies, whereas Chinese sacbrood bee virus (CSBV) and sacbrood bee virus (SBV) were the primary viruses found in *A. cerana*. The percentage infection of BQCV and CSBV were 84.6% and 61.6% in all detected samples. We first detected the occurrences of *Varroa destructor* virus-1(VDV-1, DWV-B) and DWV-C/KV in China but not ABPV in both *A. mellifera* and *A. cerana*.

Introduction

Viruses, bacteria, fungi and parasites were all confirmed to be capable of inducing reductions in colony populations [1–4]. Among these pathogens, more than 20 viruses were identified to infect honey bee, most of them belonged to the family Picornaviridae and possessed a single-strand positive-sense RNA genome. These viruses were demonstrated to not only have severe impacts on individual bees by damaging their physiology [2], interfering with their productivity [5] and contributing to behavioral disorders [6] but also on the colony growth rate and colony fitness (i.e., decrease of foraging activity at the colony level [7]).

However, most honey bee viruses cause covert infections without significant clinical symptoms, which is a barrier to their study. A few viruses can be recognized during the late stage of infection by their appearance, such as chronic bee paralysis virus (CBPV), black queen cell virus (BQCV), deformed wing virus (DWV), sacbrood virus (SBV) and Israeli acute paralysis virus (IAPV). Nevertheless, little is known about DNA viruses of honey bees compared to RNA viruses. Recently, the DNA virus *Apis mellifera* filamentous virus (AmFV) was reported to be associated with crawling bees. The AmFV viral loads were reported to be higher in the bees in spring in Switzerland and demonstrated a significant correlation with RNA viruses in infected honey bees [8]. Owing to the constant discovery of emerging viruses, many investigations have reported the occurrence and prevalence of honey bee viruses as well as other viruses that may infect honey bees, especially since IAPV was found to be associated with colony collapse disorder (CCD) [9]. Ai et al. [10] investigated the occurrence of 7 honey bee viruses in 2011 in China, and found that DWV was the most prevalent of the viruses. Subsequently, a report that demonstrated the prevalence of 6 viruses in *A. cerana* found that BQCV had an infection rate of 98% in *A. cerana* [11]. In contrast, Yang et al. [12] found that DWV was the most prevalent among the 7 viruses in China regardless of whether *A. mellifera* or *A. cerana* was investigated.

Despite the prevalence of honey bee viruses that have been extensively investigated around the world, several neglected and new honey bee viruses have not been described in China. To obtain a broader spectrum of information about honey bee viruses, we investigated the presence of viruses in *A. mellifera* and *A. cerana* bee samples from different provinces that reported large bees crawling in front of the hives and undergone colony losses. We screened bee samples from 56 apiaries from 13 provinces to identify the presence and distribution of 9 honey bee viruses in China. We found that BQCV and CSBV were the most prevalent in *A. mellifera* and *A. cerana*, respectively. Additionally, we discovered that a high percentage of colonies were infected by AmFV and DWV-C/KV in many regions in China for the first time.

Methods

Bee samples

Honey bees were sampled from 56 apiaries in 13 provinces from March to November, 2015. Worker samples of *A. mellifera* were collected from 38 apiaries in 8 provinces and samples of *A. cerana* were obtained from 18 apiaries in 10 provinces (Table S**1**). Ten colonies were randomly selected in each apiary per province. Clinical signs of virus infection were not found in these colonies; thus, these colonies were thought to be capable of honey production, although the colonies were reported to have undergone large losses or were identified with crawling bees. All of the above colonies were maintained consistent with guidelines for beekeeping practice manipulation and regularly monitored and treated for mites (only for *A. mellifera*).

DNA and RNA extraction

Briefly, for the detection of *Apis mellifera* filamentous virus (AmFV), total DNA was isolated from pooled honey bee samples (~ 50 bees) with the TissuePrep homogenizer (Gening Scientific, Beijing, China). Then, genomic DNA was extracted from the filtrate using the DNA Purification Kit (Promega Corp., Madison, WI, USA) following the manufacturer's instructions. The resulting DNA was eluted into 20 μL of nuclease-free water and stored at -20 °C.

For the other 8 RNA viruses (IAPV, SBV, ABPV, BQCV, CBPV, CSBV, KBV DWV-A, DWV-B, and DWV-C/KV), total RNA was extracted from approximately 50 pooled honey bee samples using the TRIzol Kit (Invitrogen, USA) according to the manufacturer's protocol in the TissuePrep homogenizer (Gening Scientific, Beijing, China). The obtained RNA was dissolved in 20 μL of sterile water and stored at -80 °C prior to analysis. The quantity and purity of the RNA were measured using a Nanodrop spectrophotometer (Thermo Scientific, Beijing, China). The cDNA was synthesized using the M-MLV reverse transcriptase with the oligo dT primer according to the manufacturer's instructions. The cDNA samples were used as templates for PCR amplification with specific primers corresponding to viral genes.

