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APPLICATION OF PCR FOR THE DETECTION OF VIRAL INFECTIONS IN THE HONEYBEE *Apis mellifera* IN THE NORTHERN REGIONS OF UKRAINE

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Aim. This study investigated viruses of the western honeybee (*Apis mellifera*) from the northern regions of Ukraine using polymerase chain reaction (PCR).

Methods. To identify honeybee viruses, oligonucleotide primers, which are specific to a gene fragment encoding the capsid protein and a fragment of the RNA-dependent RNA polymerase gene, were used. The reaction mixture contained 12.5 µL of master mix solution (DNA polymerase, dNTPs, and buffer), 1 µL of each primer (20 pmol/µL), 2 µL of cDNA, and water to a total volume of 25 µL. PCR products were visualized in a 2% agarose gel.

Results. In the Zhytomyr region in *Apis mellifera* was revealed for the first time the presence of four viruses representing different taxonomic groups, in particular Israeli Acute Paralysis Virus (IAPV), Sacbrood Virus (SBV), Deformed Wing Virus (DWV) and Chronic Bee Paralysis Virus (CBPV). Three of these viruses (SBV, DWV, and CBPV) were detected in the Kyiv region, while two (SBV and DWV) were identified in the Chernihiv region.

Conclusions. The obtained data indicate the widespread distribution of *Apis mellifera* honeybee viruses in the northern regions of Ukraine. The use of PCR to detect bee viruses will contribute to the development of effective methods for improving the health of bee colonies.

Key words: *Apis mellifera*, bee viruses, diagnosis, PCR .

Bees play a crucial role in ensuring global food security. It has been estimated that 36% of global agricultural crop production depends on bees and other pollinating insects [1]. Moreover, bees are producers of honey, a valuable and delicious product. In recent years, despite ongoing military actions, Ukraine has maintained a stable positive trend in honey exports to the global market. For the third consecutive year, Ukraine has ranked second after China among honey exporters to the EU. In 2022, China's share of honey purchased by Europeans was 36%, followed by Ukraine with 24%. Argentina (10%), Mexico (7%), Turkey (4%), Cuba, and Vietnam (3% each) lagged significantly behind. According to the *Agro Perspective* journal, Ukraine exported 55,357 tons of natural honey worth 121.384 million USD in 2023. Furthermore, according to the State Customs Service, Ukraine exported 64,918 tons of honey worth 124.8 million USD between January and September 2024. Ukrainian honey was also present in other markets, including the USA, Turkey, the United Kingdom, Japan, Switzerland, Canada, Qatar, Jordan, and Ireland.

However, the economic and ecological value of honeybees has faced severe challenges in recent years due to the sharp decline in honeybee colonies both in Europe and globally [2–4]. This issue is closely related to several biotic and abiotic factors [5, 6], including parasitic mites such as *Varroa destructor* and a number of pathogenic viruses [7–9]. Bee viral diseases are among the most damaging compared to other infections. Due to their high evolutionary plasticity and exponential replication speed, these pathogens can quickly adapt to new hosts and cause large-scale epidemics [4, 9]. Moreover, increasing evidence suggests that viruses infecting honeybees can impact a wide range of other insect species [10]. In nature, various bee viruses have been isolated from bumblebees (*Bombus* species, family *Apidae*), bees from the families *Andrenidae*, *Halictidae*, *Megachilidae* [11], as well as from *Myrmica rubra* ants [12] and other insects. Thus, bee viruses are capable of posing more significant threat to our ecosystems than previously thought. The most common viruses in European beekeeping are flaviviruses [13, 14], particularly the Deformed Wing Virus (DWV). Additionally, other bee viruses have been identified in Europe, including the Sacbrood Virus (SBV), Acute Bee Paralysis Virus (ABPV), Israeli Acute Bee Paralysis Virus (IAPV), Kashmir Bee Virus (KBV), Black Queen Cell Virus (BQCV), Slow Paralysis Virus (SBPV), Chronic Bee Paralysis Virus (CBPV), Bee Maculavirus (BeeMLV), Amaryllis Filamentous Virus (AmFV), Rhabdovirus (BRV), and other viruses [15, 16].

In Ukraine, viruses of the western honeybee *Apis mellifera* have been detected in the Kyiv and Cherkasy regions [17], as well as in many other areas of the country [18]. This study focused on investigating bee viruses from the northern regions of Ukraine.

Materials and Methods

Sample Collection. Samples were collected from private apiaries in the Kyiv, Zhytomyr, and Chernihiv regions (Table 1). In the laboratory, material for RNA extraction was selected from a brood with abnormal color patterns, sunken cells (in the center), partially perforated, or uncapped cells.

