MOLECULAR DETECTION OF HONEY BEE VIRUSES IN AN *OSMIA BICORNIS* POPULATION IN THE CZECH REPUBLIC AND THEIR PREVALENCE IN THE PROXIMITY OF COMMERCIAL HIVES

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ABSTRACT

The global decline in pollinators, particularly honeybees (*Apis mellifera*) and solitary bees such as *Osmia bicornis*, has raised significant concerns due to the increasing threats from environmental stressors and pathogen spillover. This study aimed to detect the presence of honeybee-associated viruses in an *O. bicornis* population in the Czech Republic and investigate the potential for viral transmission between *A. mellifera* and *O. bicornis*. Molecular techniques were used to determine the presence of five common viruses: Deformed Wing Virus (DWV), Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Lake Sinai Virus (LSV) and Apis mellifera Filamentous Virus (AmFV). Sampling was done at two locations: an apiary where *O. bicornis* coexisted with *A. mellifera* and a remote site without commercial hives.

The results confirmed the presence of all five viruses in *O. bicornis* at the apiary, while only BQCV and DWV were consistently detected in bees from the remote site. Interestingly, the viral load at the apiary increased over time, particularly, that of ABPV and DWV, indicating that proximity to *A. mellifera* hives facilitates virus transmission to *O. bicornis*. Moreover, the presence of virus was confirmed in all developmental stages of *O. bicornis*, from larvae to adults, indicating potential for vertical transmission. Despite high viral incidence, no visible morphological deformities were observed in *O. bicornis*, indicating that these viruses may exist asymptomatically in solitary bees. These findings underscore the risks posed by managed bee populations to wild pollinators and the need for further investigations into the ecological effect of viral spillover.

Keywords: ABPV; AmFV; DWV; LSV; mason bee

Introduction

The decline in pollinator populations has become a significant global concern, with both managed and wild bees facing increasing threats from various environmental and anthropogenic factors (McCallum and Dobson 2002; Potts et al. 2010; Goulson and Hughes 2015). Among the species providing pollination services, honeybees (*A. mellifera*) have attracted much attention due to their critical role in agriculture (Klein et al. 2007) and ecosystem stability (Tarpy 2003; Whitehorn et al. 2011). However, solitary bees such as *O. bicornis* also play an essential role in agricultural productivity (Ladurner et al. 2004). *O. bicornis*, commonly known as the red mason bee, is a solitary bee widely distributed across Europe, including the Czech Republic, and is highly efficient at pollinating in early spring as they forage when temperatures are low. However, when they share habitats with commercial bees like *A. mellifera*, solitary bees such as *O. bicornis* can become infected with pathogens, which pose a risk to their health and populations (Babin et al. 2024).

Various stressors, including habitat loss, pesticide exposure, and pathogen transmission increasingly threaten the health of both wild and managed bee populations. Viral pathogens, in particular, are recognized as a major contributor to the decline in bee health. Numerous viruses, including Deformed Wing Virus (DWV) (Genersch et al. 2006), Acute Bee Paralysis Virus (ABPV), Apis mellifera Filamentous Virus (AmFV), Black Queen Cell Virus (BQCV) (Peng et al. 2011) and Lake Sinai Virus (LSV) are well studied in *A. mellifera* populations (Singh et al. 2010; McMahon et al. 2015; Mráz et al. 2021), where they are associated with colony collapse and significant reductions in bee vitality, often in conjunction with the parasitic mite *Varroa destructor* (Martin and Brettell 2019).

This study aims to investigate the molecular presence of common honeybee viruses in the *O. bicornis* population in the Czech Republic and evaluate their prevalence in relation to the proximity of commercial hives. Utilizing molecular techniques, the presence of viruses typically associated with honey bees were identified in *O. bicornis* from various locations. In addition, whether the distance from commercial hives correlates with the incidence and load of these viruses in *O. bicornis* was assessed, which indicates the potential for virus spillover and its implications for wild bee conservation.

Materials and Methods

Sample collection

Samples *A. mellifera* were collected from a private apiary in South Bohemia, Czech Republic, between April and July 2019. Pooled samples of 10 bees were taken at regular intervals from four hives at a single location, which housed a total of 48 colonies. The presence of infections was determined by screening 10 individuals from each hive. For experiments involving *O. bicornis*, cocoons and

Ryba, Š., Lencová, J., Havrdová, N., Hýbl, M., Mráz, P.: Molecular detection of honey bee viruses in an *Osmia bicornis* population in the Czech Republic and their prevalence in the proximity of commercial hives
European Journal of Environmental Sciences, Vol. 14, No. 2, pp. 83–90
https://doi.org/10.14712/23361964.2024.10

queen bees were purchased from Luper s.r.o., Slovakia (primaverahouse.sk). The cocoons were maintained at room temperature (20–25 °C) to induce hatching. Due to the scarcity of *O. bicornis*, 2–5 individuals were taken from each hive, whereas for *A. mellifera* 10 individuals were always collected. Samples of *O. bicornis* were collected at specific time intervals. The first collection of *O. bicornis* and *A. mellifera* samples was carried out immediately after the emergence of adult *O. bicornis* on April 10th, followed by subsequent collections at intervals (see Table 2). The solitary bee hives containing *O. bicornis* were placed approximately 1 meter away from the *A. mellifera* hives, which were positioned in groups of four on pallets. Two additional *O. bicornis* hives were placed in isolated locations in forest, with the nearest *A. mellifera* colonies being 9 km away. At both locations monitored, approximately 50 *O. bicornis* cocoons hatched, with identical nesting success and the number of nesting tubes was the same at all sites. Subsequent collections of *O. bicornis* at the specified intervals included the following developmental stages: adults, larvae and cocoons (Table 2).

