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Phylogenetic and epidemiological characteristics of H9N2 Avian Influenza Viruses from 2020 to 2022 in Egypt

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ABSTRACT

The H9 low-pathogenic avian influenza (LPAI) viruses cause enormous economic harm despite their low pathogenicity. It became common in Egypt in 2011 and has undergone ongoing genetic evolution since then. To limit the virus's transmission, regular monitoring of its evolution is essential. The current study concentrated on the frequency and molecular characteristics of LPAI H9N2 viruses spreading throughout different Egyptian areas between 2020 and 2022. Using real-time PCR, 503 positive LPAI H9 cases were detected out of 29,319 cases, for a total prevalence rate of 1.7%. However, live bird market (LBM) had the highest LPAI H9N2 prevalence rate (10.6%), followed by household sector and farm (2 % and 1.3% respectively). The 33 samples were isolated in 11-day-old embryonated chicken eggs (ECEs) before being sequenced for partial hemagglutinin (HA). The H9 isolates were phylogenetically related to the Egy-2 G1-B branch (pigeon-like), which has been the prevalent circulating H9N2 genotype in Egypt since 2016. The findings of the sequence analysis revealed a clear genetic evolution compared to the original virus (A/quail/Egypt/113413v/2011), which shared 93.2–95.4% and 94.7-97.1% homology at the nucleotide and amino acid levels, respectively. In comparison to the reference Egyptian strain, the molecular analysis found 12 alterations in amino acid residues with genetic stability in the major locations. The majority of examined strains had five glycosylation sites in HA. However, some strains had an extra sites at position 105, 145, 258. Comparable to A/quail/Hong Kong/G1/97, and all strains had the substitutions H191and L234 in the HA gene, indicating a predilection for binding to human-like receptors. Because of continues genetic development of H9 viruses reported in this work, frequent viral surveillance is required for better management.

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INTRODUCTION

H9N2 low pathogenic avian influenza is a subtype of influenza viruses type A belonging to family Orthomyxoviridae. Since the first isolation of H9N2 prototype strain A/turkey/ Wisconsin/1966 (Tu/WS/66) in 1966 in USA from turkey (Homme and Easterday, 1970), it was widely distributed among domestic and wild bird worldwide. The widespread and continuous circulation of H9N2 LPAI among different bird species cause persistent problems for the poultry industry (Alexander, 2000; Nili and Asasi, 2002), besides its ability to infect human beings causing threat to public health **(Butt et al. 2005 Li et al. 2003).**

Based on phylogenetic analysis, H9N2 was classified into two major genetic lineages; the North American and Eurasian lineages, the Eurasian lineage was further divided into 3 sub lineages; Korean like (A/chicken/ Korea/38349), Y280-like (A/ duck/Hong Kong/Y280/9), and G1-like (A/quail/ Hong Kong/G1/97) (**Guan et al. 2000),** the LPAIV H9N2 from 1998 to 2010 in Central Asia and the Middle East, were clustered into four distinct groups (A, B, C, and D) **(Fusaro et al. 2011).** The H9N2 LPAI genome is unstable and continuously mutates through antigenic drift in the HA gene arising H9N2 variants **(Peacock et al. 2018)** as well as the antigenic shift as The H9N2 viruses frequently donate their internal genes to other AIVs during the co -infection (**Hagag et al. 2019 Kandeil et al. 2017 Peacock et al. 2019).**

In Egypt, Since the first detection of H9N2 LPAI from quail in 2011 **(El-Zoghby et al. 2012),** Egypt has been endemic with H9N2 LPAIV causing severe economic losses in poultry production, the circulated virus belonged to Asian G1-like and closely related to Israel strains (**Monne et al. 2013).** Genetic variability was detected in amino acid levels in the surface genes indicated continuous evolution of H9N2 AIVs with complicated genetic reassortment in Egypt **(Elsayed et al. 2021).** In 2014, new antigenically distinct variant of H9N2 LPAIV was detected in quail (quail/2014 variant) **(Adel et al. 2017).** As well as a novel reassortament variant was reported in pigeon containing five internal gene segments (PB2, PB1, PA, NP, and NS) from wild bird like AIVs (Eurasian AIV) subtypes and (HA, NA, M) from Egyptian H9N2/2011 virus **(Kandeil et al. 2017).** The same genotype was found in backyard chickens in three Egyptian governorates in 2015 **(Samir et al. 2019).** Another reassortant virus has been evolved in 2014-2015 from the pigeon H9N2 virus with an Egyptian virus in late 2014, sharing PB2, PB1, PA, and NS genes **(Hassan et al. 2020).**

