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Molecular Identification of Honey Bee Viruses in some Egyptian Governments during 2021-2022

Dalia M.A. Elmasry[1]*, Dalia M. El-Husseini[1], Asmaa A. Eissa[1], Zakaria R. Elkanawati[2], Momtaz A. Shahein[3], Amany Adel[4]

[1]Nanotechnology Research Unit, Animal Health Research Institute, Agricultural Research Center (ARC), Giza, Egypt; [2]Beekeeping Department Plant protection Research Institute, Agriculture Research Center Giza, Egypt; [3]Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agriculture Research Center P.O. Box 264-Dokki, Giza 12618, Egypt; [4]Virology Research Department Animal Health Research Institute, Agriculture Research Center P.O. Box 264-Dokki, Giza 12618, Egypt.

Abstract | Numerous illnesses, including bacteria, viruses, and protozoa, endanger the health and survival of honey-bee colonies. Viruses, among ailments, pose a significant danger to the health and well-being of honeybees, causing beekeepers to be concerned about economic losses. One hundred samples has been collected from bee colony farms had bees loss 20% or more during 2021-2022 from some Egyptian governorates. The samples have been prepared and suspected viruses detection for Sacbrood virus (SBV), Israeli acute paralysis virus (IAPV), Acute bee paralysis virus (ABPV), Lake Sinai Viruses (LSV) ,Black queen cell virus (BQCV), Kashmir bee virus (KBV), Deformed wing virus (DWV) and Chronic bee paralysis virus (CBPV) by Reverse transcription polymerase chain reaction (RT-PCR). Then the positive samples have been genetically characterized by partial sequencing of respective genes of each virus. The results illustrated the domination of the DWV-B and LSV-4 viruses' distribution in Egypt during all seasons with high incidence up to 88% and 82%, respectively. However, the other viruses have been detected with different incidence rate of BQCV, IAPV, SBV were 15%, 23%, 22%, respectively. KBV and CBPV were low incidence 4% and 1% without any spatial correlation. The obtained results for genetic diversity and relationships of bee viruses that are circulating in Egypt compared to earlier studies, show notable similarities and differences that provide insight into the genetic evolution and temporal dynamics of these viruses. As conclusion, the identification of mixed infections highlights the intricacy of viral interactions in honey bee colonies and highlights the necessity of more investigation into the processes responsible for co-infections.

Keywords: Egypt, SBV, IAPV, ABPV, LSV, BQCV, KBV, DWV, CBPV

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***Correspondence** | Dalia M.A. Elmasry, Nanotechnology Research Unit, Animal Health Research Institute, Agricultural Research Center (ARC), Giza, Egypt; Email: dr_daliaelmasry@yahoo.com
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INTRODUCTION

The Western honey bee, scientifically known as *Apis mellifera,* belongs to the Apis genus and is globally recognized for its production of honeybee products (Engel, 1999). Furthermore, it plays a crucial role in plant pollination (Chapman *et al*., 2019). Honey bees are a key species that provide crucial ecological services by pollinating crops, and they are nevertheless valued commercially as pollinators worldwide. Honey bees are estimated to be able to pollinate between 90 and 130 crops. Compared to other pollinators, honey bees are inexpensive, adaptable, and readily available. Around the world, animal pollination produces 65% of the food that is generated independently, and 15% to 30%, or around 35%, of the food that is produced for humans. Without these pollinators, the safety of our food may be seriously compromised (Naz *et al*., 2022).

However, a recent study revealed that the loss of natural habitat and the overuse of pesticides reduced bee visits to that region, which led to an extreme decline in pollination. If there are merely a lot of bees, pollination is guaranteed. Thus, by using honey bees along with natural pollinators, we can control the pollination service (Gallant *et al*., 2014). Honey bees are ideal for pollinating native crops such as watermelon, which have a high pollination need. In certain countries, farmers can rent pollination services at prescribed prices, particularly for crops such as almonds, sunflowers, canola (seeds), grapes, apples, sweet cherries, watermelons, avocados, and so on (Bond *et al*., 2014).

Viruses are ubiquitous, the factors that influence viral abundance in wild host populations is generally inadequate. Biotic factors, such as sympatric host species, and abiotic factors, such as climatic variables, are both likely to influence viral prevalence. Managed and wild bees, which carry multiple multi-host viruses with a mostly fecal-oral between-species transmission mechanism, are a good paradigm for testing the influence of biotic and abiotic variables on viral prevalence in wild host populations (Poit *et al*., 2022).

Honey bee colonies are susceptible to a variety of illnesses caused by diverse infections (Chen *et al*., 2006; Alburaki *et al*., 2016; Gisder and Genersch, 2017) and can be impacted by altered environmental conditions in numerous ways (Hao and Li, 2016). The replication and transmission of viral particles may occur within and between several species of bees, with the extent of infection influenced by factors such as the specific viral strain or the characteristics of the host organism (Tehel *et al*., 2016). Additionally, factors like sex, nutritional condition, genetic composition, and age of the host organism can also affect the infection rate (McMenamin and Flenniken, 2018).

One of the risk factors impacting the health of these economically and ecologically significant insects, honey bees, is viral infections. Numerous colonies are lost as a result of these illnesses. The ectoparasitic mite Varroa destructor poses the greatest danger to the health of western honey bees (Apis mellifera), mostly due to its capacity as a virus vector. The existence of black queen cell virus (BQCV) and deformed wing virus (DWV) infections, which result in major colony losses in honey bees in three separate Balıkesir districts. The bee colonies, 18% (9/50) of the samples had multiple infections with both viruses. (Karapınar and Özüiçli, 2024).