PCR detection

The primer sequences, orientation and references are provided in Table S**2**. For the 8 RNA viruses, the initial cycle for reverse transcription was 80 °C for 30 min, followed by the PCR cycles. The PCR reaction

for AmFV and the other 10 RNA viruses consisted of a total 20 μl volume containing 2 × GoTaq reaction buffer (Promega, USA), 1 μM of the sense and antisense primers, 1 μL of cDNA or DNA, and nuclease-free water. The cycling conditions were as follows: 1 min at 95 °C; 33 cycles of 30 s at 94 °C, 30 s at 55 °C and 72 °C for 1 min; a final extension of 10 min at 72 °C; and cooling to 4 °C. The PCR amplification products were separated in a 2% agarose gel stained with GV II (Biomec, China) and photographed with a FR-200A luminescent and fluorescent biological image analysis system (Furi, China). The product size was determined using a 100-bp molecular size ladder.

Statistical analysis

The results were expressed as the presence or absence of virus in an apiary. We considered virus to be present in an apiary if samples from any colony were positive for any virus. Differences in the viral infection percentages of different provinces and virus co-infection were estimated using the Chi-square test with the SPSS 21 software.

Results

Virus prevalence and occurrence

As shown in Table **1**, different types of viruses were detected in all provinces with the exception of Jiangsu province where no virus was detected. All of the viruses were present in the different provinces with the exception of KBV and ABPV, which were not detected. BQCV was the most prevalent virus and was present in 18 % of the *A. mellifera* apiaries and in seven provinces. CSBV was found in 38.9 % of the *A. cerana* apiaries and in seven provinces. In contrast, BQCV was detected in 22 % of the *A. cerana* apiaries and CSBV was found in 2.6 % of the *A. mellifera* apiaries. The following frequencies were obtained for the remainder of the viruses: IAPV was detected in 7.9 % of the *A. mellifera* apiaries and 5.6 % of the *A. cerana* apiaries; CBPV was found in 15.8 % of the *A. mellifera* apiaries and was not detected in *A. cerana*; SBV was identified in 5.3 % of the *A. mellifera* apiaries and 16.7 % of the *A. cerana* apiaries; DWV-A was found in 13.2 % of the *A. mellifera* apiaries and 5.6 % of the *A. cerana* apiaries; DWV-B was found in 10.5 % of the *A. mellifera* apiaries and was not detected in the *A. cerana* apiaries; AmFV was found in 13.2 % of the *A. mellifera* apiaries and 11.1 % of the *A. cerana* apiaries; and DWV-C/KV was found in 10.5 % of the *A. mellifera* apiaries and 11.1 % of the *A. cerana* apiaries.

BQCV was the most common virus and accounted for 84.6 % among the tested samples in each province (Figure **1A**). The second highest frequency virus was CSBV (61.6%), followed by another 3 viruses at equal frequency (53.8 %; DWV-A, AmFV and DWV-C/KV). The least frequent viruses were IAPV and DWV-B with 30.8 %. The number of honey bee viruses in *A. mellifera* was higher than that in *A.cerana* in the same province (Figure **1B**) with the exception of Chongqing and Yunnan, which gave priority to keeping *A. cerana*. The number of viruses in *A. mellifera* in Zhejiang was the same as that of *A. cerana*. Liaoning province had the highest number of viruses, with 9 viruses in *A. mellifera* and only one in *A. cerana*. Similarly, there were 7 viruses in *A. mellifera* in Inner Mongolia and Heilongjiang and no viruses were detected in *A. cerana*.