Furthermore, a novel reassortant H5N2 HPAI virus emerged in late 2017–2018, with 7 genes of the Egyptian LPAI H9N2 virus and only the HA gene from the Egyptian HPAI H5N8 virus **(Hagag et al. 2019; Hassan et al. 2020).**

Based on the phylogenetic analysis, the EgyptianH9N2 LPAIVs have been further diversified into three groups clustered within G1 -B lineage based on their HA gene segment **(Kandeil et al. 2014; Naguib et al. 2017).** So, this study aimed to molecular detection, isolation and Genetic characterization of H9N2 LPAI currently circulating in Egypt. The current study was aimed to monitor the LPAI H9N2 virus. The collected LPAIH9N2viruses samples during 2020-2022 in different governorate in Egypt.

MATERIALS and METHODS

Samples collection

Total of 496,166 Cloaca and oropharyngeal swabs were collected from 29319 cases from different poultry sectors (307 Household, 27762 Farm and 1250 LBM, for regular screening at the Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQP, Egypt) in 2020-2022. The samples have been collected during active and passive surveillance from 27 Egyptian governorates. Several poultry species were involved in the surveillance, including chicken (no. of cases: 25947), ducks (no. of cases: 1466), mixed flocks (no. of cases: 1150 turkey (no. of cases: 698), wild birds (no. of cases:5) , other species including geese, pigeon , quail, ostrich and environmental samples (no. of cases: 53)

Virus detection and isolation

For each collected sample, RNA was extracted from the supernatant liquid using QIAamp viral RNA Mini kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. Using specific primers and probes **(Adel et al. 2017; Adel et al. 2019; Shabat et al. 2010),** the RNA was examined against the Matrix (M) gene of influenza A viruses. Positive sample was further tested against AIV subtypes using real-time reverse transcriptase quantitative polymerase chain reaction RT-qPCR. Further, all samples were screened against Newcastle disease virus (NDV), infectious bronchitis virus (IBV) to explore the possibility for co-infection with other viruses. Reaction mixes were prepared using RT-qPCR Verso 1-step™ Real Time PCR kit (Catalog no.AB4101A) and performed using Stratagene MX3005real-time PCR machine.

For each positive sample 0.1ml of the supernatant fluid was injected into three separate specific pathogen free embryonated chicken eggs (SPF-ECE) of 9-11 days of age. The inoculated eggs were then incubated at 37 °C and monitored daily for 3–5 days. Allantoic fluid was retrieved from the collected or dead eggs and tested for virus haemagglutination activity by HA assay **(Manual, 2015).**

Nucleotide sequencing and phylogenetic analysis

The HA gene of selected positive samples were amplified using specific primers. The PCR was carried out using applied biosystem thermal cycler (ProFlexTM PCR System) using an Easyscript one-step RT-PCR kit (Trans Gen Biotech)). Size-specific PCR products for each gene were separated by gel electrophoresis and purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany).

Further, purified products were using for nucleotide sequencing using Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) and purified using Centrisep spin column, (Thermo Fisher,Waltham, MA, USA). Sequencing was performed using ABI PRISM® 3100 Genetic Analyzer (Life Technologies, USA). Further, the obtained nucleotide sequences were assembled and edited using Bio-edit programme version 7.2.5 **(Hall et al. 2011).** Generated sequences in this study were deposited at the GenBank database under accession numbers provided in **(Table 1).** A Blast search was performed using (http://www.ncbi.nlm.nih.gov/blast/) on the NCBI website

The nucleotide sequences were aligned using BioEdit version 7.0 **(Hall, 2004)** with other AIV strains representing different clades as well as vaccine strains against H5N1 used in Egypt, obtainedfrom the National Center for Biotechnology Information (NCBI). The Phylogenetic analyses were conducted out using MEGA 6 **(Tamura et al. 2013).** best models were the General Time Reversible (GTR) substitution with Gamma distribution (G) and estimate of proportion of invariable sites (I), a moderate strength neighbor-joining approach, and 1000 bootstrap repeats **(Kumar et al. 2016).** The pairwise nucleotide percent identity was calculated using BioEdit version 7.0 (Hall, 2004). Further, the N-linked glycosylation pattern on HA gene H9N2 AIVs were analyzed via by NetNGlyc 1.0Server http://www.cbs.dtu.dk/services/NetNGlyc/.