According to Ray *et al*. (2020), viruses may pose significant threats to honey bees compared to other pathogens, as a substantial number of infections occur without the development of observable illness symptoms, as noted by Chen *et al*. (2006) and Martin *et al*. (2012). Viral proliferation can occur through various means, including transfer from queens or drones to their progeny during mating, as well as horizontal transmission across colony members of the same age generation and between individuals of the same or different castes (Chen *et al*., 2006; de Miranda *et al*., 2012; Chagas *et al*., 2019). According to studies, the majority of infectious agents that affect honey bees are single-strand positive (SS+) sense RNA viruses (Ullah *et al*., 2021). Among these, the viruses most harmful to bee health are classified into Dicistroviruses, including Acute Bee Paralysis Virus (ABPV), Kashmir Bee Virus (KBV), Israeli Acute Paralysis Virus (IAPV), and Black Queen Cell Virus (BQCV); Iflaviruses, which encompass Deformed Wing Virus (DWV), Slow Bee Paralysis Virus (SBPV), Varroa destructor virus (VDV1), and Sacbrood virus (SBV); and taxonomically unsystematic viruses such as Lake Sinai viruses (LSV1-7) and Chronic Bee Paralysis Virus (CBPV) (Žvokelj *et al*., 2020; Schläppi *et al*., 2020).

Viral diseases can infect bee populations at any breeding level, and they can also affect honey bee colonies and hives. For instance, viruses like ABPV, KBV, IAPV, DWV, and LSV2 have adverse effects on honeybee colonies and hives, leading to Colony Collapse Disorder (CCD), which can infect adult bees, hives, and queens (Ullah *et al*., 2021). Increased mortality of adult bees is often attributed to severe infections with KBV and ABPV. Consequently, infected pupae and larvae emerge, leading to fewer adults available to care for the young offspring. ABPV, in particular, accumulates in the brain and hypo pharyngeal glands (Chagas *et al*., 2019).

SBPV primarily affects the forelegs of honeybees but is also present in various other body parts. Prior to the emergence of parasitic mites in the UK, ABPV and DWV were occasional causes of colony collapse in honeybee colonies

(Sánchez-Bayo *et al*., 2016). DWV attacks brain regions responsible for scent regulation, adversely affecting honeybee foraging behavior. DWV-infected bees fly shorter distances and for shorter periods compared to non-infected bees. DWV also reduces gene counts regulating memory, wing growth, and olfactory learning. Additionally, DWV can infect both adult and young queens, resulting in malformed wings and ovarian degeneration (Wells *et al*., 2016; Amiri *et al*., 2017).

CBPV can cause severe symptoms in adult bees and shorten workers' lifespans when virus titers reach their maximum level. Conversely, SBV, DWV, and BQCV can continue to grow even in honeybees that show no external symptoms of illness. However, when favorable conditions for replication are present, these viruses can become problematic (Chen and Siede, 2007; Dainat *et al*., 2012; Amiri *et al*., 2015).

In Egyptian honey bee hives, DWV-A and BQCV were extensively dispersed in the winter and summer of 2021. The DWV variation currently circulating worldwide, but DWV-B was not. Our information on the incidence of viruses in honey bees at the moment may help safeguard Egypt's beekeeping sector. Furthermore, by addressing a knowledge gap about the presence of honey bee viruses in Egypt, our work contributes to the comprehensive evaluation of the worldwide honey bee virome (Kandel *et al*., 2023).

The coexistence of multiple viruses within the same population, especially viruses of the same species, may lead to inter- and intra-species genetic recombination (Meznar, 2009; Wang *et al*., 2013). This study aims to provide a comprehensive and in-depth overview of the epidemiological distribution and genetic evolution of bee viruses commonly circulating in Egypt from 2020 to 2022 through cooperative surveillance efforts.

MATERIALS AND METHODS

Sample Collection and Preparation

A total of 20–30 dead or recently deceased bees from the area close to the entrance of the hive, then transfer them into a 15ml sterile falcon tubes from bees farm had be 20% or more mortality. To preserve the samples' freshness until they are sent to the lab, chill them in a refrigerator. This will assist in halting the formation of bacterial and fungus. One hundred bee samples were collected from bee farms where bee infections and mortality occurred, depending on the presence of these apiaries in Egypt governorates and sent to Animal Health Research Institute in sterile ice box.

Preparation of samples was done by bee tissue homogenization, following the steps in molecular methods in Bee Book (Evans, 2001). Ten bees have been homogenized and treated as one samples, the homogenization was done by mechanical shaker with steel beads then diluted in PBS for uniform and perfect tissue homogenates. The homogenized samples were centrifuged for 10 min at 5,000 g to be clarified.

Viral RNA Extraction

Viral RNA extraction from the homogenized samples was accomplished following the work instruction of Patho Gene-spin™ DNA/RNA Extraction Kit – iNtron – Lot. No. 16GC0011088

Conventional Reverse Transcriptase-Polymerase Chain Reactions (RT-PCR)

The RT-PCR reactions were conducted using EasyScript® One-Step RT-PCR Super Mix (+dye) – Trans – cat. No. AE411-02, following the manufacturer's instructions. Primers specific for each virus, along with their annealing temperatures and expected product sizes, were utilized for amplification, as detailed in Table 1 (Stoltz *et al*., 1995; Ribie`re *et al*., 2000; Palacios *et al*., 2008; Siede *et al*., 2008; Runckel *et al*., 2011; Kumar *et al*., 2018). The PCR reactions were prepared according to the protocol provided by the manufacturer. Subsequently, the prepared PCR reactions underwent incubation under the following thermal conditions: 45°C for 30 minutes for the RT step, followed by initial denaturation and RT enzyme inactivation at 94°C for 5 minutes. This was followed by 40 cycles of amplification, including denaturation at 94°C for 45 seconds, annealing at 53-60°C for 45 seconds, and extension at 72°C for 45 seconds. The incubation process concluded with a final extension step at 72°C for 10 minutes. The results were visualized using gel electrophoresis. PCR products were loaded onto a 1.5% agarose gel, alongside a GeneRuler 100 bp ladder (Fermentas, Thermo Scientific, Germany), to allow for size comparison. Subsequently, the results were documented using a gel documentation system (Alpha Innotech, Biometra).

