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Short communication

Chinese sacbrood virus infection in *Apis mellifera*, Shandong, China, 2016

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ABSTRACT

The Chinese sacbrood virus (CSBV) was first isolated from *Apis cerana* in 1972. However, the biological characteristics of the CSBV that naturally infects *Apis mellifera*, causing larval death, have not been reported yet. In the present study, natural CSBV infection was evaluated using clinical symptoms of *A. mellifera* larvae, RT-PCR, electron microscopy, agar gel immunodiffusion assays, and virus analysis in inoculated *A. cerana* larvae. The isolated CSBV strain was named AmCSBV-SDLY-2016. Subsequently, AmCSBV-SDLY-2016 was analyzed by constructing a phylogenetic tree using VP1. Data from the phylogenetic tree suggested that AmCSBV-SDLY-2016 is evolutionarily close to JLCBS-2014. It was also observed that CSBV crossed the species barrier, causing the death of *A. mellifera* larvae.

The sacbrood virus (SBV) is characterized by its ability to spread rapidly and widely (Yan and Han, 2008; Liu et al., 2010). Since it was first identified in *Apis mellifera L.* in the United States in 1913, SBV infection has been detected in almost all honeybee colonies worldwide (Allen and Ball, 1996; Ellis and Munn, 2005; Thi et al., 2008; Xia et al., 2015). SBV mainly infects 2–3-day-old larvae, and leads to their death. The SBV that infects the Chinese honeybee was named the Chinese SBV (CSBV). CSBV was first described in Guangdong, China in 1972, and re-emerged in Liaoning, China in 2008 (Ma et al., 2010), causing lethal disease in individual bees and the collapse of entire colonies. Since then, the virus has frequently infected *Apis cerana* in this region of China, which has destroyed apiculture in the region. SBV belongs to the small RNA virus family, *Picornaviridae*. The genome contains one large open reading frame (ORF); the ORFs of SBV and CSBV encode three and four structural proteins, respectively (Bailey et al., 1964; Shufa et al., 2011; Ghosh et al., 1999); however, there is no distinct difference between the nonstructural proteins of SBV and CSBV. Among the structural proteins of the different SBV strains, VP1 has the greatest variation in amino acid sequence, and contains the common and major epitope of SBV (Jian et al., 2011). Changes in this protein may be responsible for the diversity of SBV and changes in its host specificity. Compared with VP1, which is one of the non-structural proteins of CSBV, RdRp is highly conserved, and is mainly used to distinguish between the species or genus specificity of the virus. VP1 is often used to distinguish between the type or subtype specificity of the virus, and based on the SBV-VP, SBV is divided into two major groups (AC genotype SBV, which infects *Apis cerana,* and AM genotype SBV, which infects *Apis mellifera*) (Ma et al., 2013). The AC genotype could be further divided into subgroups. The differences between the AC and AM genotypes may be due to the adaptation of the virus to a different host, and the fact that different subgroups of the AC genotype exist based on regional variations (Grabensteiner et al., 2001; Choe et al., 2012; Ma et al., 2013). CSBV and SBV differ in their molecular biological characteristics, pathogenicity, and immunogenicity (Zhang et al., 2001; Choe et al., 2012; Gong et al., 2016). In the paper, the biological characteristics of CSBV from *A. mellifera* have been reported.

In May 2016, an infectious disease was detected in two *A. mellifera* apiaries in Linyi City, Shandong Province, China. Initially, the larvae of the honeybees failed to pupate, and ecdysial fluid accumulated beneath the unshed skin of the larvae. The color of the infected larvae changed from white to pale yellow, and they died. Shortly after they dried out, the larvae formed a dark brown gondola-shaped scale, and showed a mortality rate of approximately 85%–90%. The adult bees showed no obvious clinical symptoms. A considerable number of dead adult worker bees and larvae were observed in the hives or near the entrances.

To identify the causative agent of the disease, we examined 50 bee larvae that had died in the hives or near the entrances of the 2 above mentioned apiaries.

After weighing, the larvae were completely homogenized in sterile water (1.5-fold by weight) using a pestle and mortar. CSBV purification was performed by cesium chloride gradient centrifugation as described by Ma et al. (2011a,b). The supernatant was passed successively through a 0.45-μm cell filter and a 0.22-μm cell filter. The samples were homogenized in phosphate-buffered saline, and analyzed by RT-PCR and electron microscopy for presence of the following viruses: black

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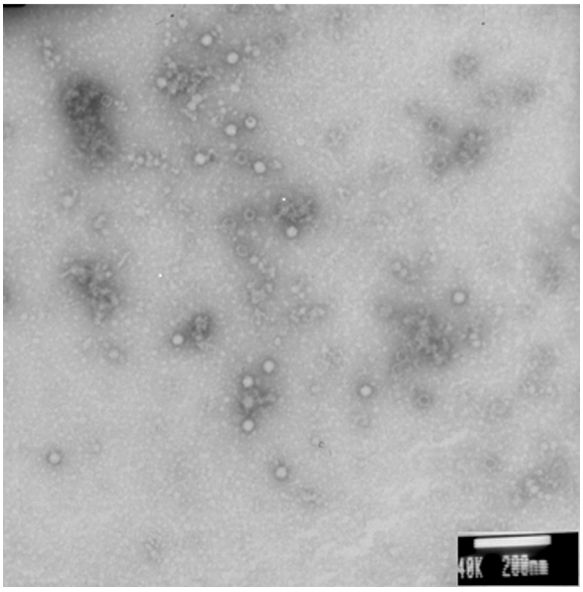

Fig. 1. Observed virus particles of CSBV by electron microscopy. electron microscopy revealed the presence of large amounts of empty and filled icosahedral virus particles of about 26 nm in diameter in virus preparations from infected larvae.

queen cell virus (BQCV; Grabensteiner et al., 2007), acute bee paralysis virus (ABPV; Grabensteiner et al., 2007), chronic bee paralysis virus (CBPV; Tentcheva et al., 2004), deformed wing virus (DWV; Tentcheva et al., 2004), Kashmir bee virus (KBV; Blanchard et al., 2014), Israeli acute paralysis virus (IAPV; Miranda et al., 2010, and CSBV (Ma et al., 2013).