Table 1. Epidemiological data for the viruses of the study:

NO	Isolates ID	Governorates	TYPE	Date Of Collection	Accession No.
1	A/Egypt/chicken/FAO/S15/2020	Al Giza	market	Februer /2020	OR144575
2	A/Egypt/chicken/FAO/S54/2020	Al Giza	market	October /2020	OR144576
3	A/Egypt/chicken/FAO/S34/2020	Al Gharbia	market	Septamber/2020	OR144577
4	A/Egypt/chicken/V6/2020	Al Monofiya	farm	Junury/2020	OR144578
5	A/Egypt/Turky/V16/2020	Al Giza	farm	Junury /2020	OR144579
6	A/Egypt/chicken/S63/2020	Al Giza	farm	Septamber /2020	OR144580
7	A/Egypt/chicken/V71/2020	Al Monofiya	farm	Junury /2020	OR144581
8	A/Egypt/chicken/V78/2020	Al Qaliobia	farm	Junury /2020	OR144582
9	A/Egypt/chicken/VD44/2020	Al Gharbia	farm	Junury /2020	OR144583
10	A/Egypt/chicken/V424/2020	Al Giza	farm	Februer /2020	OR144584
11	A/Egypt/chicken/V905/2020	Al Qaliobia	farm	March/2020	OR144585
12	A/Egypt/chicken/V997/2020	Al Qaliobia	farm	March/2020	OR144586
13	A/Egypt/chicken/V1980/2020	Al Qaliobia	farm	June/2020	OR144587
14	A/Egypt/chicken/V2090/2020	Al Monofiya	farm	June/2020	OR144588
15	A/Egypt/chicken/V2687/2020	Al Minia	farm	Septamber //2020	OR144589
16	A/Egypt/chicken/V3049/2020	Asyut	farm	Septamber /2020	OR144590
17	A/Egypt/chicken/V3100/2020	Al Qaliobia	farm	Septamber /2020	OR144591
18	A/Egypt/chicken/V3162/2020	Al Monofiya	farm	Septamber /2020	OR144592
19	A/Egypt/chicken/V3416/2020	Asyut	farm	Septamber /2020	OR144593
20	A/Egypt/chicken/V3562/2020	Asyut	farm	October /2020	OR144594
21	A/Egypt/chicken/V3831/2020	Al Qaliobia	farm	October /2020	OR144595
22	A/Egypt/chicken/V4165/2020	Al Qaliobia	farm	November/2020	OR144596
23	A/Egypt/chicken/V4654/2020	Al Monofiya	farm	December/2020	OR144597
24	A/Egypt/chicken/ FAO/S1/2021	Al Qaliobia	market	Junury /2021	OR144598
25	A/Egypt/chicken/F1032/2021	Al Daqahlia	farm	October /2021	OR144599
26	A/Egypt/chicken/V3102/2021	Al Qaliobia	farm	Septamber /2021	OR144600
27	A/Egypt/chicken/FAO/S14/2021	Al Fayioum	market	November/2021	OR144601
28	A/Egypt/chicken/FAO/S64/2021	Al Qahera	market	October /2021	OR144602
29	A/Egypt/chicken/FAO/S68/2021	Al Giza	market	Septamber /2021	OR144603
30	A/Egypt/chicken/FAO/SG79/2021	Asyut	market	October /2021	OR144604
31	A/Egypt/chicken/FAO/S51/2021	Al Monofiya	market	Septamber /2021	OR144605
32	A/Egypt/chicken/F635/2022	Al Giza	farm	April /2022	OR144606
33	A/Egypt/chicken/VG109/2022	Domiatt	farm	Februer /2022	OR144607

RESULTS

Surveillance of H9N2-AIV in Egypt

The epidemiological analysis of the given data revealed that surveillance in the farm sector the most intense sector by 27762 cases, while the Live Bird Market and Household were 1250 and 307 respectively. Consequently, we have recorded a total of 503 positive cases of LPAI H9N2 outbreak during 2020-2022 with total prevalence rate 1.7%. However the highest LPAI H9N2 prevalence rate were recorded in LBM (10.6%), followed by household sector and farm (2 % and 1.3% respectively), (fig.1-A). among the examined species, mixed flocks had the highest prevalence (11.5 %) (fig.1-B).we observed remarkable increase in H9N2 relatively mild seasonality, with larger number of positive samples during winter and spring from January to mid-May, Positivity declined gradually during the summer and autumn in June to October (fig.1-C).