Sample preparation

All the samples of *A. mellifera* and *O. bicornis* were immediately stored on ice in the field, transported to the laboratory and then frozen at −80 °C until processing.

Nucleic acids isolation and reverse transcription

Bees, larvae and cocoons were each homogenized in 5 ml of PBS in the presence of glass beads. Total RNA was extracted from 100 µl of supernatant using the RNeasy Tissue Kit (Qiagen). Using random hexamer primers, 1 µg of RNA was reverse-transcribed with the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). DNA was extracted from 120 µl of supernatant using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's instructions for animal tissues. RNA was resuspended in 20 µl of DEPC-treated water. The quantity and purity of RNA were measured using a spectrophotometer, and both RNA and DNA were stored at −80 °C until further use.

PCR assays

All PCR reaction mixtures contained 2 µM of each primer (Table 1); 1.0 mM MgCl2; 0.2 mM dNTPs; 1.25 U Hotstar Taq DNA polymerase (Qiagen); and 1 µl of cDNA (for RNA viruses) or 3 µl of DNA product (for *A. mellifera* Filamentous Virus). For the primers developed in this study, the PCR protocol involved an initial denaturation at 94 °C for 15 minutes, followed by 35 cycles of 94 °C for 30 seconds, 52 °C for 30 seconds, and 72 °C for 1 minute, with a final extension at 72 °C for 10 minutes. Positive and negative controls were included

Table 1 Primers used in this study.

Target gene	Primers	Sequence (5-3)	Size (bp)	References
DWV	DWVQ-F1	TAG TGC TGG TTT TCC TTT GTC	145	(Highfield et al. 2009)
	DWVQ-R1	CTG TGT CGT TGA TAA TTG AAT CTC		
ABPV	ABPVQ_F2	GGA TGA GAG AAG ACC AAT TG	169	(Highfield et al. 2009)
	ABPVQ_R2	CCA TAG GAA CTA ATG TTT ATT CC		
BQCV	BQCVQ_F1	CCA ATA GTA GCG GTG TTA TCT GAG	177	(Highfield et al. 2009)
	BQCVQ_R1	AGC GTA TAA TAT GTC GGA CTG TTC		
LSV	LSV1765-F	TCAAYCTKGAGCGATTTCGTGCTG	603	(Ravoet et al. 2014)
	LSV2368-R	GAGGTGGCGGCGCSAGATAAAGT		
Actin	Act_F1	CCT GGA ATC GCA GAT AGA ATG C	120	(Highfield et al. 2009)
	Act_R1	AAG AAT TGA CCC ACC AAT CCA TAC		
AmFV	AmFV rrSSUF	ACG AAC GAC TAT CTA GCC ATG AAC	591	(Cornman et al. 2010)
	AmFV rrSSUR	GTC CGT TTC GGA GTG CAT GAC		

Table 2 Sampling of *A. mellifera* and *O. bicornis* bees placed in an apiary close to bees and at a remote site without *A. mellifera* bees.

Date	Sampling of *A. mellifera* and *O. bicornis*		
	A. mellifera in apiary	*O. bicornis* close to apiary	*O. bicornis* remote location
April 10	10 bees	3 pupae hatching	3 pupae hatching
April 25	10 bees	3 adults	3 adults
May 5	10 bees	2 adults + 3 larvae	2 adults + 3 larvae
May 24	10 bees	2 adults + 3 larvae	2 adults + 3 larvae
June 10	10 bees	1adult + 3 larvae	1 adult + 2 larvae + 2 pupae
July 1	10 bees	3 pupae	3 pupae
July 27	10 bees	3 pupae	3 pupae

in each PCR reaction. A negative control lacking template DNA and a positive cDNA control were created, with the positive control enhanced by including the detection of actin (Table 1). PCR products were electrophoresed using 1.4% agarose gels, stained with ethidium bromide and visualized under UV light.

Results

Detection of viruses

In this study, the presence of five different viruses in two key bee species: *A. mellifera* and *O. bicornis,* were detected. The sample sites included an apiary where both *A. mellifera* and *O. bicornis* occurred, as well as a remote location where *O. bicornis* was isolated from *A. mellifera*. PCR analysis revealed the consistent presence of Black Queen Cell Virus (BQCV), Deformed Wing Virus (DWV), Acute Bee Paralysis Virus (ABPV), Lake Sinai Virus (LSV), and Apis mellifera Filamentous Virus (AmFV) in both species of bee. Importantly, in *O. bicornis* viruses were present in larvae, pupae and adults. In particular, the BQCV virus was detected first in the pupal stage and then throughout adulthood. This indicates infection at an early stage and the potential for vertical transmission from larval to adult stages in *O. bicornis*. In *A. mellifera*, all viruses, except for AmFV, were always present, which confirms the apiary as a reservoir of many viral pathogens. Fig. 1 illustrates the presence of these viruses over time.

Presence of viruses in larval stages

Viruses were not restricted to adult bees. As listed in Table 2, viruses were present in the larval stages of *O. bicornis*, especially BQCV and DWV. Both these viruses were consistently present in larvae of *O. bicornis* sampled in the area close to the apiary. This finding is significant as it demonstrates that infection can occur early in a bee's development. The detection of DWV in larvae may indicate viral spillover from *A. mellifera* to *O. bicornis* at the apiary, which supports the idea of interspecies viral transmission in shared habitats (Nanetti et al. 2021a). The results highlight the importance of viral presence in the early developmental stages, which may contribute to the persistence and spread of viruses within solitary bee populations. These findings are in accordance with previous studies, which indicate that solitary bees, like *O. bicornis*, can harbour honeybee-associated viruses throughout their life cycle (Levitt et al. 2013).