Spatial and temporal analysis of the epidemiological data involved collecting and entering the data into an Excel spreadsheet (version 2010) for analysis. The spatial distribution of the samples and the distribution of positive viruses in Egypt were analyzed using Tableau Public 2020.1 software.

Nucleotide sequence reactions, the amplified PCR products of appropriate size were purified using a QIA quick Gel Extraction Kit (QIAGEN, Hilden, Germany) following the kit manual. Subsequently, the purified PCR products underwent sequencing reactions using a Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's specifications.

Table 1: The primers used for detection of Bee viruses.

Virus	Primers	Molecular weight	Annealing temperature	
Sacbrood virus (SBV)	F-SBV: GGATGAAAGGAAATTACCAG	426	50	Stoltz *et al.*, 1995
	R- SBV: CCACTAGGTGATCCACACT			
Deformed wing virus (DWV)	F- DWV: TTTGCAAGATGCTGTATGTGG	395	55	
	R- DWV: GTCGTGCAGCTCGATAGGAT			
Kashmir bee virus (KBV)	F- KBV: GATGAACGTCGACCTATTGA	393	53	Palacios *et al.*, 2008
	R- KBV: TGTGGGTTGGCTATGAGTCA			
Black queen cell virus (BQCV)	F- BQCV: GGACGAAAGGAAGCCTAAAC	424	55	Palacios *et al.*, 2008
	R- BQCV: ACTAGGAAGAGACTTGCACC			
Chronic bee paralysis virus (CBPV)	F- CBPV: AGTTGTCATGGTTAACAGGATACGAG	455	53	
	R- CBPV: TCTAATCTTAGCACGAAAGCCGAG			
Acute bee paralysis virus (ABPV)	F- ABPV: TGAGAACACCTGTAATGTGG	452	53	Ribie *et al.*, 2000
	R- ABPV: ACCAGAGGGTTGACTGTGTG			
Israeli acute bee paralysis virus (IAPV)	F- IAPV: AGACACCAATCACGGACCTCAC	475	60	Siede *et al.*, 2008
	R- IAPV: AGATTTGTCTGTCTCCCAGTGCACAT			
Lake Sinai virus (LSV)	Common LSV-F: CKTGCGGNCCTCATTTCTTCATGTC	365	60	Palacios *et al.*, 2008)
	Common LSV-R: CATGAATCCAAKGTCAAAGGTRTCGT			

(A = Adenine; C = Cytosine; G = Guanine; T = Thymidine; I = Inosine; R = A/G; Y = C/T; K = G/T; M = A/C; S = C/G; W = A/T; H = A/C/T; B = C/G/T; V = A/C/G; D = A/G/T; N = A/C/G/T)

The reaction product was then purified by exclusion chromatography using a DyeEX 2.0 Spin Kit. The recovered materials were sequenced using a 3500 XL DNA Analyzer (Applied Biosystems).

Sequence analysis and phylogenetic tree construction were conducted by submitting the partial sequences of the suspected viruses to NCBI – Gene bank. Multiple sequence alignment for the sequenced viruses was performed using Bio Edit version 7.0 with the Clustal W method, determining percent identity matrices between different viruses. Neighbor-joining phylogenetic trees were constructed using the distance-based method in MEGA version 7 (Stoltz *et al.*, 1995; Evans, 2001; Ribie`re *et al.*, 2000; Palacios *et al.*, 2008; Siede *et al.*, 2008; Runckel *et al.*, 2011; Kumar *et al.*, 2018). These trees included the sequenced samples from this study along with sequences of strains downloaded from GenBank (NCBI).

GenBank accession numbers (65) for nucleotide sequences of viruses detection for ascension numbers from OR591533 to OR591548 and from OR581103 to OR581153

RESULTS AND DISCUSSION

Detection of Suspected Viruses by Conventional RT-PCR Reactions

The detection of the suspected viruses in the collected samples was performed by specific primers for DWV, BQCV, CBPV, ABPV, SBV, KBV, LSV, and IAPV as mentioned in Table 1.

According to Table 2, both DWV and LSV viruses have the highest positivity in detection with 88 and 82 positive cases out of 100 samples, respectively. ABPV has also high number of positive cases as 66 cases, followed by IAPV, SBV, and BQCV with number of positive cases equal 23, 22, and 15 respectively. However, the lowest detection rate was recorded for KBV and CBPV, encountering 4 and 1 positive cases, respectively.

Spatial Analysis of the Examined Viruses

The spatial analysis has been done based on the epidemiological data and geographical distribution of the examined samples, in addition to the distribution of positive samples for the spotted different viruses. As shown in Table 2, Giza, New valley and Gharbia have the major participation in sample collection, provided 10 and 11 and 12 samples, respectively. Most of the governorates had detection for more than one virus among the examined viruses. The spatial distribution of the collected and positive samples has been illustrated in Figure 1, the governorates pointed with large and dark circles were that of high density in sample numbers and high positivity for the examined viruses such as Sohag and New valley in Upper Egypt and Gharbia and Dakahlia in Lower Egypt.