Spatially, the surveillance of this study included some of the Egyptian governorates. As shown in (Fig.1-D). LPAI H9N2 was showed high Geo-prevalence (85.1%) during 2020-2022. The disease was recorded in 23 governorates (Al Monofiya, Al Giza, Al Behera, Al Daqahlia, Al Gharbia, Benisueif, Al Fayioum, Al Minia, Al Qaliobia, Al WadiAlgidid , Port said, Qena, Ismaliya, Cairo, Asuit, Al Sharqia, Alexandria, Aswan, Damietta, KafrAlsheikh, South Sinai, Luxor and Mersa Matrouh) during 2020-2022. A high density of suspected and positive cases, with the highest incidence during the surveillance period was recorded in Asyuit (17%) followed by Al Fayioum (7.2%) Giza (5%), Al Monofyia (2.5%) finally Al Qalioubia (2.4%).

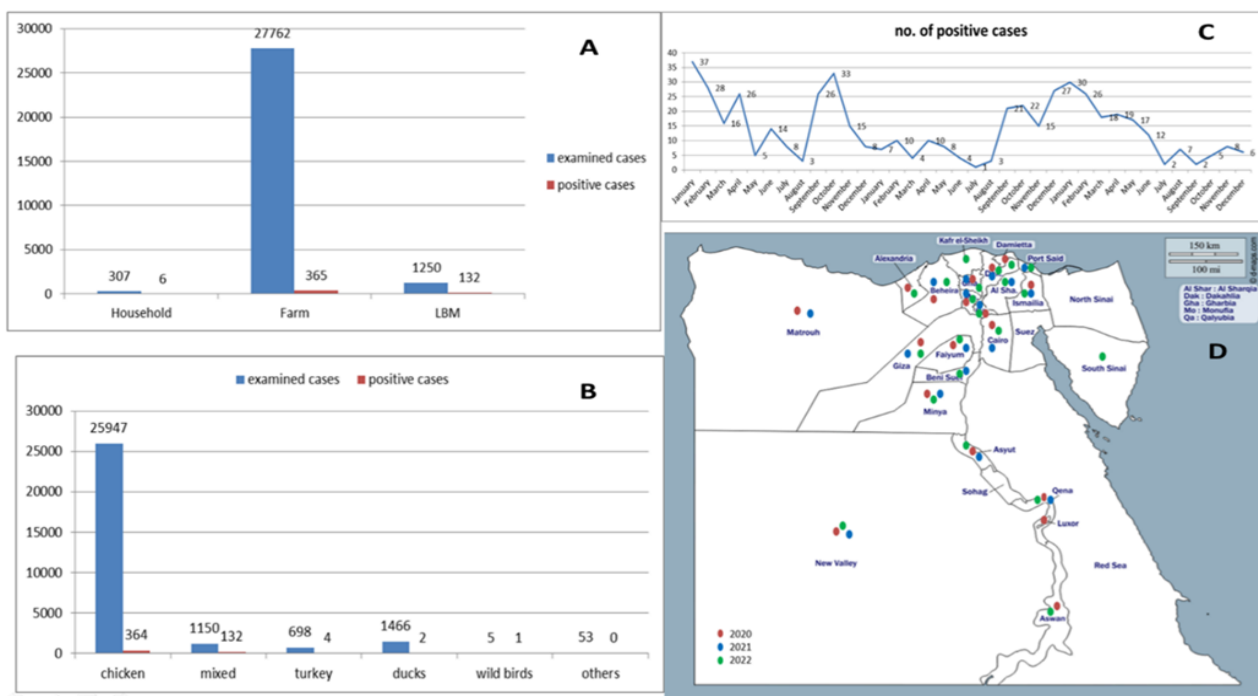

Fig. 1. Showing (A): the number of examined and positive cases of LPAI H9N2 outbreak during 2020- 2022, the highest LPAI H9N2 prevalence rate were recorded in LBM followed by household sector and farm. (B): Showing the prevalence of LPAI with in different species flocks. (C)The distribution of the H9N2 in different seasons during 2020–2022 collectively. Winter and spring record the highest incidences. (D): showing Map of Egypt illustrates the geographic distribution of positive cases.

HA gene Phylogenetic analysis

Phylogenetic analysis showed that HA gene of all studied AIV H9N2 grouped in the G1 sub-lineage of the Eurasian lineage. The highest phylogenetic relationship and nucleotide identity were shown with the prototype A/quail/Hong Kong/G1/97 rather than other AIV H9N2prototypes such as A/Duck/Hong Kong/Y280/97 and A/Chicken/Hong Kong/G9/1997. All Egyptian strains were also related to each other (nucleotides identity ranging from 99.98 to 100 % for the HA and grouped together in the phylogenic tree (Fig. 2). Our strains were also monophyletic with recently published Egyptian AIV H9N2 sequences published during 2021 with high nucleotide sequence identities 99% .All sequenced virus strains were 95 –99% identical to the nucleotide sequence of A/pigeon/Egypt/S10408B/2014 (accession No. KX000876).

