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

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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.

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.

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         |

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

| Virus   | Forward Primer          | Reverse Primer          | Reference                     |
|---------|-------------------------|-------------------------|-------------------------------|
| AmFV    | CAGAGAATTCCGTTTTTGTGAGTG| CATGGTGCGCAATGCTTGCT    | Hartmann et al., 2015         |
| IAPV    | AGACACCAAATCCGGACCTCAG | AGATTGTCTGTTCCCAAGTGCAC | Maori et al., 2007           |
| SBV     | ATATACGTGGGCAGAAGCTGC  | TCTGGTAAATAAGGCGCACTG   | Hou et al., 2014              |
| ABPV    | TTATGTCTGACAGAGCTGTAT | GCTGCTATGTCGTTTTTTC     | Blanchard et al., 2007        |
| BQCV    | TGGTCAGCTCCACCTACCTAAAACG| GCAACACAAACAGTAAACACCAC | Benjeddou et al., 2001       |
| CBPV    | TCAGACACCGAATCTGATTATTG| ACTACTAGAAACTCGTCGCTTCG | Berényi et al., 2006          |
| VDV-1   | CATAGCGAATTACGGTGCAA   | GAGGGTGCCCTACTCTACC     | Hou et al., 2014              |
| DWV     | TTATCTGCTGGCGCCCA     | CCCTAGAGAAGCTTCATTCGCG  | Chen et al., 2005             |
| CSBV    | CCTGGGAAGTTTGCTAGTATTTACG| CCTATCACATCCATGCTGTCAG  | Ma et al., 2013               |
| KBV     | TATGCTGAAAACAGCAAAGA | ACAACACAGATGCTGGGGTTT   | Stolz et al., 1995            |
| KV      | GACTGAACCAAATCCGATGTC| TCTCAAGTTCCGAGCGCATTC   | 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    |

(Table S1 contd.....
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