The prevalence of the bee's viral diseases in Egypt has been illustrated in Figure 2. LSV and DWV possessed the highest

Table 2: The total number of collected samples in different governorates and number of positive samples in each suspected viruses.

Governorates	NO. of collected samples	Examined suspected viruses							
		Dwv	BQCV	CBPV	ABPV	SBV	KBV	LSV	IAPV
Alexandria	2	2	0	0	1	0	0	2	0
Monufia	3	3	0	0	3	0	0	3	0
Sharqia	3	3	1	0	1	1	0	3	1
Gharbia	12	12	5	0	7	0	2	10	3
Qalyubia	3	3	0	0	2	0	1	3	1
Beheira	3	3	0	0	1	0	0	3	1
Dakahlia	7	7	2	0	5	1	0	0	7
Fayoum	3	1	0	0	1	0	0	1	0
Beni Suef	8	4	4	0	6	4	0	5	3
Giza	10	8	0	0	6	0	0	9	0
Sohag	9	9	0	1	8	4	1	9	3
Luxor	1	1	0	0	1	0	0	1	0
Minya	7	7	1	0	4	5	0	7	0
Assuit	2	1	0	0	1	0	0	1	0
New Valley	11	11	1	0	8	0	0	11	1
Qena	5	4	0	0	5	2	0	5	1
North Sinai	3	2	0	0	0	0	0	2	0
Ismailia	5	5	1	0	4	4	0	4	0
Suez	3	2	0	0	2	1	0	3	2
Total	100	88	15	1	66	22	4	82	23

Figure 1: Map of Egypt elaborated the density of the collected samples and positive viruses in each governorate during the study. The sizes of circles indicate the intensity of the collected samples, while the density of color indicates the number of detected viruses in each governorate.

prevalence in Egypt = 88% and highly distributed all over the Egyptian strains especially new valley, Minia, Giza, and Sohag in Upper Egypt, and Gharbia and Dakahlia in Lower Egypt. APBV also had a high prevalence in Egypt = 66% and dominated in New valley, Giza, and Gharbia. IAPV and SBV had nearly the same prevalence with 21% and 23%, respectively, followed by BQCV with 12%. The lowest prevalence belonged to CPBV and KBV with percentage = 2% and 6%, respectively, as shown in Figure 2. Concerning the spatial analysis of each virus individually, as shown in Figure 2 and Table 2, Newvally governorate occupies the top position among the Egyptian governorates in recording positive cases of ABPV, DWV, and LSV, followed by Giza, Minya, Beni-suif, Gharbia and Dakahlia.

Temporal Analysis of the Examined Viruses

The analysis of viral distribution and detection during the seasons of the year through 2021 and 2022 has been shown in Figure 3. The positivity% of ABPV, DWV, and LSV were relatively high in all seasons, with no relation to the climatic conditions, while BQCV, CBPV, IAPV, KBV, and SBV were of low detection rate in all seasons, except BQCV and IAPV which were detected in spring 2022 obviously.

Analysis of Partial Nucleotide Sequence of the Bee Viruses

These accession numbers appear in Gene bank accession numbers. The data are simultaneously made available to other INSDC databases, the European Nucleotide Archive (ENA) and the DNA Data Bank of Japan (DDBJ) as shown in Table 3.