Thus, RT-PCR analyses did not show the presence of viruses other than CSBV in the viral suspensions, and *Iflavirus*-like particles were identified by electron microscopy (Fig. 1), and named AmCSBV-SDLY-2016. The presence of AmCSBV-SDLY-2016 was further confirmed by agar gel immunodiffusion assays using antisera against CSBV LNQY-2008 (Hu et al., 2016). This result indicated cross-immunogenicity between AmCSBV-SDLY-2016 and the CSBV antisera.

A phylogenetic tree was constructed based on the high variability among the partial amino acid sequences of VP1 (Hu et al., 2016; Mingxiao et al., 2013), which was obtained from China, Korea, Vietnam, India, Australia, and the United Kingdom, in order to illustrate the probable genetic relationships among the selected SBV/CSBV strains by the neighbor-joining method (p = distances) and the use of up to 1000 bootstrapping replicates in the MEGA 5.0 software.

The VP1-based phylogenetic tree (Fig. 2) revealed that AmCSBV-SDLY-2016, JLCBS-2014, CQ-2012, JXNC-2013, and SXXA-2015 were classified into the same group; except for AmCSBV-SDLY-2016, all the other strains were obtained from *A. cerana*. These results demonstrated that AmCSBV-SDLY-2016 belongs to CSBV, and is likely to have originated from JLCBS-2014 (Table 1).

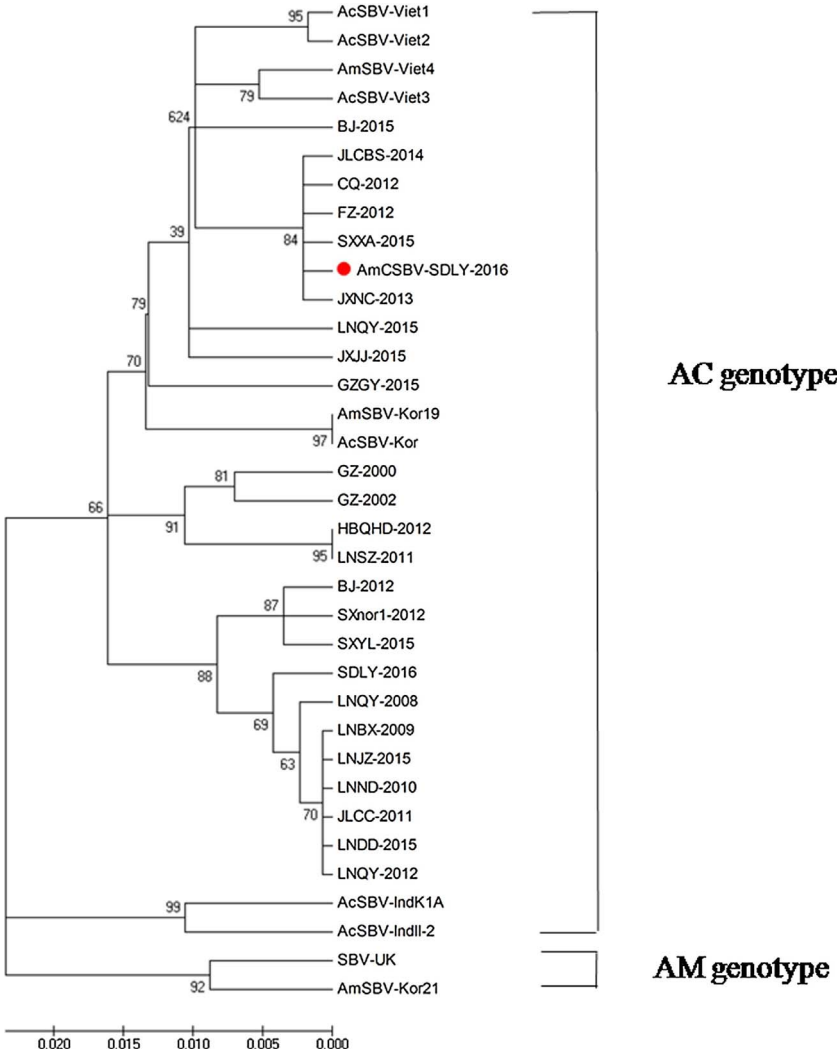

Fig. 2. Phylogenetic analysis of the amino acid sequences of VP1 of the virus obtained from China, Korea, Vietnam, India, Australia, and the United Kingdom. The numbers on the nodes indicate clade credibility values. The sequence names are in the following format: area of isolation, accession number, year of collection (Table 1). The tree reveals two clusters, namely the AC and AM genotypes (AC genotype SBV, which infects *Apis cerana,* and AM genotype SBV, which infects *Apis mellifera*) (Ma et al., 2013). AmCSBV-SDLY-2016 consists of the AC genotype, and is likely to have originated from JLCBS-2014. The bar represents a genetic distance of 0.005.