Higher viral incidence close to the apiary

The data reveal a notable difference in viral incidence close to and distant from the apiary. At the apiary, where

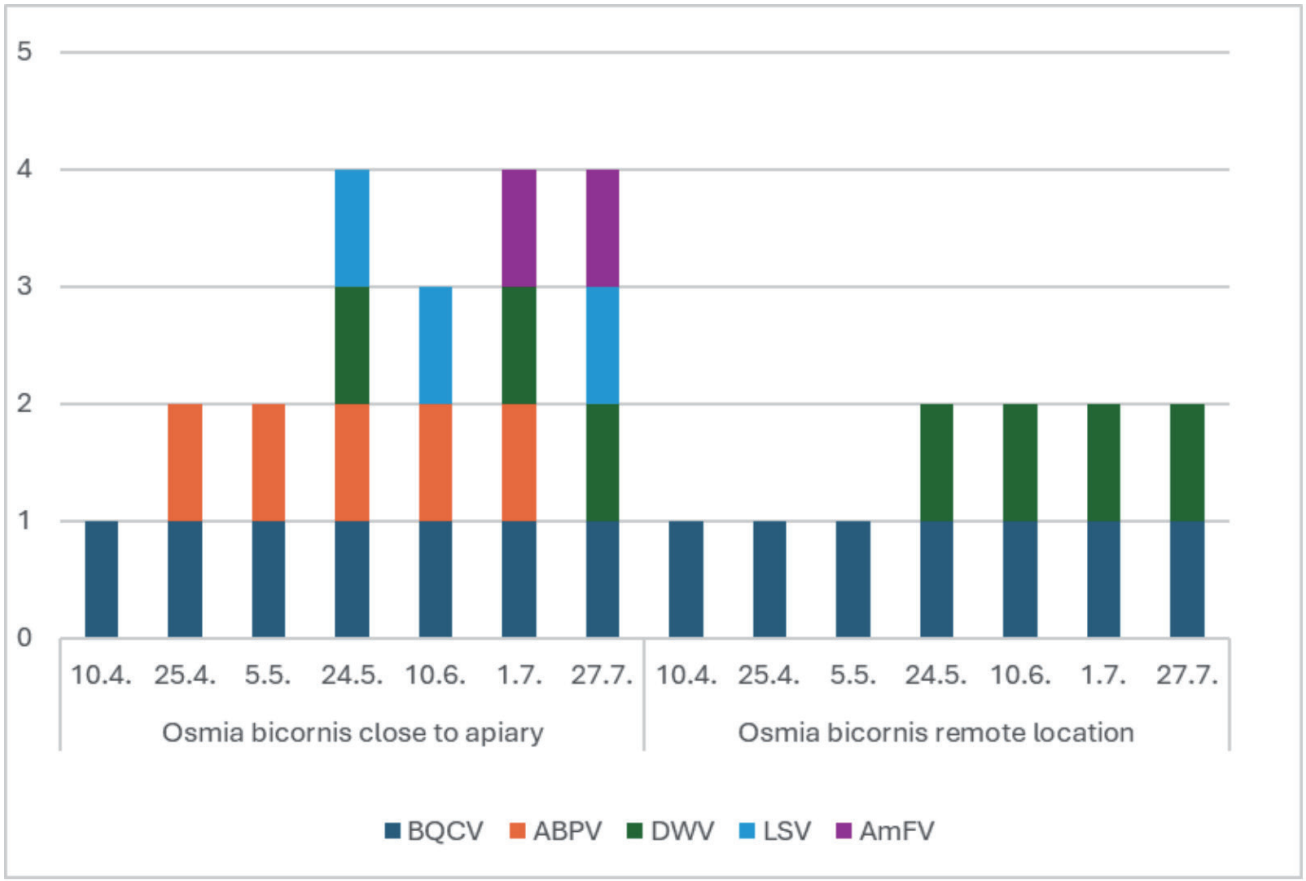

Fig. 1 Comparison of the Presence of Virus in *O. bicornis* at Two Locations Over Time; BQCV – Black Queen Cell Virus, ABPV – Acute Bee Paralysis Virus, DWV – Deformed Wing Virus, LSV – Lake Sinai Virus, AmFV – Apis mellifera Filamentous Virus.