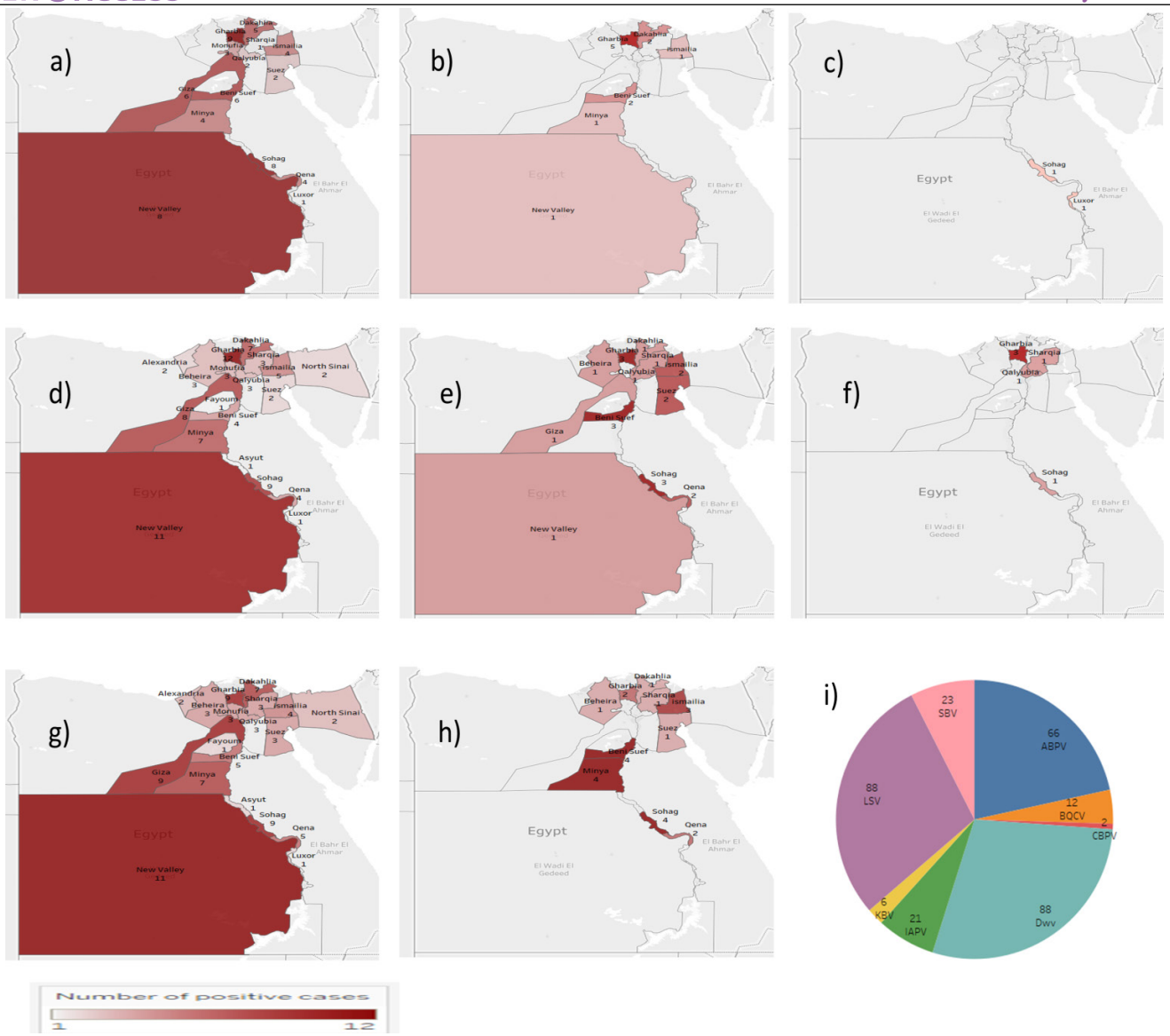

Figure 2: Prevalence of the bee's viral diseases in Egypt. (a-h) Geographical distribution in all the Egyptian governorates for the positive cases of each virus, ABPV, BQCV, CBPV, DWV, IAPV, KBV, LSV, and SBV, respectively. i) The percentage of the positive cases of each virus in 100 collected samples during the surveillance in 2021-2022.

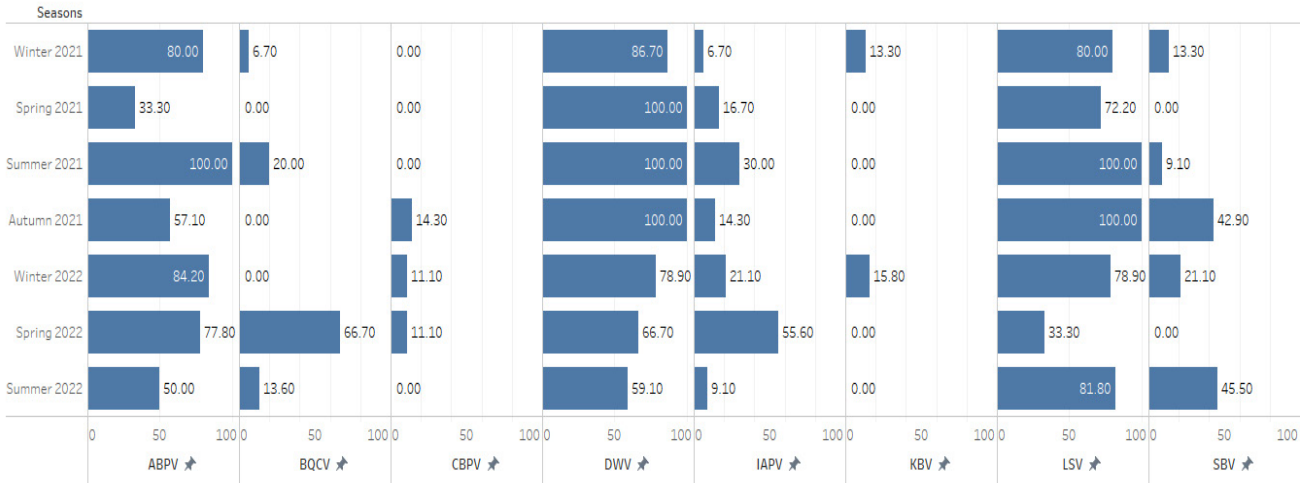

Figure 3: The temporal analysis of the positive cases% of the bee viral diseases in Egypt through 2021- 2022. The positivity% of ABPV, DWV, and LSV were relatively high in all seasons. BQCV, CBPV, IAPV, KBV, and SBV were of low detection rate in all seasons, except BQCV and IAPV which were detected in spring 2022 obviously.

Table 3: The Gene Bank accession numbers approved for collected samples in different governorates and number of positive samples in each suspected viruses.