Phylogenetic relatedness was determined for all sequenced strains. Phylogenetic analysis of the HA gene revealed that the Egyptian LPAI H9N2 virus was divided into many groups in the G1-B sub-lineage: EGY-1a-c (2011-like) and EGY-2 (pigeon-like). Since 2015, the EGY-2 strain has been the most prevalent strain, and no more EGY-1 strains have been reported after 2016. The HA gene was shown to be associated with the EGY-2 (pigeon-like) gene (Fig. 2). These findings indicate that the EGY-2 (pigeon-like) genotype has been the most prevalent LPAI H9N2 genotype in Egypt since 2016.

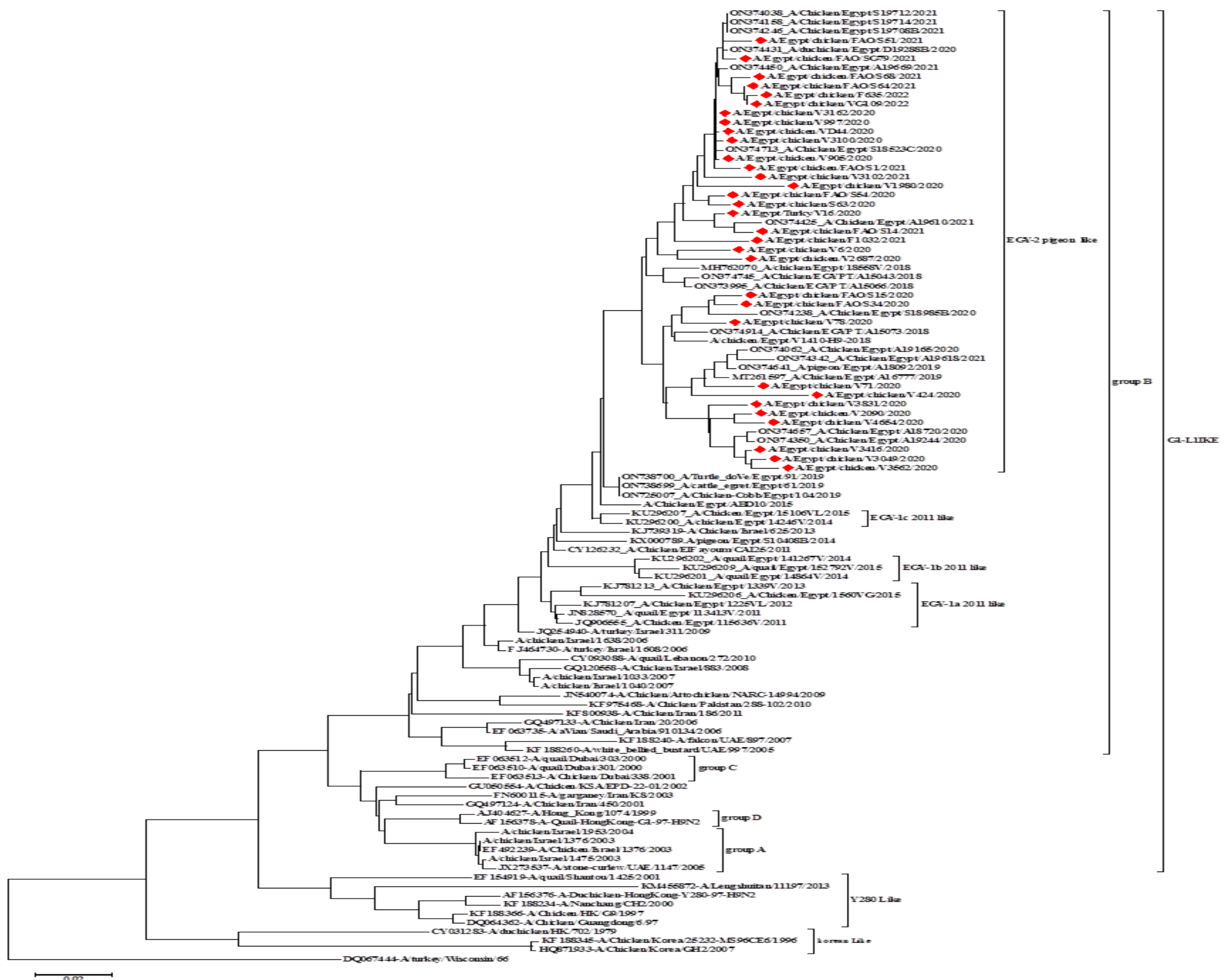

Figure (2): Phylogenetic tree of the HA gene of avian influenza subtype H9N2 viruses isolated in Egypt during 2020–2022 and reference isolates from Gen Bank. Phylogenetic analysis was conducted by using the neighbor-joining algorithm with the Kimura 2-parameter model. Strain A/Turkey/Wisconsin/1/1966 was used as the root for the tree and the reliability of Phylogenetic tree inference at each node was estimated by the bootstrap method with 1,000 replications. Evolutionary analysis was conducted by using MEGA6.A red rhomboid indicates isolates sequenced specifically for this study.

