




Detection patterns of honey bee diseases in clinically healthy colonies under long-term surveillance

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ABSTRACT

Honey bee (*Apis mellifera* L.) health is increasingly threatened by pathogens, yet little is known about their circulation in clinically healthy, closely monitored colonies. We screened 820 adult bees from 22 colonies in Northern Greece using PCR-based assays for major viral, bacterial, and parasitic agents. Acute Bee Paralysis Virus (ABPV) and Black Queen Cell Virus (BQCV) were universally detected, while Varroa Destructor Virus 1 (VDV1/DWV-B) (18.2%) and *Vairimorpha ceranae* (4.5%) appeared sporadically. All other pathogens tested were absent. These results support a classification of baseline, risk, and exclusion markers, offering a diagnostic reference that strengthens risk-based surveillance and sustainable apiculture.

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Honey bee (*Apis mellifera* L.) populations face significant pathogen-related pressures worldwide (De Jong & Lester, 2023). While surveillance efforts often focus on symptomatic or collapsing colonies, less is known about pathogen circulation in clinically healthy, treatment-free populations (Kunat-Budzyńska et al., 2025; Matthijs et al., 2020). This study aimed to characterize detection patterns in monitored asymptomatic colonies and to distinguish baseline from risk-associated markers.

In this context, the study was conducted between July and August 2020 on a population of 250 stationary colonies located at the apiary site of the Hellenic Agricultural Organization DEMETER (Ag. Mamas, Chalkidiki), which has been under continuous veterinary surveillance for more than 10 years. Colonies included in the study were clinically healthy at sampling and had shown no visible signs of disease during the preceding six months. No antimicrobial or antiparasitic treatments were applied during this period. *V. destructor* infestation was monitored over the same six-month interval using the powdered sugar roll method, and no mites were detected. A total of 820 adult worker bees collected from 22 randomly selected colonies were screened for Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Chronic Bee Paralysis Virus (CBPV),


Deformed Wing Virus genotype A (DWV-A), Varroa destructor Virus 1 (also known as DWV genotype B, VDV1/DWV-B), Sacbrood Virus (SBV), European foulbrood (EFB, *Melissococcus plutonius*), *Vairimorpha* spp. (formerly *Nosema*), Small Hive Beetle (SHB, *Aethina tumida*), *Tropilaelaps* spp., and *Varroa destructor* (Supplementary Material). This suite of agents reflects both the World Organisation for Animal Health (WOAH) list of notifiable diseases and the most commonly reported microbial threats to managed honey bee colonies worldwide (World Organisation for Animal Health (WOAH-OIE), 2024).

The results revealed a distinct pattern of pathogen distribution within the sampled population (Figures 1 and 2). ABPV and BQCV were detected in all colonies (22/22), consistent with their widespread occurrence in both symptomatic and asymptomatic populations (Brzoskowski Chagas et al., 2023; Molineri et al., 2017). Their universal detection in clinically healthy colonies supports their classification as baseline components of the honey bee virome.

By contrast, VDV1/DWV-B was detected in 4/22 colonies (18.2%). This genotype has been associated with increased virulence in the presence of *V. destructor* and has displaced DWV-A in several regions (Kevill et al., 2021; Paxton et al., 2022). In the present study, colonies remained negative for

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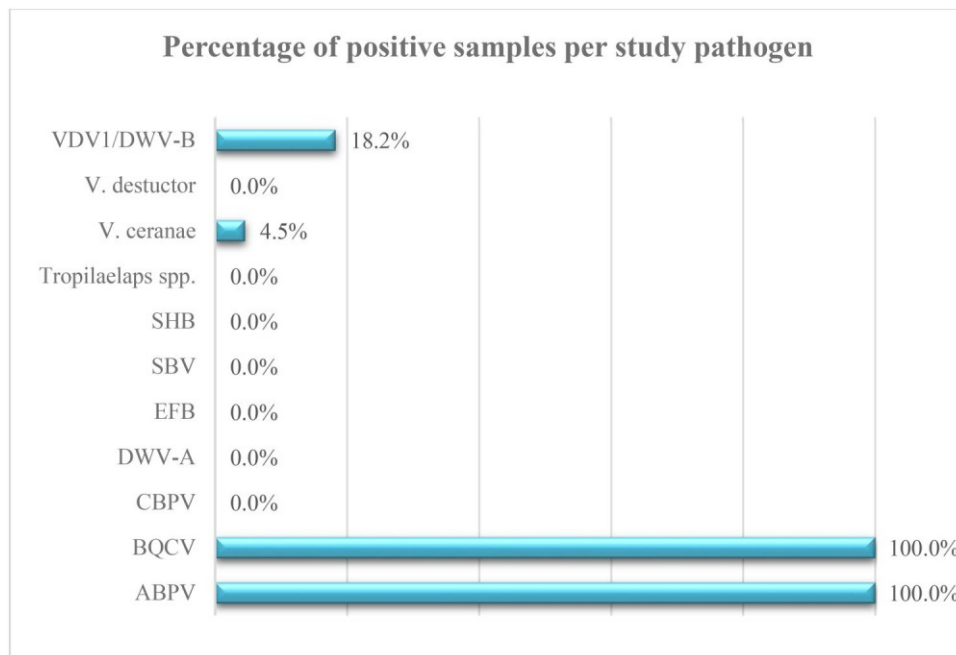


Figure 1. Prevalence of major microbial and parasitic pathogens detected in clinically healthy honey bee (*Apis mellifera* L.) colonies under veterinary surveillance in Northern Greece ($n = 22$).