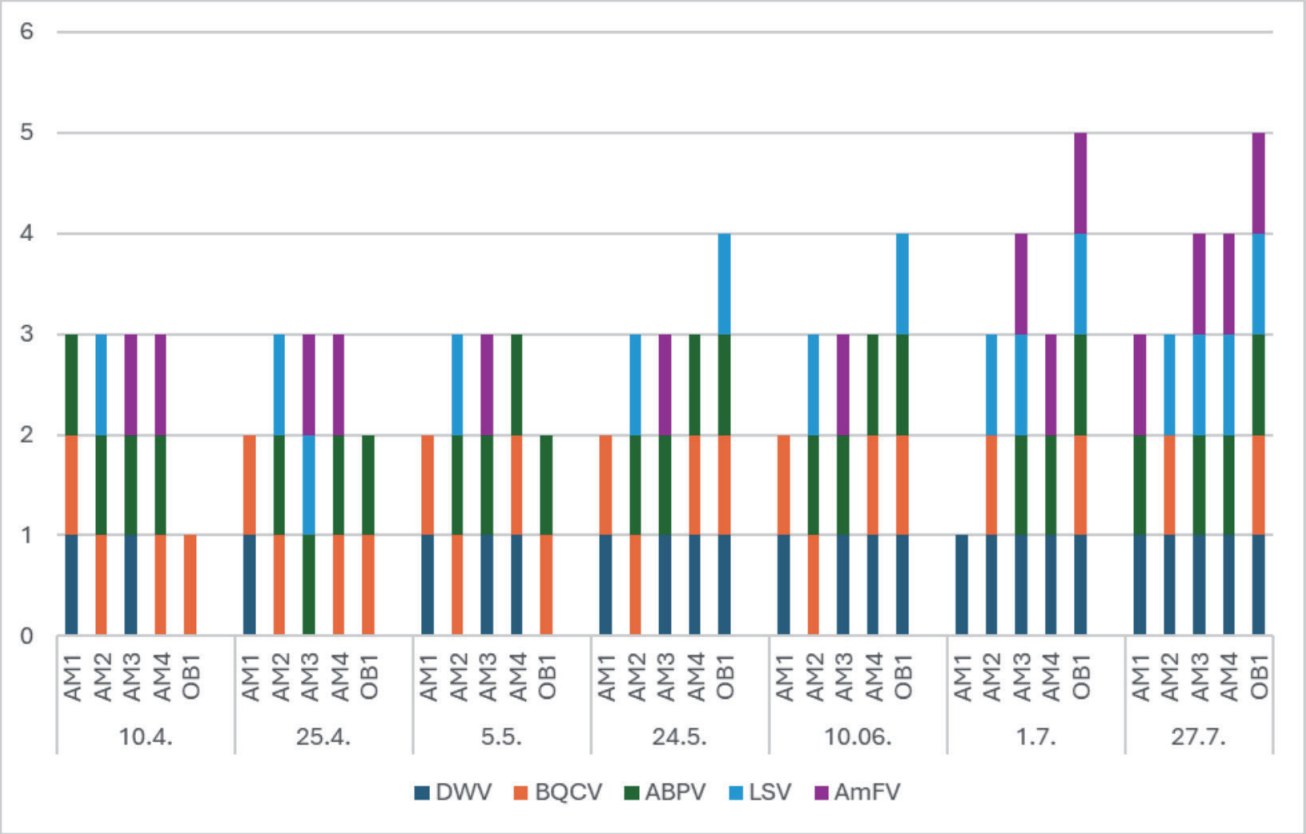

Fig. 2 Comparison of the presence of viruses in *O. bicornis* and four colonies of *A. mellifera* in an apiary. AM (1.4) – *A. mellifera* (hives 1–4), OB1 – *O. bicornis* close to apiary. BQCV – Black Queen Cell Virus, ABPV – Acute Bee Paralysis Virus, DWV – Deformed Wing Virus, LSV – Lake Sinai Virus, AmFV – Apis mellifera Filamentous Virus.

O. bicornis was in close proximity to *A. mellifera*, viral infections were more frequent and abundant. In contrast, *O. bicornis* collected from the remote location had fewer virus infections, with only BQCV and DWV present consistently. Over time, viral infections of *O. bicornis* at the apiary steadily increased, particularly those by ABPV and DWV, which were detected more frequently in samples collected later in the season. The progression of viral load in *O. bicornis* at the apiary suggests that viral transmission dynamics are influenced by proximity to *A. mellifera* populations (Graystock et al. 2016). This pattern is further supported by the detection of LSV and AmFV in *O. bicornis* at the apiary, but not in individuals from the remote location. The consistent presence of many viruses in *O. bicornis* populations near commercial hives raises concerns about pathogen spillover and the effect of managed bee colonies on solitary bee health. These findings concur with those of other studies that document virus transmission between managed and wild bee species in mixed habitats (Singh et al. 2010).

Morphological deformaties of adults, pupae, and larvae

Interestingly, despite the widespread infection of both *A. mellifera* and *O. bicornis*, no morphological deformities were observed in any of the developmental stages of *O. bicornis*. Pupae, larvae, and adults appeared morphologically normal, despite high viral incidence in certain cases, particularly by DWV and ABPV. This indicates that, at least in *O. bicornis*, these viruses may exist asymptomatically, with no immediate detrimental effects on bee morphology. In contrast, other studies have reported symptoms such as wing deformities in *A. mellifera* colonies heavily infected with DWV, particularly when they are also infested with mites (Highfield et al. 2009). In this study, no notable morphological symptoms were observed in either species. However, previous studies report significant symptoms in *A. mellifera* under certain conditions, such as wing deformities in colonies heavily infected with DWV and infested with mites. This absence of visible symptoms in *O. bicornis* infected with similar viral loads indicates that solitary bees may have a different or less visible response to these viruses compared to honeybees, warranting further investigation into the sub-lethal effects of honey bee viruses on wild bees like *O. bicornis*.

Discussion

Recent research indicates that honeybee viruses, such as DWV, ABPV, BQCV, AmFV and LSV are not limited to *A. mellifera*, but also infest wild bees, including soli-

tary bees like *O. bicornis* (Ravoet et al. 2014; Schoonvaere et al. 2016; Nanetti et al. 2021a). This interspecies transmission raises concerns, particularly in areas where commercial beekeeping is widely practised (Li et al. 2014). More than 20 viruses are reported infesting honeybees (Ryba et al. 2012a), mainly belonging to the families *Dicistroviridae* and *Iflaviridae*, which are characterized by positive-sense, single-stranded RNA genomes that form "mutant clouds" of dynamically evolving variants (Lauring and Andino 2010; Li et al. 2014).