Genetic and Phylogenetic analysis of influenza A H9N2 viruses

The existence of multiple amino acid changes at the antigenic epitope sites of the HA has been identified by genetic investigation of several antigenic sites in the HA gene of H9N2 viruses. According to genetic analysis of the HA cleavage motif, all strains under investigation owned RSSR*GLF (Table 2). Several receptor binding sites (RBS) of the HA of H9N2 viruses were examined at positions 166, 191, 197, 198, 232, 234, 235, and 236 (H9 numbering), which are determining variables related with the virus's capacity to attach to mammalian host cellular receptors. In summary, all H9N2 isolates included N166, as previously discovered in A/quail/Hong Kong/G1/97 (Table 1). In contrast, all analyzed viruses had H, T, N, L, I, and G at positions 191, 197, 232, 234, 235, and 236 (H9 numbering), respectively. Moreover, (A) was detected at position 198 (H9 numbering) in all chicken and turkey isolates, except for A/Egypt/chicken/V6/2020 and A/Egypt/chicken/FAO/S14/2021were carried (T) at position 198 (H9 numbering). The isolates from chickens (A/Egypt/chicken/FAO/S15/2020 and A/Egypt/chicken/FAO/S54/2020) show E198 (H9 numbering) was previously observed in A/quail/Hong Kong/G1/97 (H9N2). All analyzed H9N2 viruses had H191and L234 (H9 numbering).

The Egyptian H9N2 viruses have five N-linked glycosylation sites at positions 29, 141, 298, 305, and 492 (H9 numbering), according to genetic study of HA sequences. However, viruses A/chicken/Egypt/V1980/2020, and A/chicken/Egypt/V3831/2020, and A/chicken/Egypt/V4654/2020 had six glycosylation sites by additional sit at position 145. There were eight glycosylation sites in A/Egypt/chicken/V424/2020, by additional sit located at 105, 145, and 258. Compared to G1-like viruses, all isolates lacked the glycosylation sites at positions 206 and 218.

DISCUSSION

Continuous surveillance of LPAI H9N2 in domestic poultry is required since it adds another risk factor to Egypt's poultry industry, which is already under pressure. Because LPAI H9N2 is an endemic disease in Egypt, monitoring its presence in the country is critical. Since its introduction in 2011 **(El-Zoghby et al. 2012)**, because of the pattern of its genetic evolution. Annual surveillance is done routinely in RLQP. Commercial farms and live bird market (LBM) are the primary population focus for surveillance because they represent the main sector for poultry production in Egypt, the highest LPAI H9N2 prevalence rate were recorded in LBM (9.5%), followed by household sector and farm, this verified that live bird markets in the continue to be high risk locations for AIVs due to the existence of several AIV subtypes specially H9 and H5 in poultry species from different breeding sectors in Egypt. This mixing permits transmission of the disease from infected areas to non-infected ones **(Helal et al. 2017).** Thus, control actions towards AIVs should include live bird markets as a critical threat source of the disease transmission.

The highest incidence of LPAI H9N2 was recorded in chickens, followed by ducks, turkeys, then quails, as reported in previous studies**(Adel et al. 2019; Kandeil et al. 2019).** LPAI H9N2 has different patterns of spread throughout it Furthermore, due to the cocirculation of other influenza subtypes in Egypt, H9N2 AIV poses a danger of human transmission **(El-Sayed et al. 2021).** LPAI H9N2 has different patterns of spread throughout the year **(Abdelwhab and Abdel-Moneim, 2015; Arafa et al. 2012).** From 2020 to 2022, H9N2 showed higher incidence in winter and spring than in summer and autumn, as shown in (Fig. 1-c). This finding accords with the most recent study on H9N2 in Egypt **(Adel et al. 2021),** and supports the theory that the distribution of the virus is positively correlated with colder weather **(Park and Glass, 2007)** Benisueif , Al Fayioum, Cairo, South Sinai and finally Al Giza possessed the highest incidence rates, and these governates had high densities for positive cases as shown in (Fig. 1-d). While other governorates had a low density of suspected cases among poultry, with low incidence. The geographical data confirm the theory that the Delta governorates and the neighbouring governorates (Giza and Fayioum) are crucial in the spread of the virus across the

nation **(Adel et al. 2021).** Based on the HA gene, Egyptian H9N2 viruses have genetic ties to the G1- subgroup B lineage (Fusaro et al., 2011), Egypt's LPAI H9N2 viruses are constantly changing **(Adel et al. 2017; El-Sayed et al. 2021; Kandeil et al. 2019).**