ABPV: Acute Bee Paralysis Virus; BQCV: Black Queen Cell Virus; CBPV: Chronic Bee Paralysis Virus; DWV-A: Deformed Wing Virus; SBV: Sacbrood Virus; VDV1/DWV-B: Varroa Destructor Virus 1; EFB: European Foulbrood; SHB: Small Hive Beetle.

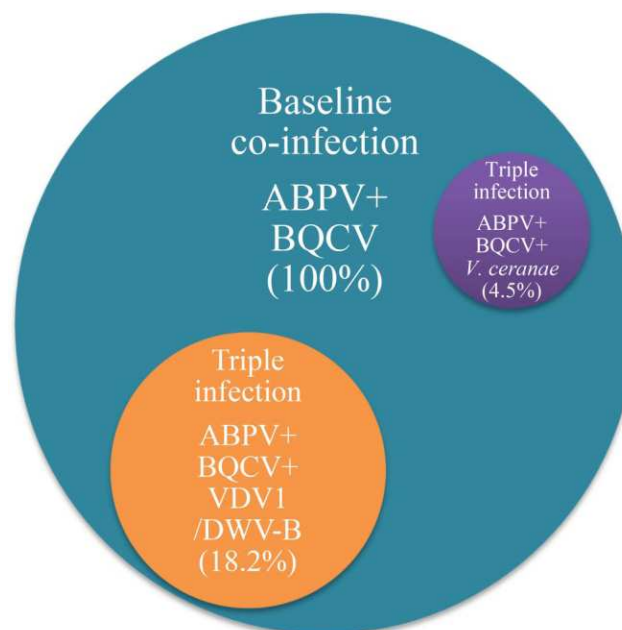


Figure 2. Colony-level co-infection structure of detected pathogens in clinically healthy honey bee colonies ($n = 22$). All colonies (22/22; 100%) exhibited baseline co-infection with ABPV and BQCV. Additional triple infections were observed in distinct colonies: ABPV+BQCV+VDV1/DWV-B in 4/22 (18.2%) colonies and ABPV+BQCV+*V. ceranae* in 1/22 (4.5%) colony. No colony exhibited simultaneous VDV1/DWV-B and *V. ceranae* infection. Sphere size is proportional to the percentage of positive colonies.

ABPV: Acute Bee Paralysis Virus; BQCV: Black Queen Cell Virus; VDV1/DWV-B: Varroa Destructor Virus 1.

V. destructor during surveillance, and VDV1/DWV-B occurred only sporadically, suggesting limited amplification under monitored conditions. Additionally, *V. ceranae* was detected in 1/22 colonies (4.5%). Although globally prevalent and often associated with colony stress (Grupe & Quandt, 2020), its low detection rate here indicates limited circulation within this monitored population.

No colonies tested positive for DWV-A, CBPV, SHB, SBV, EFB, *Tropilaelaps* spp., or *V. destructor*. These negative findings define exclusion markers within the sampled population. Given that colonies remained negative for *V. destructor* during surveillance, the universal presence of ABPV and BQCV suggests that these viruses were detectable without evidence of active mite infestation.

It is important to recognize the limitations of this study. Sampling involved a relatively small proportion of the total apiary that may have reduced sensitivity for detecting low-prevalence pathogens, and pathogen screening was performed at a single time point, which may have underestimated seasonal variability and temporal dynamics. Nevertheless, the study provides a rare dataset from a long-term monitored, treatment-free population, offering insights into pathogen ecology under certain conditions.

Collectively, despite limitations, these findings support a tripartite framework for interpreting pathogen surveillance in clinically healthy honey bee colonies. ABPV and BQCV were universally detected and may serve as baseline markers of the monitored virome, whereas VDV1/DWV-B and *V. ceranae* occurred sporadically and represent potential risk indicators whose epidemiological relevance may increase under changing conditions. The absence of other major pathogens defines exclusion markers that delineate the current health boundaries of this population. Together, these results illustrate the value of classifying detected agents according to baseline, risk, and exclusion roles rather than viewing surveillance solely as a catalog of presence or absence.

Author contributions

Am: Data curation; investigation; methodology; validation; visualization; formal analysis; software; writing–review and editing; writing–original draft. P.K.: Methodology; data curation. F.H.: Conceptualization; project administration; resources. L.C.: Conceptualization; project administration; resources. M.K.: Methodology; data curation. J.I.: Conceptualization; data curation; supervision; writing–review and editing; writing–original draft.

Ethics statement

Ethical approval for this study was obtained from the Research Ethics Committee of the Agricultural University of Athens (33/2025).

Disclosure statement

The authors report there are no competing interests to declare.

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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