Deformed Wing Virus (DWV) is the most widespread and well-studied honeybee pathogen, classified as a non-enveloped, single-stranded RNA (+) virus within the *Iflavirus* genus of the family *Picornaviridae* (De Miranda and Genersch 2010). DWV consists of three distinct genomotypes: A, B and C (McMahon et al. 2016; Mordecai et al. 2016) and is frequently associated with the parasitic mite *Varroa destructor*, in which it can asymptomatically replicate (Ryabov et al. 2014). This virus is not only found in species closely associated with honeybees, such as *Aethina tumida* (Eyer et al. 2009, Nanetti et al. 2021b), *Galleria mellonella* (Levitt et al. 2013) and *Vespa* spp. (Forzan et al. 2017), but also in a variety of *Apis* and non-*Apis* species that can act as incidental hosts (Singh et al. 2010; Levitt et al. 2013). In some *Bombus* species DWV reduces individual lifespan and deforms their wings (Fürst et al. 2014; Graystock et al. 2016).

Acute Bee Paralysis Virus (ABPV) is another widespread honeybee pathogen, classified as a non-enveloped single-stranded RNA (+) virus within the *Apavirus* genus of the family *Dicistroviridae* (Benjeddou et al. 2001; Chen et al. 2006). ABPV is genetically similar to Kashmir Bee Virus (KBV) and Israeli Acute Paralysis Virus (IAPV) (De Miranda and Genersch 2010). While ABPV does not replicate in *Varroa destructor* (Berényi et al. 2006; Genersch et al. 2010), it has been reported as present in various *Bombus* species since at least 1964 (Bailey and Gibbs 1964). This virus has a broad host range and is reported infecting numerous bee species, including *Bombus* species and others (Alvarez et al. 2018; Dalmon et al. 2021).

Black Queen Cell Virus (BQCV) belongs to the *Cripavirus* genus in the family *Dicistroviridae*. It is a non-enveloped, single-stranded RNA (+) virus that frequently infects adult honeybees (Benjeddou et al. 2001; Berényi et al. 2006; Chen et al. 2006). BQCV primarily causes symptomatic infections in queen pupae, resulting in the decomposition of these pupae (Siede and Büchler 2003). The virus is widespread and affects several *Apis* species and subspecies, including *A. mellifera*, *A. cerana indica*, *A. cerana japonica*, *A. dorsata* and *A. florea* (Mookhploy et al. 2015). In addition to honeybees, BQCV has been detected in a wide range of other organisms, including small hive beetles, hoverflies, roaches, spiders and wax moths (Bailes et al. 2018). The presence early in the year of Black Queen Cell Virus (BQCV) in both *O. bicornis* populations is noteworthy. It raises the possibility that the virus may have already been present in these bees prior to the experiment, either due to latent infection or previous exposure. One plausible hypothesis is that BQCV could have been introduced during overwintering, as the virus was consistently detected in *O. bicornis* pupae and persisted into adulthood. This indicates the potential for vertical transmission of BQCV in *O. bicornis*, which could explain the early-season presence of this virus.

Lake Sinai Virus (LSV) is a single-stranded RNA (+) virus classified within the *Sinaivirus* genus of the family *Sinhaliviridae*, with two strains identified so far: LSV-1 and LSV-2 (Runckel et al. 2011; Daughenbaugh et al. 2015). Cases of LSV spillover are reported in *Andrena* spp. (Ravoet et al. 2014), *Bombus* spp. (Parmentier et al. 2016) and species of the familes *Halictidae* and *Megachilidae* (Dolezal et al. 2016). Active viral replication of LSV is confirmed in *O. cornuta* (Ravoet et al. 2015), while in *Varroa destructor*, only the positive-sense strand of the virus genome is present and no replication confirmed. Oral transmission of LSV via contaminated pollen is also plausible (Ravoet et al. 2015).

Apis mellifera Filamentous Virus (AmFV) is an unclassified double-stranded DNA virus that primarily infects honeybees (Gauthier et al. 2015; Hartmann et al. 2015). Severe infections result in milk-white haemolymph due to a high concentration of virions, which results in weakness in bees and a tendency for them to gather near hive entrances. Despite these symptoms, AmFV is weakly pathogenic and has little effect on the lifespan of bees (Hou et al. 2016; Quintana et al. 2021). Spillover cases are reported in other hosts, such as, *Andrena* spp., *Bombus* spp. (Plischuk et al. 2021) and *Osmia* spp. (Ravoet et al. 2014).

In countries like the Czech Republic (with an average of 10.1 hives per km^2 in 2022), the proximity of commercial hives to natural habitats heightens the risk of disease transmission to native pollinators (Ryba et al. 2009). Interestingly, despite the close proximity of *A. mellifera* colonies, there was no evidence of transmission of BQCV between hives, particularly hive 1, which was infected with very few viruses. The strength of the colonies and the age of the bees might have accounted for this. Hive 1, for instance, could have had a stronger colony with a higher number of foragers and nurse bees, which are known to play a crucial role in colony immunity. The exposure of young foraging bees to virus may have been less and potentially limited internal colony transmission. Strong colonies are typically better equipped to manage virus infections through social immunity mechanisms, such as hygienic behaviour and robust brood care, which could account for the variation in the incidence of viral infections in the hives.