Co-infection of viruses in each province

The co-occurrence of more than one virus in one province was fairly common in both the *A. mellifera* and *A. cerana* apiaries (**Table S3** and **Table** S4). In *A. mellifera*, virus co-infection varied from 2 to 6 viruses. Co-infection with 2 viruses represented 28.9 % of the occurrences, 3 viruses represented 31.6 %, 4 viruses represented 15.7 %, 5 viruses represented 10.5 % and 6 viruses represented 7.89 %. Among the multiple co-infections, BQCV and CBPV co-infection was the most common 2 virus co-infection and accounted for 10.53 %. IAPV, SBV and CBPV was the highest percentage 3 virus co-infection with 7.89 %, followed by BQCV, CBPV and AmFV, IAPV, SBV and CSBV, and IAPV, DWV-A and DWV-B. IAPV, BQCV, DWV-A and DWV-B was the most common 4 virus co-infection with 13.16 %. In contrast, fewer co-infections were detected in *A. cerana* compared to *A. mellifera* (**Table S4**). Virus co-infections in *A. cerana* primarily involved 2 and 3 viruses. Simultaneous infections with 2 viruses were detected in 61.11% and with 3 viruses in 16.67% of the apiaries. The most prevalent co-infection was SBV and CSBV in *A. cerana*, accounting for 22.22 %. Co-infection with 4 viruses (IAPV, BQCV, DWV-A, and DWV-B) was significantly higher than the other co-infections in *A. mellifera,* (χ^2=12.61, P=0.00038, df=1) (Table **S5**), whereas in *A. cerana* co-infection with 2 viruses was significantly more common than the other co-infection types (e.g., BQCV and CSBV; χ^2=0.0009, P=0.02, df=1) (Table **S6**).

Discussion

The results showed that several viruses with a high prevalence and wide distribution in China were not previously reported, such as DWV-B, DWV-C/KV and AmFV. Our surveys indicated that DWV-C/KV and AmFV were widely distributed in China in both *A. mellifera* and *A. cerana*. Additionally, neither ABPV nor KBV was found in any province. This primary investigation helps to narrow future efforts to identify the cause of colony decline.

In addition, we found multiple virus co-infections in honey bee colonies of both *A. mellifera* and *A. cerana*. The widespread distribution of honey bee viruses in the two honey bee species suggests a stable interaction between the viruses and their hosts. Simultaneous infection with more than 3 viruses (65.78%) occurred in more than 12 apiaries of *A. mellifera*, which was more than occurred in *A. cerana*, suggesting that multiple infections were very common in *A. mellifera*; in contrast, most cases of virus co-infection in *A. cerana* involved 2 viruses. Data from experiment studies indicated that multiple virus infections will increase the virulence [13]. A recent report showed that IAPV could be elevated to a higher level when several viruses were mixed to infect honey bees, although the initial IAPV infection level was not the highest [14]. Thus, colony losses might be due to a combination of multiple viruses.

Conclusion

We demonstrated for the first time the presence of DWV-B and DWV-C/KV. The most prevalent viruses were BQCV, CBPV, DWV-C/KV and AmFV in *A. mellifera* and CSBV and SBV in *A. cerana*. The results of

this study suggest that future studies should focus on these viruses to narrow our objectives and help us understand the differences in different areas and hosts.

Limitations

A main limitation of current study is that we did not investigate the honey bee samples from all provinces. Therefore, we cannot draw conclusions regarding the prevalence of honey bee viruses in China. In addition, the differences in the distributions and levels of different viruses among the different tissues of honey bees also require further study, although multiple virus co-infections were found.

Declarations

Availability of data and materials

All data generated or analysed during this study are included in this published article and Additional file.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

Authors have no potential conflict or competing interests to declare.

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Authors' contributions

CSH conceived and designed the experiments. YCD, LZ, XJJ, SY and SD performed the experiments. CSH analyzed the data and wrote the manuscript.

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Tables

Table 1 Presence of IAPV, BQCV, CBPV, ABPV, SBV, CSBV, DWV-A, DWV-B, AmFV, KBV and DWV-C/KV in *A. mellifera* and *A. cerana* samples from 56 apiaries

Apiary region	IAPV[b]		BQCV		CBPV		ABPV		SBV		CSBV		DWV-A		DWV-B		AmFV		KBV		DWV-C	
	A.m	A.c	A.m	A.c	A.m	A.c	A.m	A.c	A.m	A.c	A.m	A.c	A.m	A.c	A.m	A.c	A.m	A.c	A.m	A.c	A.m	A.c
Zhejiang JH[a]	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	-
Henan PY/ZK	-	-	+	+	+	-	-	-	-	+	-	+	+	-	+	-	-	+	-	+	-	-
Hubei SNJ/ES	×	-	×	-	×	-	×	-	×	-	×	+	×	-	×	-	×	-	×	-	×	-
Anhui HF	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Guangdong GZ	×	-	×	+	×	-	×	-	×	-	×	-	×	-	×	-	×	-	×	-	×	-
Liaoning HLD/CY	+	-	+	-	+	-	-	-	+	-	+	+	+	-	+	-	+	-	-	+	-	-
Hunan YY	×	-	×	-	×	-	×	-	×	+	×	-	×	-	×	-	×	-	×	-	×	-
Beijing HD	-	-	+	+	+	-	-	-	-	-	-	+	+	-	+	-	+	-	-	-	+	-
Inn Mon AH	+	×	+	×	+	×	-	×	+	×	-	×	+	×	-	×	+	×	-	×	+	×
Chongqing NC	×	-	×	-	×	-	×	-	×	-	×	+	×	+	×	-	×	-	×	-	×	-
Jiangsu XZ	-	×	-	×	-	×	-	×	-	×	-	×	-	×	-	×	-	×	-	×	-	×
Heilongjiang YC	+	×	+	×	+	×	-	×	-	×	-	×	+	×	+	×	-	×	+	×	+	×
Yunnan WX	×	+	×	-	×	-	×	-	×	+	×	+	×	-	×	-	×	-	×	-	×	+
No. (%) of positive apiaries	7.9 (38)	5.6 (18)	18.4 (38)	22.2 (18)	15.8 (38)	0 (18)	0 (38)	0 (18)	5.3 (38)	16.7 (18)	2.6 (38)	38.9 (18)	13.2 (38)	5.6 (18)	10.5 (38)	0 (18)	13.2 (38)	11.1 (18)	0 (38)	0 (18)	15.8 (38)	5.6 (18)