Sequence ID	Accession No.	Sequence ID	Accession No.
9-LSV-New Valley-Egypt-2021	OR591533	56-ABV-Sohag-Egypt-2021	OR581120
31-LSV-Luxor-Egypt-2021	OR591534	31-ABV-Luxor-Egypt-2021	OR581121
33-LSV-Gharbia-Egypt-2021	OR591535	61-ABV-Giza-Egypt-2022	OR581122
36-LSV-Alexandria-Egypt-2021	OR591536	63-ABV-Qaliobia-Egypt-2022	OR581123
51-LSV-Menofia-Egypt-2021	OR591537	65-ABV-Suez-Egypt-2022	OR581124
61-LSV-Giza-Egypt-2022	OR591538	70-ABV-Qena-Egypt-2022	OR581125
64-LSV-Qaliobia-Egypt-2022	OR591539	80-ABV-Benisuef-Egypt-2022	OR581126
66-LSV-Suez-Egypt-2022	OR591540	81-ABV-Benisuef-Egypt-2022	OR581127
67-LSV-Sharqia-Egypt-2022	OR591541	96-BQCV-Dakahlia-Egypt-2022	OR581129
69-LSV-Sohag-Egypt-2022	OR591542	20-DWV-Giza-Egypt-2021	OR581130
77-LSV-Benisuef-Egypt-2022	OR591543	11-DWV-Newvalley-Egypt-2021	OR581131
82-LSV-NorthSinai-Egypt-2022	OR591544	12-DWV-Newvalley-Egypt-2021	OR581132
87-LSV-Minia-Egypt-2022	OR591545	26-DWV-Gharbia-Egypt-2021	OR581133
88-LSV-Asyut-Egypt-2022	OR591546	6-DWV-Dakahlia-Egypt-2021	OR581134
93-LSV-Ismailia-Egypt-2022	OR591547	27-DWV-Behera-Egypt-2021	OR581135
96-LSV-Dakahlia-Egypt-2022	OR591548	2-DWV-Gharbia-Egypt-2021	OR581136
8-SBV-Newvalley-Egypt-2021	OR581103	21-DWV-Giza-Egypt-2021	OR581137
37-SBV-Suez-Egypt-2021	OR581104	36-DWV-Alexandria-Egypt-2021	OR581138
39-SBV-Qena-Egypt-2021	OR581105	41-DWV-Minya-Egypt-2021	OR581139
53-SBV-Sohag-Egypt-2021	OR581106	50-DWV-Menofia-Egypt-2021	OR581140
79-SBV-Benisuef-Egypt-2022	OR581107	57-DWV-Sohag-Egypt-2021	OR581141
81-SBV-Benisuef-Egypt-2022	OR581108	31-DWV-Luxor-Egypt-2021	OR581142
87-SBV-Minia-Egypt-2022	OR581109	64-DWV-Qaliobia-Egypt-2022	OR581143
91-SBV-Ismailia-Egypt-2022	OR581110	66-DWV-Suez-Egypt-2022	OR581144
95-SBV-Gharbia-Egypt-2022	OR581111	67-DWV-Sharqia-Egypt-2022	OR581145
96-SBV-Dakahlia-Egypt-2022	OR581112	71-DWV-Sohag-Egypt-2022	OR581146
18-ABV-Giza-Egypt-2021	OR581113	73-DWV-Qena-Egypt-2022	OR581147
11-ABV-NewValley-Egypt-2021	OR581114	79-DWV-Benisuef-Egypt-2022	OR581148
4-ABV-Gharbia-Egypt-2021	OR581115	88-DWV-Asyut-Egypt-2022	OR581149
7-ABV-Sharqia-Egypt-2021	OR581116	89-DWV-NorthSinai-Egypt-2022	OR581150
1-ABV-Gharbia-Egypt-2021	OR581117	90-DWV-Ismailia-Egypt-2022	OR581151
42-ABV-Minia-Egypt-2021	OR581118	94-DWV-Gharbia-Egypt-2022	OR581152
51-ABV-Menofia-Egypt-2021	OR581119	38-IAPV-Behera-Egypt-2021	OR581153

Acute bee paralysis virus (ABPV): Partial nucleotide sequences for the non-structure gene (Replicase gene) have been done for 6 positive samples. The Egyptian viruses revealed high similarities, showing identity % range 97.1% - 99%. The Egyptian strain 42-ABPV-Minya-Egypt-2021 is highly related to the Hungary virus with identity% =97.1%. Phylogenetically, we have observed that all the Egyptian viruses have been gathered in the same group closely related to each other and genetically related to CR-026-Zimbabwe virus -2017, except the Egyptian strain 42-ABPV-Minya-Egypt-2021 which is genetically related to viruses from china, Poland and Hungary, as shown in Supplementary Figure 1.

Deformed wing virus (DWV): Twenty three positive samples have been sequenced partially for replicase gene showed high similarities among the Egyptian viruses and viruses from Israel, Uruguay, and Syria have presented identity % rang 94.5%- 99%. The Egyptian viruses 20-DWV-Giza-Egypt-2021 and 11-DWV-New valley-Egypt-2021 showed the lowest similarity with the other Egyptian viruses, with range 89.9%-93%. Phylogenetically, as shown in Supplementary Figure 2, all the Egyptian viruses classified as DWV- genotype A, however, they

have distributed in different groups, clustering with the worldwide viruses and not related to each other.

Sacbrood virus (SBV): The replicase gene of SBV for ten positive samples has been sequenced. All the Egyptian viruses have shared high similarity, with an identity percent range of 96.3% - 98.5%, while all the Egyptian viruses showed low similarity with other worldwide viruses with identity % range 86% - 88.6%. Phylogenetically, the Egyptian strains cluster into distinct group away from the other world strains as shown in Supplementary Figure 3.

Israeli acute paralysis virus (IAPV): Only one strain was successful to be sequenced for replicase gene of the IAPV virus. 38-IAPV-Behera-Egypt-2021 was highly similar to the other world circulated viruses in USA, Korea, China, Japan, Australia, and Israel with an identity % range of 96.1% - 99%. As well as, it was genetically related to the Chinese and Korean strains as shown in Supplementary Figure 4.

Lake sinai virus: Sixteen positive samples have been sequenced partially for replicase gene of LSV. Those Egyptian strains were phylogenetically related to the genotype LSV-4 (Supplementary Figure 5), with identity % ranges from 91.6% to 100% among the Egyptian strains and the other world strains in China and Korea belong to genotype LSV-4. However, they showed less similarity with the other genotypes of LSV, with identity% ranges from 80.9% to 91.2%.