This could indicate the presence of barriers to viral transmission, such as variations in colony health, immune responses, or hive-specific management practices. It is also possible that some colonies had pre-existing resistance or tolerance of certain viruses, which could limit the spread within the apiary. In contrast, weak or old

colonies might be more susceptible to viral infections, resulting in a higher diversity and prevalence of viruses in their hives. These observations indicate that further research is needed to explore the dynamics of viral transmission both in and between species in mixed pollinator habitats, considering factors like colony strength and the age distribution of bees. Understanding the prevalence and distribution of these viruses in wild bee populations, such as *O. bicornis*, is essential for assessing the effect on pollinator health and developing mitigation strategies (Alger et al. 2019). It is possible that these viruses were present in *O. bicornis* populations long before molecular tools made detection possible. These viruses may have existed asymptomatically or at low levels, only becoming apparent with advanced diagnostics. This indicates that *O. bicornis* might have developed tolerance to such infections, maintaining viral presence without visible symptoms. Monitoring the prevalence of these viruses through environmental nucleic acid detection is essential for identifying newly introduced pathogens, which frequently occur at a low incidence and prevalence (Ryba et al. 2012b), and for promptly implementing measures to safeguard bees and maintain ecosystem stability (Gisder and Genersch 2017).

Conclusions

The above findings indicate that *O. bicornis* is not only susceptible to honeybee viruses, but may act as a reservoir for these pathogens, particularly in environments where it coexists with *A. mellifera*. The absence of visible symptoms when infected highlights the complexity of host-virus interactions in solitary bees, raising important questions about the long-term health implications for these species in shared habitats. Further research is required to elucidate the full ecological and evolutionary consequences of viral spillover from managed honeybee populations to wild pollinators like *O. bicornis*.

REFERENCES

Alger SA, Burnham PA, Brody AK (2019) Flowers as viral hot spots: Honey bees (*Apis mellifera*) unevenly deposit viruses across plant species. PLOS One 14: e0221800. doi: 10.1371/journal.pone.0221800.

Alvarez LJ, Reynaldi FJ, Ramello PJ, Garcia MLG, Sguazza GH, Abrahamovich AH, Lucia M (2018) Detection of honey bee viruses in Argentinian stingless bees (Hymenoptera: Apidae). Insectes Sociaux 65: 191–197. doi: 10.1007/s00040-017-0587-2.

Babin A, Schurr F, Delannoy S, Fach P, Huyen Ton Nu Nguyet M, Bougeard S, De Miranda JR, Rundlöf M, Wintermantel D, Albrecht M, Attridge E, Bottero I, Cini E, Costa C, De La Rúa P, Di Prisco G, Dominik C, Dzul D, Hodge S, Klein A-M, Knapp J, Knauer AC, Mänd M, Martínez-López V, Medrzycki P, Pereira-Peixoto MH, Potts SG, Raimets R, Schweiger O, Senapathi D, Serrano J, Stout JC, Tamburini G, Brown MJF, Laurent M, Rivière M-P, Chauzat M-P, Dubois E (2024) Distribution of infectious and parasitic agents among three sentinel bee species across European agricultural landscapes. Sci Rep-UK 14: 3524. doi: 10.1038/s41598-024-53357-w.

Bailes EJ, Deutsch KR, Bagi J, Rondissone L, Brown MJF, Lewis OT (2018) First detection of bee viruses in hoverfly (syrphid) pollinators. Biol Lett 14: 20180001. doi: 10.1098/rsbl.2018.0001.

Bailey L, Gibbs AJ (1964) Acute infection of bees with paralysis virus. J Insect Pathol: 395–407.

Benjeddou M, Leat N, Allsopp M, Davison S (2001) Detection of Acute Bee Paralysis Virus and Black Queen Cell Virus from honeybees by reverse transcriptase PCR. Appl Environ Microbiol 67: 2384–2387. doi: 10.1128/AEM.67.5.2384-2387.2001.

Berényi O, Bakonyi T, Derakhshifar I, Köglberger H, Nowotny N (2006) Occurrence of six honeybee viruses in diseased austrian apiaries. Appl Environ Microbiol 72: 2414–2420. doi: 10.1128/AEM.72.4.2414-2420.2006.

Chen YP, Pettis JS, Collins A, Feldlaufer MF (2006) Prevalence and transmission of honeybee viruses. Appl Environ Microbiol 72: 606–611. doi: 10.1128/AEM.72.1.606-611.2006.

Cornman RS, Schatz MC, Johnston JS, Chen Y-P, Pettis J, Hunt G, Bourgeois L, Elsik C, Anderson D, Grozinger CM, Evans JD (2010) Genomic survey of the ectoparasitic mite Varroa destructor, a major pest of the honey bee *Apis mellifera*. BMC Genomics 11: 602. doi: 10.1186/1471-2164-11-602.

Dalmon A, Diévart V, Thomasson M, Fouque R, Vaissière BE, Guilbaud L, Le Conte Y, Henry M (2021) Possible spillover of pathogens between bee communities foraging on the same floral resource. Insects 12: 122. doi: 10.3390/insects12020122.

Daughenbaugh K, Martin M, Brutscher L, Cavigli I, Garcia E, Lavin M, Flenniken M (2015) Honey bee infecting Lake Sinai Viruses. Viruses 7: 3285–3309. doi: 10.3390/v7062772.

De Miranda JR, Genersch E (2010) Deformed wing virus. J Invertebr Pathol 103: S48–S61. doi: 10.1016/j.jip.2009.06.012.

Dolezal AG, Hendrix SD, Scavo NA, Carrillo-Tripp J, Harris MA, Wheelock MJ, O'Neal ME, Toth AL (2016) Honey Bee Viruses in wild bees: Viral prevalence, loads, and experimental inoculation. PLOS One 11: e0166190. doi: 10.1371/journal.pone.0166190.

Eyer M, Chen YP, Schäfer MO, Pettis J, Neumann P (2009) Small hive beetle, *Aethina tumida*, as a potential biological vector of honeybee viruses. Apidologie 40: 419–428. doi: 10.1051/apido:2008051.