RNA Extraction. RNA was extracted from larvae, and underdeveloped adult bees were removed from the brood. For this, a homogenate was prepared using 10 larvae and underdeveloped adult bees, mixed with phosphate-buffered saline (PBS) at pH 7.4 in a 1:2 ratio (2 parts buffer). For RNA extraction from the homogenate (200 µL), the commercial Quick-RNA Miniprep Kit (Zymo Research) was used according to the manufacturer's instructions. For cDNA synthesis, the ProtoScript First Strand cDNA Synthesis Kit (NEB) was employed according to the manufacturer's guidelines.

Polymerase chain reaction (PCR). Oligonucleotide primers specific to the gene fragment encoding the capsid protein were used for the identification of IAPV, SBV, and BQCV. In contrast, primers targeting the RNA-dependent RNA polymerase gene fragment were used for identifying other viruses (Table 2). The reaction mixture contained:

- 12.5 µL of master mix solution (DNA polymerase, dNTPs, and buffer).
- 1 µL of each primer (20 picomoles/µL).
- 2 µL of cDNA.
- Water to a total volume of 25 µL.

The reaction parameters included an initial denaturation cycle for 30 seconds at 94 °C,

Table 1

Bee Samples Apis mellifera for RNA Extraction

Sample No.	Sample type for RNA extraction	Sample collection location
1	Brood [larvae and undeveloped imago]	Zhytomyr region
2	Brood [larvae]	Zhytomyr region
3	Brood [larvae]	Kyiv region, Kyiv-Svyatoshyn district, Novoselky village
4	Imago	Kyiv region, Kyiv-Svyatoshyn district, Novoselky village
5	Brood [larvae]	Kyiv, National Scientific Center Institute of Beekeeping
6	Imago	Kyiv, National Scientific Center Institute of Beekeeping
7	Brood [larvae]	Kyiv region, Fastiv district, village of Gatne
8	Imago	Chernihiv region, Nizhyn district, Bakhmach town

Table 2

Oligonucleotide primers to bee viruses

Virus	Primer name	Sequence 3′-5′	Product length, mm	Annealing temperature [Ta], °C	Target gene
ABPV	ABPVfor	TGAGAACACCTGTAATGTGG	451	55–56	RdRp [RNApol]
	ABPVrev	ACCAGAGGGTTGACTGTGTG			
IAPV	IAPVfor	AGACACCAATCACGGACCTCAC	137	57–59	Capsid protein VP1
	IAPVrev	GAGATTGTTTGAGAGGGGTGG			
KBV	KBVfor	GATGAACGTCGACCTATTGA	413	55–56	RdRp [RNApol]
	KBVrev	TGTGGGTTGGCTATGAGTCA			
AKI	AKIfor	CTTTCATGATGTGGAAACTCC	100	57–59	RdRp [RNApol]
	AKIrev	AAACTGAATAATACTGTGCGTA			
SBV	SBVfor	ACCACCCGATTCCTCAGTAG	469	55–56	Capsid protein
	SBVrev	CCTTGGAACTCTGCTGTTA			
DWV	DWVfor	TTTGCAAGATGCTGTATGTGG	395	55–56	RdRp [RNApol]
	DWVrev	GTCGTGCAGCTCGATAGGAT			
CBPV	CBPVfor	CGCAAGTACGCCTTGATAAAGAAC	300	55–56	RdRp [RNApol]
	CBPVrev	ACTACTAGAAACTCGTCGCTTCG			
BQCV	BQCVfor	AGTGGCGGAGATGTATGC	293	55–56	Capsid protein
	BQCVrev	GGAGGTGAAGTGGCTATATC			

Note: * AKI primer identical to ABPV, KBV, and IAPV.

followed by 40 cycles of 94 °C for 30 seconds, 56 °C for 30 seconds, 72 °C for 1 minute, and a final synthesis cycle at 72 °C for 2 minutes. PCR products were visualized in a 2% agarose solution.

Results and Discussion

The results of the conducted studies showed that several viruses, belonging to different taxonomic groups were detected in the honeybee *Apis mellifera* in the northern regions of Ukraine (Table 3).

Among the known representatives of the genus *Dicistroviruses* (Israeli Acute Paralysis Virus (IAPV), Kashmir Bee Virus (KBV), Acute Bee Paralysis Virus (ABPV), and Black Queen Cell Virus (BQCV)), only one virus, IAPV, was detected. This virus was found in both Kyiv and Zhytomyr regions. In these regions, the Chronic Bee Paralysis Virus (CBPV), which is still unclassified, was also detected. Two viruses, Sacbrood Virus (SBV) and Deformed Wing Virus (DWV), belonging to the genus *Iflaviruses*, were found in all three regions. The analysis of the obtained results shows that four bee viruses (IAPV, SBV, DWV, and CBPV) were detected for the first time in the Zhytomyr region. These data may indicate the widespread distribution of honeybee viruses (*Apis mellifera*) in the northern regions of Ukraine.

