1874-2858/21 *Send Orders for Reprints to reprints@benthamscience.net*

The Open Microbiology Journal

Content list available at: https://openmicrobiologyjournal.com

Supplementary Material

An Investigation of Honey Bee Viruses Prevalence in Managed Honey Bees (*Apis mellifera* and *Apis cerana*) Undergone Colony Decline

Chunying Yuan[1,†], Xuejian Jiang[2,†], Man Liu[3,†], Sa Yang[4,5], Shuai Deng[4,5] and Chunsheng Hou[4,5,*]

[1]*Liaoning Agricultural Development Service Center, Xingcheng, China*
[2]*Guangxi Zhuang Autonomous Region Forestry Research Institute, Nanning, China*
[3]*Guizhou Institute of Biology, Guiyang, China*
[4]*Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, China*
[5]*Key Laboratory of Pollinating Insect Biology, Ministry of Agriculture and Rural Affairs, Beijing, China*

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.

Methods:

We conducted a screening of 8 honey bee viruses on *A. mellifera* and *A. cerana* samples collected from 54 apiaries from 13 provinces in China using RT-PCR.

Results:

We found that the types and numbers of viral species 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-A/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 or DWV-B) and DWV-A/KV in China but not ABPV in both *A. mellifera* and *A. cerana*.

Conclusion:

This study showed that BQCV and CSBV are the major threat to investigated *A. mellifera* and *A. cerana* colonies.

Keywords: Honey bee viruses, BQCV, CBPV, AmFV, CSBV, *A. mellifera*, *A. cerana*.

Article History	Received: November 11, 2020	Revised: April 1, 2021	Accepted: April 6, 2021

Table S1. Numbers of apiaries selected in different province.

Virus	*A. mellifera*	*A. cerana*
Zhejiang	1	1
Henan	11	3
Hubei	0	3
Anhui	1 1	
Guangdong	0	2
Liaoning	13	2
Hunan	0	1
Beijing	2	1
Neimenggu	7	0
Heilongjiang	2	0

(Table S1) contd.....

Virus	*A. mellifera*	*A. cerana*
Chongqing	0	2
Jiangsu	1	0
Yunnan	0	2

Table S2. Primers used for PCR detection in present study.

Virus	Forward Primer Reverse Primer	Reference
AmFV	CAGAGAATTCGGTTTTTGTGAGTG CATGGTGGCCAAGTCTTGCT	Hartmann *et al*., 2015
IAPV	AGACACCAATCACGGACCTCAC AGATTTGTCTGTCTCCCAGTGCAC	Maori *et al*., 2007
SBV	ATATACGGTGCGAGAACTGC CTCGGTAATAACGCCACTGT	Hou *et al*., 2014
ABPV	TTATGTGTCAGAGACTGTAT GCTCCTATTGCTCGGTTTTTC	Blanchard *et al*., 2007
BQCV	TGGTCAGCTCCCACTACCTTAAAC GCAACAAGAAGAAACGTAAACCAC	Benjeddou *et al*., 2001
CBPV	TCAGACACCGAATCTGATTATTG ACTACTAGAAACTCGTCGCTTCG	Berényi *et al*., 2006
VDV-1	CATAGCGAATTACGGTGCAA GAGGGGTCCCTACTCTACCG	Hou *et al*., 2014
DWV	CTTACTCTGCCGTCGCCCA CCGTTAGGAACTCATTATCGCG	Chen *et al*., 2005
CSBV	CCTGGGAAGTTTGCTAGTATTTACG CCTATCACATCCATCTGGGTCAG	Ma *et al*., 2013
KBV	TATGCTGAACAACGCAAAGA ACAACACGATGTCTGGGTTT	Stolz *et al*., 1995
KV	GACTGAACCAAATCCGATGTC TCTCAAGTTCGGGACGCATTC	Fujiyuki *et al*., 2009

Table S3. Results of chi-square test for all types of co-infection in *A. mellifera*.

Number of Virus	Type of Co-infection	Chi-square (df=1)	P Level
2	BQCV; KV	1.81	0.18
-	BQCV; CBPV	0.22	0.64
-	CBPV; DWV	0.51	0.48
-	BQCV; DWV	1.82	0.18
-	BQCV; AmFV	0.42	0.48
-	IAPV; DWV	0.05	0.18
3	BQCV; AmFV; KV	2.56	0.11
-	BQCV; CBPV; DWV	2.56	0.11
-	BQCV; CBPV; AmFV	1.01	0.32
-	BQCV; CSBV; CBPV	2.56	0.11
-	IAPV; SBV; CSBV	1.01	0.32
-	IAPV; SBV; CBPV	0.19	0.66
-	IAPV; DWV;VDV-1	1.01	0.32
4	IAPV; SBV; CSBV; DWV	0.0026	0.95
-	IAPV; BQCV; DWV; VDV-1	12.61	**0.00038**
5	IAPV; DWV; VDV-1; CBPV; AmFV	4.61	**0.031**
-	IAPV; SBV; BQCV; DWV; CSBV	4.61	**0.031**
6	IAPV; BQCV; CBPV; DWV; VDV-1; AmFV	18.88	<10-5

Table S4. Results of chi-square test for all types of co-infection *A. cerana*.

Number of Viruses	Type of Co-infection	Chi-square (df=1)	P Level
2	BQCV; AmFV	0.0009	**0.02**
	BQCV; CSBV	0.0009	**0.02**
	SBV; CSBV	0.061	0.19
	BQCV; DWV	<10-5	**0.004**
	BQCV; CBPV	0.0009	**0.02**
3	BQCV; CSBV; KV	0.04	0.15
	SBV; CSBV; AmFV	0.04	0.15
	IAPV; SBV; CSBV	0.04	0.15

REFERENCES

[1] Maori E, Tanne E, Sela I. Reciprocal sequence exchange between non-retro viruses and hosts leading to the appearance of new host phenotypes. Virology 2007; 362.

[2] Stolz D, Shen XR, Boggis C, Sisson G. Molecular diagnosis of Kashmir bee virus infection. J Apic Res 1995; 34.

[3] Blanchard P, *et al.* Evaluation of a real-time two-step RT-PCR assay for quantitation of Chronic bee paralysis virus (CBPV) genome in experimentally-infected bee tissues and in life. Appl Environ Microbiol 2007; 73.

[4] Benjeddou M, Leat N, Allsopp M, Davison S. Detection of acute bee paralysis virus and black queen cell virus from honeybees by reverse transcriptase PCR. Appl Environ Microbiol 2001; 67.

[5] Berényi O, Bakonyi T, Derakhshifar I, Köglberger H, Nowotny N. Occurrence of six honeybee viruses in diseased Austrian apiaries. Appl Environ Microbiol 2006; 72.

[6] Chen Y, Pettis JS, Feldlaufer MF. Detection of multiple viruses in queens of the honey bee *Apis mellifera* L. J Invertebr Pathol 2005; 90.

[7] Fujiyuki T, *et al.* Distribution of Kakugo virus and its effects on the gene expression profile in the brain of the worker honeybee *Apis mellifera* L. J Virol 2009; 22.