Forzan M, Felicioli A, Sagona S, Bandecchi P, Mazzei M (2017) Complete genome sequence of Deformed Wing Virus isolated from *Vespa crabro* in Italy. Genome Announc 5: e00961-17. doi: 10.1128/genomeA.00961-17.

Fürst MA, McMahon DP, Osborne JL, Paxton RJ, Brown MJF (2014) Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature 506: 364–366. doi: 10.1038/nature12977.

Gauthier L, Cornman S, Hartmann U, Cousserans F, Evans J, De Miranda J, Neumann P (2015) The Apis mellifera Filamentous Virus Genome. Viruses 7: 3798–3815. doi: 10.3390/v7072798.

Genersch E, Evans JD, Fries I (2010) Honey bee disease overview. J Invertebr Pathol 103: S2–S4. doi: 10.1016/j.jip.2009.07.015.

Genersch E, Yue C, Fries I, De Miranda JR (2006) Detection of Deformed wing virus, a honey bee viral pathogen, in bumble bees (*Bombus terrestris* and *Bombus pascuorum*) with wing deformities. J Invertebr Pathol 91: 61–63. doi: 10.1016/j.jip.2005.10.002.

Gisder S, Genersch E (2017) Viruses of commercialized insect pollinators. J Invertebr Pathol 147: 51–59. doi: 10.1016/j.jip.2016.07.010.

Goulson D, Hughes WOH (2015) Mitigating the anthropogenic spread of bee parasites to protect wild pollinators. Biol Conserv 191: 10–19. doi: 10.1016/j.biocon.2015.06.023.

Graystock P, Meeus I, Smagghe G, Goulson D, Hughes WOH (2016) The effects of single and mixed infections of *Apicystis bombi* and Deformed Wing Virus in *Bombus terrestris*. Parasitology 143: 358–365. doi: 10.1017/S0031182015001614.

Hartmann U, Forsgren E, Charrière J-D, Neumann P, Gauthier L (2015) Dynamics of Apis mellifera Filamentous Virus (AmFV) infections in honey bees and relationships with other parasites. Viruses 7: 2654–2667. doi: 10.3390/v7052654.

Highfield AC, El Nagar A, Mackinder LCM, Noël LM-LJ, Hall MJ, Martin SJ, Schroeder DC (2009) Deformed Wing Virus implicated in overwintering honeybee colony losses. Appl Environ Microb 75: 7212–7220. doi: 10.1128/AEM.02227-09.

Hou C, Li B, Luo Y, Deng S, Diao Q (2016) First detection of Apis mellifera Filamentous Virus in *Apis cerana cerana* in China. J Invertebr Pathol 138: 112–115. doi: 10.1016/j.jip.2016.06.011.

Klein A-M, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. P Roy Soc B: Biol Sci 274: 303–313. doi: 10.1098/rspb.2006.3721.

Ladurner E, Recla L, Wolf M, Zelger R, Burgio G (2004) *Osmia cornuta* (Hymenoptera Megachilidae) densities required for apple pollination: a cage study. J Apicult Res 43: 118–122. doi: 10.1080/00218839.2004.11101121.

Lauring AS, Andino R (2010) Quasispecies Theory and the Behavior of RNA Viruses. PLoS Pathog 6: e1001005. doi: 10.1371/journal.ppat.1001005.

Levitt AL, Singh R, Cox-Foster DL, Rajotte E, Hoover K, Ostiguy N, Holmes EC (2013) Cross-species transmission of honey bee viruses in associated arthropods. Virus Res 176: 232–240. doi: 10.1016/j.virusres.2013.06.013.

Li JL, Cornman RS, Evans JD, Pettis JS, Zhao Y, Murphy C, Peng WJ, Wu J, Hamilton M, Boncristiani HF, Zhou L, Hammond J, Chen YP (2014) Systemic spread and propagation of a plant-pathogenic virus in European honeybees, *Apis mellifera*. mBio 5: e00898-13. doi: 10.1128/mBio.00898-13.

Martin SJ, Brettell LE (2019) Deformed Wing Virus in honeybees and other insects. Ann Rev Virol 6: 49–69. doi: 10.1146/annurev-virology-092818-015700.

McCallum H, Dobson A (2002) Disease, habitat fragmentation and conservation. Proceedings of the Royal Society of London. Series B: Biological Sciences 269: 2041–2049. doi: 10.1098/rspb.2002.2079.

McMahon DP, Fürst MA, Caspar J, Theodorou P, Brown MJF, Paxton RJ (2015) A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees. Altizer S (ed) J Anim Ecol 84: 615–624. doi: 10.1111/1365-2656.12345.

McMahon DP, Natsopoulou ME, Doublet V, Fürst M, Weging S, Brown MJF, Gogol-Döring A, Paxton RJ (2016) Elevated virulence of an emerging viral genotype as a driver of honeybee loss. P Roy Soc B: Biol Sci 283: 20160811. doi: 10.1098/rspb.2016.0811.

Mookhploy W, Kimura K, Disayathanoowat T, Yoshiyama M, Hondo K, Chantawannakul P (2015) Capsid gene divergence of Black Queen Cell Virus Isolates in Thailand and Japan honey bee species. J Econ Entomol 108: 1460–1464. doi: 10.1093/jee/tov102.

Mordecai GJ, Wilfert L, Martin SJ, Jones IM, Schroeder DC (2016) Diversity in a honey bee pathogen: first report of a third master variant of the Deformed Wing Virus quasispecies. The ISME Journal 10: 1264–1273. doi: 10.1038/ismej.2015.178.