Previous research divided Egyptian H9N2 AIVs into two subgroups based on phylogenetic study of the HA gene **(Afifi et al. 2013 Kandeil et al. 2014);** nevertheless, the Egyptian viruses were classified as one major group with many minor subgroups, A through E for the HA gene **(Elsayed et al. 2021)** The most recent Israeli viruses shared a close relationship with the Egyptian viruses of the new cluster in 2017 –2020. In light of this discovery, the most recent research demonstrates evidence for bilateral diffusion between Egypt and Israel. The LPAI H9N2 virus was first spread to Egypt from Israel in mid-2009 through migrating birds, and the converse happened in 2015 when the Egyptian viruses were spread to Israel **(Li et al. 2020).**

The viruses in this investigation have HA sequences that were genetically related to the most recent viruses in Egypt that were circulating (Egy-2 Pigeon like) **(Elsayed et al. 2021; Kandeil et al. 2019).** The motif of HA cleavage site of the analyzed four isolates was found to be $^{(335}$RSSR↓GLF$^{341)}$. This motif is a characteristic of LPAIVs due to absence of multibasic amino acids, however, it continues to be a key pathogenic hallmark for these viruses. Concerns regarding the effects of substitutions in the cleavage sites of LPAI H9N2 were raised in a recent study as several H9Nx viruses acquired tri- and dibasic amino acids that enabled trypsin-independent replication in chicken egg embryos **(Parvin et al. 2020)**

The viruses that were examined share (L) with the majority of Egyptian H9N2 AIVs and HK/G1/97 strains. H9N2 AIVs may pose a risk to the public health if Q234 at the left edge of the RBS is changed to L234, as this could alter the host specificity from the avian type sialic receptors to the human type. **(Peiris et al. 1999).**

The addition of HA glycosylation sites may play a role in the adaptation of avian influenza viruses to certain hosts via its influence on host cell receptors, concealing antigenic epitopes that aid the virus in evading the host immune response, and altering the virus pathogenicity site of H9 HA **(Escorcia et al. 2010)** .H9N2 viruses in this study have five sites of glycosylation. In addition, isolates A/Egypt/chicken/V424/2020, A/Egypt/chicken/V1980/2020, A/Egypt/chicken/V3831/2020, A/Egypt/chicken/V4654/2020 have glycosylation site at position 145 like that found in A/qu/Egypt/141267V/2014.Isolate A/Egypt/chicken/V424/2020 has eight glycosylation sites as it has two additional sites (NGT) at positions 105 and 258 like that found inA/quail/Hong Kong/G1/97. Presence of glycosylation sites close to the RBS of the HAs may affect receptor binding and increases host range variability **(Lin et al. 2004)**

CONCLUSION

The incidence of H9N2 infections in Egypt's backyards and poultry farms suggested that the disease is pervasive throughout the nation. To reduce the risk factors for the poultry business and the potential for new pandemic variants to emerge, it is necessary to support effective efforts to manage H9N2 AIV in poultry in Egypt. LPAI H9N2 viruses are constantly changing, the HA gene alterations may play a significant effect in changing the natural features of H9N2 viruses. Further antigenic and replication studies are strongly advised to assess the impact of the newly developed viruses' acquired mutations.