Black Queen Cell Virus (BQCV)

There is only one strain which has been sequenced for the replicase gene of BQCV. The Egyptian strain 96Dakahlia-Egypt-2022 was related phylogenetically to the UK lineage which includes strains from UK, Israel and Europe, shown in Supplementary Figure 6. The similarity between the Egyptian strain and the strains belong to the UK lineage is very high, with identity% ranges from 96.5% to 97.6%, while the identity between the Egyptian strain and the other lineage of BQCV ranges from 86.5% to 88.4%.

In the current surveillance, LSV, DWV, and ABPV were the predominant viruses in Egypt, with incidence rates of 82%, 88%, and 66%, respectively. The wide distribution of DWV and ABPV in Egypt is consistent with previous studies conducted in Egypt and other countries, supporting the global distribution of *Nosema ceranae* species viruses (de Miranda *et al*., 2010; Beaurepaire *et al*., 2020; Abd-El-Samie *et al*., 2021). These viruses were detected in all samples collected from 19 governorates during the surveillance, with high incidences in several governorates. Despite the low incidence of BQCV, it was detected in Gharbia and Beni-suif only, while IAPV was mainly detected in Dakahlia, with minor occurrences in other governorates.

Regarding the temporal distribution of positive cases throughout the surveillance years, there was no significant pattern related to climatic changes in the distribution of LSV, DWV, and ABPV. These viruses were detected at high incidences during all seasons of 2021-2022, consistent with previous studies conducted in Egypt in 2021 (Kandel *et al*., 2023). However, the majority of positive cases of IAPV and BQCV were recorded in spring 2022. This contrasts with a recent study conducted in Egypt in 2021, which reported a high detection rate for BQCV (Kandel *et al*., 2023). Additionally, KBV was rarely detected, only in winter during 2021-2022.

Most collected samples were positive for mixed infections, mainly LSV and DWV. This finding is consistent with numerous worldwide studies conducted on honeybees, particularly worker bees (Rüstemoğlu *et al*., 2019; Kalayci *et al*., 2020; Güller *et al*., 2021).

In a study on honeybee diseases and parasites in South Korea during 2017-2021, Truong *et al*. (2023) found that the three most prevalent viruses were DWV (52.63%), BQCV (55.26%), and SBV (52.63%). Avcı *et al*. (2022) identified BQCV, DWV, and ABPV in honey bees from Konya, Karaman, Aksaray, Niğde, and Isparta. Güller *et al*. (2022) identified SBV and BQCV infections in honey bees in Bingöl province. Utkan and Eroğlu (2023) identified two viruses (DWV, CBPV) in honey bees in Amasya province. In addition, Eroglu (2023) discovered that honey bee viruses (BQCV and KBV) were present in several wasps (Vespula germanica) discovered dead in Erzurum. Aglagane *et al*. (2024) reported that honeybee viruses epidemiological assessment was done on 87 clinically healthy beehives in southeastern Morocco that were found at varying frequencies; DWV had the highest prevalence (89.65%), followed by BQCV (17.24%), ABPV (8.04%), CBPV (4.59%), and SBV (2.29%). In contrast, single infection was discovered in 64.37% of colonies, 21.8% had mixed infection with two viruses, and 4.6% had three.

LSV is a positive-sense, single-stranded RNA virus belonging to the Sinaivirus genus. It was first detected in the USA during 2007-2008 and has since been distributed worldwide. LSV has been genetically classified into seven genotypes (LSV1-LSV7). In our study, LSV-4 genotype was detected in the examined bee population, showing high incidence, temporal, and spatial distribution in Egypt. The genetic characterization and phylogenetic analysis of honeybee viruses provide valuable insights into their epidemiology, evolution, and potential impacts on bee health. In this study, we conducted sequence analysis and phylogenetic tree construction to elucidate the genetic diversity

and relationships of bee viruses circulating in Egypt from 2020 to 2022. Our findings reveal significant similarities and differences compared to previous research, shedding light on the temporal dynamics and genetic evolution of these viruses.

Acute Bee Paralysis Virus (ABPV)

Our analysis of ABPV sequences showed high similarities among Egyptian strains, with a range of 97.1% to 99% identity. Phylogenetic analysis placed Egyptian viruses in a distinct group closely related to each other, with one exception genetically related to viruses from China, Poland, and Hungary. This genetic divergence suggests both local evolution and potential introductions from other regions (Tehel *et al*., 2016; Mc Menamin *et al*., 2018; Ray *et al*., 2020).

Deformed Wing Virus (DWV)

Sequencing of DWV strains revealed high similarities among Egyptian viruses, with identities ranging from 94.5% to 99% compared to strains from Israel, Uruguay, and Syria (Alburaki *et al*., 2018). Interestingly, two Egyptian strains showed lower similarity, suggesting genetic variability within local populations. Phylogenetic analysis classified Egyptian viruses as DWV genotype A, clustering with worldwide strains but exhibiting no clear genetic relationship among themselves (Chen *et al*., 2006; Gisder and Genersch, 2017). Alqurneh *et al*., (2024) reported that due to the limited sample size and low Varroa infestation levels (0.3%±0.1 mites/100 bees), other significant correlations were not detectable. However, viral loads of DWV-A were 50 times higher and DWV-B were 1000 times higher in Varroa-infested colonies compared to colonies without Varroa, as determined by qPCR (Student's t-test, log-transformed data, $p<0.05$ for both viruses; nVarroa=8, nw/o Varroa= 45 and 40, respectively). No relation between virus loads and Varroa infestation was detected for other Varroa-transmitted viruses (ABPV, KBV, and CBPV).