Mráz P, Hýbl M, Kopecký M, Bohatá A, Hoštičková I, Šipoš J, Vočadlová K, Čurn V (2021) Screening of honey bee pathogens in the Czech Republic and their prevalence in various habitats. Insects 12: 1051. doi: 10.3390/insects12121051.

Nanetti A, Bortolotti L, Cilia G (2021a) Pathogens spillover from honey bees to other Arthropods. Pathogens 10: 1044. doi: 10.3390/pathogens10081044.

Nanetti A, Ellis JD, Cardaio I, Cilia G (2021b) Detection of *Lotmaria passim*, *Crithidia mellificae* and replicative forms of Deformed Wing Virus and Kashmir Bee Virus in the small hive beetle (*Aethina tumida*). Pathogens 10: 372. doi: 10.3390/pathogens10030372.

Parmentier L, Smagghe G, De Graaf DC, Meeus I (2016) Varroa destructor Macula-like virus, Lake Sinai Virus and other new RNA viruses in wild bumblebee hosts (*Bombus pascuorum*, *Bombus lapidarius* and *Bombus pratorum*). J Invertebr Pathol 134: 6–11. doi: 10.1016/j.jip.2015.12.003.

Peng W, Li J, Boncristiani H, Strange JP, Hamilton M, Chen Y (2011) Host range expansion of honey bee Black Queen Cell Virus in the bumble bee, *Bombus huntii*. Apidologie 42: 650–658. doi: 10.1007/s13592-011-0061-5.

Plischuk S, Fernández De Landa G, Revainera P, Quintana S, Pocco ME, Cigliano MM, Lange CE (2021) Parasites and pathogens associated with native bumble bees (Hymenoptera: Apidae: *Bombus* spp.) from highlands in Bolivia and Peru. Stud Neotrop Fauna E 56: 93–98. doi: 10.1080/01650521.2020.1743551.

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impacts and drivers. Trends Ecol Evol 25: 345–353. doi: 10.1016/j.tree.2010.01.007.

Quintana S, Brasesco C, Porrini LP, Di Gerónimo V, Eguaras MJ, Maggi M (2021) First molecular detection of Apis mellifera Filamentous Virus (AmFV) in honey bees (*Apis mellifera)* in Argentina. J Apicult Res 60: 111–114. doi: 10.1080/00218839.2019.1690100.

Ravoet J, De Smet L, Meeus I, Smagghe G, Wenseleers T, De Graaf DC (2014) Widespread occurrence of honey bee pathogens in solitary bees. J Invertebr Pathol 122: 55–58. doi: 10.1016/j.jip.2014.08.007.

Ravoet J, De Smet L, Wenseleers T, De Graaf DC (2015) Genome sequence heterogeneity of Lake Sinai Virus found in honey bees and Orf1/RdRP-based polymorphisms in a single host. Virus Research 201: 67–72. doi: 10.1016/j.virusres.2015.02.019.

Runckel C, Flenniken ML, Engel JC, Ruby JG, Ganem D, Andino R, DeRisi JL (2011) Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, Nosema, and Crithidia. PLOS One 6: e20656. doi: 10.1371/journal.pone.0020656.

Ryabov EV, Wood GR, Fannon JM, Moore JD, Bull JC, Chandler D, Mead A, Burroughs N, Evans DJ (2014) A Virulent strain of Deformed Wing Virus (DWV) of honeybees (*Apis mellifera*) prevails after Varroa destructor-Mediated, or in vitro, transmission. PLoS Pathog 10: e1004230. doi: 10.1371/journal.ppat.1004230.

Ryba S, Kindlmann P, Titera D, Haklova M, Stopka P (2012b) A new low-cost procedure for detecting nucleic acids in low-incidence samples: A case study of detecting spores of *Paenibacillus larvae* from bee debris. J Econ Entomol 105: 1487–1491. doi: 10.1603/EC12010.

Ryba S, Titera D, Haklova M, Stopka P (2009) A PCR method of detecting american foulbrood (*Paenibacillus larvae*) in winter beehive wax debris. Vet Microbiol 139: 193–196. doi: 10.1016/j.vetmic.2009.05.009.

Ryba S, Titera D, Schodelbauerova-Traxmandlova I, Kindlmann P (2012a) Prevalence of honeybee viruses in the Czech Republic and coinfections with other honeybee disease. Biologia 67: 590–595. doi: 10.2478/s11756-012-0038-5.

Schoonvaere K, De Smet L, Smagghe G, Vierstraete A, Braeckman BP, De Graaf DC (2016) Unbiased RNA shotgun metagenomics

in social and solitary wild bees detects associations with eukaryote parasites and new viruses. PLOS One 11: e0168456. doi: 10.1371/journal.pone.0168456.

Siede R, Büchler R (2003) Symptomatic Black Queen Cell Virus infection of drone brood in Hessian apiaries. Berliner Und Munchener Tierarztliche Wochenschrift 116: 130–133.

Singh R, Levitt AL, Rajotte EG, Holmes EC, Ostiguy N, vanEngelsdorp D, Lipkin WI, dePamphilis CW, Toth AL, Cox-Foster DL (2010) RNA viruses in Hymenopteran pollinators: Evidence of inter-taxa virus transmission via pollen and potential impact on non-Apis Hymenopteran species. PLOS One 5: e14357. doi: 10.1371/journal.pone.0014357.

Tarpy DR (2003) Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. P Roy Soc Lond B Bio 270: 99–103. doi: 10.1098/rspb .2002.2199.

Whitehorn PR, Tinsley MC, Brown MJF, Darvill B, Goulson D (2011) Genetic diversity, parasite prevalence and immunity in wild bumblebees. P Roy Soc B: Biol Sci 278: 1195–1202. doi: 10.1098/rspb.2010.1550.