[a] The collection locations: JH, Jinhua; PY/ZK, Pingyu and Zhoukou; SNJ/ES, Shengnongjia and Enshi; HF, Hefei; GZ, Guangzhou; HLD/CY, Huludao and Chaoyang; YY, Yueyang; HD, Haidian; AH, Aohan; NC, Nanchuan; XZ, Xuzhou; YC, Yichun; WX, Weixi.[b] +, positive; -, negative; ×, samples not available

Figures

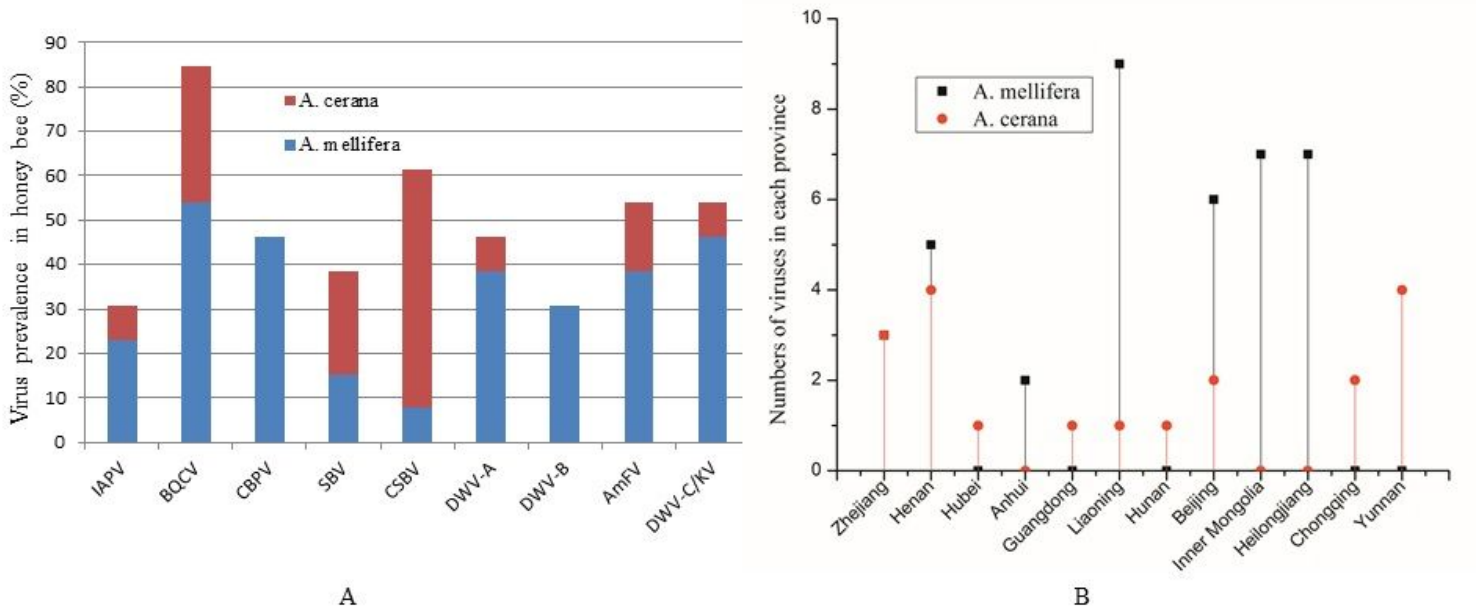

Figure 1

Virus prevalence varied in different honey bee species and provinces. The cumulative prevalence of different viruses in A. mellifera and A. cerana (A); the number of virus in A. mellifera and A. cerana in each province (B).

Supplementary Files

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