Sacbrood Virus (SBV)

Our analysis of SBV sequences showed high similarity among Egyptian strains, ranging from 96.3% to 98.5% identity (Mc Menamin *et al*., 2018). Phylogenetically, Egyptian strains formed a distinct group separate from worldwide strains, indicating localized evolution or introduction of a unique strain into the Egyptian bee population (Hao and Li, 2016).

Israeli Acute Paralysis Virus (IAPV)

Sequencing of IAPV revealed high similarity (96.1% to 99%) with viruses from the USA, Korea, China, Japan, Australia, and Israel (Martin *et al*., 2012). Phylogenetic analysis placed the Egyptian strain in a clade with Chinese and Korean strains, suggesting potential transcontinental movement of this virus (Ray *et al*., 2020).

Lake Sinai Virus (LSV)

Sequencing of LSV strains identified Egyptian strains as genotype LSV-4, showing high similarity (91.6% to 100%) with strains from China and Korea (Kumar *et al*., 2018). Phylogenetic analysis confirmed the close relationship between Egyptian and East Asian strains, indicating potential transboundary spread of this virus.

Black Queen Cell Virus (BQCV)

Sequencing of BQCV strains revealed a high similarity (96.5% to 97.6%) with the UK lineage, suggesting potential introduction from European Regions. Phylogenetic analysis placed the Egyptian strain within the UK lineage, highlighting the genetic relatedness between Egyptian and European strains (Chen *et al*., 2006). BQCV emerged as the most prevalent virus identified in the examined samples. This finding aligns with its widespread occurrence globally (Beaurepaire *et al*., 2020). Studies conducted in neighboring countries reported prevalence rates of 29% in Syria (AbuKubaa *et al*., 2018), 70% in Egypt (Kendel *et al*., 2023), and 100% in Turkey (Mayack and Hakanoglu, 2022). Currently, BQCV is regarded as a benign viral pathogen among adult honey bees, likely due to its primarily direct horizontal transmission through food exchange among nestmates, which fosters robust virus replication (AlNaggar and Paxaton, 2020). However, should BQCV transmission shift to vector-mediated, it could potentially induce adverse health effects.

Comparison with Previous Research: Our findings corroborate previous studies on honeybee viruses, demonstrating the global distribution and genetic diversity of these pathogens. However, our study provides novel insights into the genetic evolution and epidemiology of bee viruses in Egypt, highlighting both local dynamics and potential transboundary movements. The observed genetic diversity and relationships among Egyptian strains underscore the need for continued surveillance and management efforts to mitigate the impacts of bee viruses on honeybee populations.

The lack of a map indicating the sites of beekeeping and the existence of bees throughout Egypt's governorates were one of the difficulties we encountered when gathering samples. Bee sample collection was contingent upon apiaries with death rates of twenty percent or above. We require greater capabilities and an initial time for a long-term study in order to review the literature, select the suitable primers to diagnose viral causes, and attempt to isolate viruses on appropriate hives in an effort to find a treatment or immunisation against these viruses.

In the future, we will be to isolate the viruses in a setting that is conducive to bee viruses and develop s environmentally friendly therapy or vaccination.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, our comprehensive study sheds light on the epidemiological distribution, genetic evolution in Egypt. We have identified the predominant viruses, including LSV, DWV, and ABPV, with high incidence rates across various governorates. Notably, the temporal distribution of these viruses did not show significant patterns related to climatic changes, indicating their resilience across different seasons. The obtained results highlight the importance of continued surveillance and monitoring of honey bee viruses to understand their dynamics and mitigate potential threats to bee populations. Additionally, the detection of mixed infections underscores the complexity of viral interactions within honey bee colonies and emphasizes the need for further research on the mechanisms underlying co-infections. Furthermore, the identification of the LSV-4 genotype in Egypt for the first time underscores the global distribution and genetic diversity of honey bee viruses, necessitating ongoing efforts to track their spread and evolution. Overall, the current study contributes valuable insights into the epidemiology and molecular characteristics of honey bee viruses in Egypt, providing a foundation for future research and strategies aimed at protecting honey bee populations and ensuring the sustainability of pollination services. Multiple viruses in an infected hive during multiple seasons are a sign that some viruses are harmful and can cause the hive to collapse and the bee population to die off, which results in financial losses from beekeeping and honey production It is proposed that a geographical study be conducted and bee farms documented in all Egyptian governorates, with the goal of treating these viruses or developing a vaccination to avoid viral infections.

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NOVELTY STATEMENT

According to the previous studies in the past few years, the viral bees' diseases are endemic in Egypt. So, it becomes necessary to follow up their situation and distribution through the years which is considered an integrated pillar in strategies of the eradication and overcome of these diseases

AUTHOR'S CONTRIBUTION

DE devised the project, the main conceptual ideas, preparation of samples and proof outline, DM worked and verified the numerical results of the bee viruses by PCR. AE responsible for bee samples and data collected team, AA was analysis of the results of Sequence, submitted to Gene Bank and to the writing of the manuscript. ZR and MS aided in interpreting the results. All authors discussed the results and commented on the manuscript.

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SUPPLEMENTARY MATERIAL

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.aavs/2024/12.1.49.60

Conflict of Interest

There is no conflict of interest.

Ethical Approval

Consent to participate not applicable. Animal Ethical committees of AHRI-ARC, Egypt, reviewed and approved by AHRI Director

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