The viruses of the honeybee *A. mellifera* detected in the northern regions of Ukraine cause significant economic damage to domestic beekeeping. However, during evolution, honeybees have developed protective mechanisms that mitigate the harmful effects of their spread, including immune responses at the individual level [19–21] and social behaviors such as hygienic removal of sick hive mates [22]. Additionally, infected bees undergo behavioral changes that minimize contact between healthy bees and susceptible hive neighbors [23]. However, these protective mechanisms were significantly disrupted when the Western honeybee *A. mellifera* was first parasitized by

Table 3

Identification of Bee Viruses in the Northern Regions of Ukraine Using PCR Method

Virus	Zhytomyr Region		Kyiv Region					Chernihiv Region
	No.1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8
ABPV	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
IAPV	+	+	+	n/d	+	n/d	+	n/d
KBV	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
AKI	n/d	n/d	+	n/d	n/d	n/d	n/d	n/d
SBV	+	n/d	+	+	n/d	n/d	+	+
DWV	+	+	+	n/d	n/d	n/d	n/d	+
CBPV	+	+	+	n/d	+	n/d	n/d	n/d

Note: n/d – not detected.

the mite *Varroa destructor* in the last century. The impact of this mite on the reproduction of bee viruses has been described in many publications [4, 24]. Microsporidia *Nosema apis* and *N. ceranae*, which are intracellular parasites of bees [25, 26], can also influence the reproduction of another bee virus — Iridovirus [27]. Thus, over time, some bee viral infections have become transmissible. Therefore, studying the role of biological vectors in the ecology of bee viruses is an essential task of the present.

Thus, the results of the conducted studies showed that four viruses (IAPV, SBV, DWV, and CBPV) were detected for the first time in the honeybee *A. mellifera* in the Zhytomyr region, and these viruses belong to different taxonomic groups. All four viruses can cause bee colony collapse. DWV causes especially great harm to bees. It alters wing development during the pupal stage, shortening the lifespan of adult bees and rendering them flightless. At the onset of the disease, larvae turn yellowish, then light brown, followed by gray-brown, and finally dark brown or almost black [12, 14, 22]. Compared to the DWV virus, chronic paralysis is less virulent: when infected, it takes several days for CBPV to kill a bee [7, 20]. SBV affects bees at all stages of the life cycle but is most sensitive to a 2-day-old brood. Viral infection in adult bees varies and occurs without clinical manifestations but shortens the duration of life of individuals [3]. Three of these four viruses (SBV, DWV, and CBPV) were also detected in the Kyiv region, and two (SBV and DWV) in the Chernihiv region. These data indicate the widespread distribution of honeybee viruses from *A. mellifera* in the northern regions of Ukraine.

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Authors’ contribution

Rud Yu.P., Buchatsky L.P. — RNA isolation, PCR setup; Odnosum H.V., Yefimenko T.M., Vasylenko O.G. — collection of material in apiaries; Lasarenko L.M., Spivak M.Ya. — interpretation of results.

Conflicts of Interest

The authors declare no conflicts of interest.

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ЗАСТОСУВАННЯ ПЛР ДЛЯ ВИЯВЛЕННЯ ВІРУСНИХ ІНФЕКЦІЙ БДЖОЛИ *Apis mellifera* У ПІВНІЧНИХ РЕГІОНАХ УКРАЇНИ

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Мета. В роботі за допомогою полімеразної ланцюгової реакції (ПЛР) були досліджені віруси західної медоносної бджоли *Apis mellifera* із північних регіонів України.

Методи. Для ідентифікації вірусів бджіл використовували олігонуклеотидні праймери, специфічні до фрагменту гену, що кодує капсидний потеїн, та фрагменту гену РНК-залежної РНК-полімерази. Реакційна суміш містила 12,5 мкл розчину мастер-міксу (ДНК-полімераза, дНТФ та буфер), по 1 мкл кожного праймера (20 пікомоль/мкл), 2 мкл кДНК та воду, до загального об'єму суміші 25 мкл. Продукти ПЛР візуалізували в 2% -му розчині агарози.

Результати. Показано, що вперше у Житомирській області у бджоли *Apis mellifera* виявлено 4 віруси (IAPV, SBV, DWV та CBPV), які належать до різних таксономічних груп. Три з них (SBV, DWV та CBPV) були виявлені в Київській області та два (SBV та DWV) в Чернігівській.

Висновки. Отримані дані свідчать про широке розповсюдження вірусів медоносної бджоли *Apis mellifera* у північних регіонах України.

Ключові слова: *Apis mellifera*, віруси бджіл, діагностика, ПЛР.