		Receptor binding sites								Cleavage site		Glycosylation sites								
		166	191	197	198	232	234	235	236		29	105	141	145	206	218	258	298	305	492
1	A/quail/Hong Kong/G1/97	S	H	T	E	N	L	Q	G	RSSR/GLF	NSTE	NGTC	NVTY	NGTS	NDTT	NRTF	NGTS	NSTL	NISK	NGTY
2	A/duck/Hong Kong/Y280/97	N	N	-	T	-	-	-	-	RSSR/GLF	----	xxxx	----	xxxx	xxxx	----	xxxx	----	----	----
3	A/turkey/Wisconsin/1/1966	-	-	S	-	-	Q	-	-	VSSR/GLF	----	xxxx	----	xxxx	xxxx	----	xxxx	----	----	----
	A/quail/Egypt/14864V/2014	N	-	-	A	-	-	I	-	RSSR/GLF	----	xxxx	----	xxxx	NTTT	xxxx	xxxx	----	----	----
5	A/quail/Egypt/113413v/2011	N	-	-	A	-	-	I	-	RSSR/GLF	----	----	----	xxxx	xxxx	xxxx	xxxx	----	----	----
6	A/Egypt/chicken/FAO/S15/2020	-	-	-	-	-	-	-	-	RSSR/GLF	----	xxxx	----	xxxx	xxxx	xxxx	xxxx	----	----	----
7	A/Egypt/chicken/FAO/S54/2020	-	-	-	-	-	-	-	-	RSSR/GLF	----	xxxx	----	xxxx	xxxx	xxxx	xxxx	----	----	----
8	A/Egypt/chicken/FAO/S34/2020	N	-	-	A	-	-	I	-	RSSR/GLF	----	xxxx	----	xxxx	xxxx	xxxx	xxxx	----	----	----
9	A/Egypt/chicken/V6/2020	N	-	-	T	-	-	I	-	RSSR/GLF	----	xxxx	----	xxxx	xxxx	xxxx	xxxx	----	----	----
10	A/Egypt/Turky/V16/2020	N	-	-	A	-	-	I	-	RSSR/GLF	----	xxxx	----	xxxx	xxxx	xxxx	xxxx	----	----	----
11	A/Egypt/chicken/S63/2020	N	-	-	A	-	-	I	-	RSSR/GLF	----	xxxx	----	xxxx	xxxx	xxxx	xxxx	----	----	----
12	A/Egypt/chicken/V71/2020	N	-	-	A	-	-	I	-	RSSR/GLF	----	xxxx	----	xxxx	xxxx	xxxx	xxxx	----	----	----
13	A/Egypt/chicken/V78/2020	N	-	-	A	-	-	I	-	RSSR/GLF	----	xxxx	----	xxxx	xxxx	xxxx	xxxx	----	----	----
14	A/Egypt/chicken/VD44/2020	N	-	-	A	-	-	I	-	RSSR/GLF	----	xxxx	----	xxxx	xxxx	xxxx	xxxx	----	----	----
15	A/Egypt/chicken/V424/2020	N	-	-	A	-	-	I	-	RSSR/GLF	----	----	----	----	xxxx	xxxx	----	----	----	----
16	A/Egypt/chicken/V905/2020	N	-	-	A	-	-	I	-	RSSR/GLF	----	xxxx	----	xxxx	xxxx	xxxx	xxxx	----	----	----
17	A/Egypt/chicken/V997/2020	N	-	-	A	-	-	I	-	RSSR/GLF	----	xxxx	----	xxxx	xxxx	xxxx	xxxx	----	----	----
18	A/Egypt/chicken/V1980/2020	N	-	-	A	-	-	I	-	RSSR/GLF	----	xxxx	----	----	xxxx	xxxx	xxxx	----	----	----
19	A/Egypt/chicken/V2090/2020	N	-	-	A	-	-	I	-	RSSR/GLF	----	xxxx	----	xxxx	xxxx	xxxx	xxxx	----	----	----

20	A/Egypt/chicken/ V2687/2020	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
21	A/Egypt/chicken/ V3049/2020	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
22	A/Egypt/chicken/ V3100/2020	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
23	A/Egypt/chicken/ V3162/2020	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
24	A/Egypt/chicken/ V3416/2020	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
25	A/Egypt/chicken/ V3562/2020	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
26	A/Egypt/chicken/ V3831/2020	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	--- -	xx xx	xx xx	xx xx	--- -	-- --	--- -
27	A/Egypt/chicken/ V4165/2020	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
28	A/Egypt/chicken/ V4654/2020	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	--- -	xx xx	xx xx	xx xx	--- -	-- --	--- -
29	A/Egypt/chicken/ FAO/ S1/2021	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
30	A/Egypt/chicken/ F1032/2021	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
31	A/Egypt/chicken/ V3102/2021	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
32	A/Egypt/chicken/FAO/ S14/2021	N	-	-	T	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
33	A/Egypt/chicken/FAO/ S64/2021	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
34	A/Egypt/chicken/FAO/ S68/2021	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
35	A/Egypt/chicken/FAO/ SG79/2021	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
36	A/Egypt/chicken/FAO/ S51/2021	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
37	A/Egypt/chicken/ F635/2022	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -
38	A/Egypt/chicken/ VG109/2022	N	-	-	A	-	-	I	-	RSS R/ GLF	--- -	xx xx	--- -	xx xx	xx xx	xx xx	xx xx	--- -	-- --	--- -

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