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**RESEARCH ARTICLE**

# Isolation and Identification of The Highly Pathogenic Avian Influenza H5N8 Virus from Commercial Layer Chickens in Sharkia Province, Egypt During 2019-2021

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## ABSTRACT

This study aimed to characterize Egyptian H5N8 viruses that were recovered from 10 out of 15 vaccinated commercial layer flocks in Sharqia province that located in the eastern region of Delta, Egypt during 2019 and 2021. The selected flocks were reared in multitiered cage systems, suffered from respiratory problems, a sudden, sharp drop in egg production, and a fatality rate ranging from 15% to 50%. Qualitative reverse transcriptase-polymerase chain reaction (RT–qPCR) and virus isolation from embryonated chicken eggs (ECEs) for first- and second-blind passages, avian influenza virus (AIV) testing was used for this purpose. For isolates that passed the hemagglutination test, the hemagglutinin (HA) and neuraminidase (NA) genes were partially amplified using qualitative PCR. Five isolates that were representative of five multilayer flocks were then subjected to sequencing and phylogenetic analysis. Sequencing and phylogenetic analysis of the HA and NA genomes of five flocks revealed the AIV/HPAI H5N8 virus in clade 2.3.4.4 that resemble the highly pathogenic avian influenza (HPAI) virus subtype H5N8 clade 2.3.4.4 reported in Egypt in migratory birds in late 2016. The four HAs and NAs shared 99%. 99.8% and 98.1% of their nucleotide sequences with H5N8 viruses that were previously discovered in China, Iran, and Iraq, respectively. Furthermore, phylogenetic analyses focusing on the HA and NA genes revealed that Egyptian H5N8 viruses belonged to group B, with Russian-like reassorted H5N8 viruses in clade 2.3.4.4. In conclusion, birds that have received immunizations are at serious risk from HPAI H5N8 if these birds don't receive inactivated vaccine related to H5N8 clade 2.3.4.4. Detection of the HPAI H5N8 virus in domestic birds is therefore required for yearly surveillance, sporadic molecular monitoring, and evaluation of the effectiveness of immunization.

***Keywords:*** HPAI H5N8, Layer chicken, Phylogenetic analysis, RT–qPCR, Sequencing.

## Introduction

In the complex landscape of viral pathogens, the influenza A virus, nested within the Alpha influenza virus genus of the Orthomyxoviridae family, stands out as a potent trigger for pandemic influenza [1]. Its genome is composed of eight segments of negative-sense single-strand RNA and intricately encodes proteins pivotal to its pathogenicity. Among these, hemagglutinin (HA) and neuraminidase (NA), surface glycoproteins, play critical roles in this process, resulting in the diversity of 16 HA and 9 NA subtypes in avian species, with additional subtypes H17N10 and H18N11 identified in bats [2].

The stratification of avian influenza viruses (AIVs) into highly pathogenic (HPAI) and low pathogenic (LPAI) forms

is based on the structure of the HA gene and the ability of the virus to induce clinical symptoms [3]. Among the notable subtypes, the HPAI H5N8 virus first emerged in China, where in 2010, domestic ducks and chickens played a significant role in the clade 2.3.4.4 outbreaks [4]. The subsequent spread to South Korea in 2014 marked the beginning of its global journey, when it reached East Asia, North America, and Europe by the end of 2014 [5]. The ensuing years witnessed the evolution of reassorted H5N8 viruses of the clade 2.3.4.4 subtype, which were initially detected in wild birds in Russia during 2016–2017. Migratory birds became vectors, facilitating the spread of the virus across diverse regions in Europe, Asia, and Africa [6].

Wild birds, often asymptomatic carriers of various AIV subtypes, play a critical role in the global transmission of avian influenza to domesticated birds [7]. The Mediterranean and East African migration flyways were identified as potential routes for the introduction of the HPAI H5N8 clade 2.3.4.4b into Egyptian poultry [8]. Evidence of HPAI H5N8 isolates from clade 2.3.4.4 in Egypt dates to November 29, 2016, with isolates originating from Europe and Asia exhibiting different internal gene rearrangements. These introductions have led to substantial economic losses within the Egyptian poultry production industry [9].

Given the persistent threat posed by HPAI H5N8, there is an urgent need for vaccination programs to mitigate further financial losses in the Egyptian poultry industry. The virus manifests in layer chickens through respiratory symptoms, severe egg drops, lethargy, eyelid edema, and comb cyanosis, ultimately culminating in mortality [10]. In response to the significant deaths associated with respiratory symptoms and erratic egg laying, this study specifically focused on the governorate of Sharqia in Egypt. Tissue samples were systematically collected from vaccinated layer chicken farms in this region over the period spanning 2019 to 2021, these flocks had received vaccinations with inactivated H5N2 with clade 2.2.1 and recombinant H5 on baculovirus with clade 2.3.2.

This investigation adopted a comprehensive approach involving molecular detection, virus isolation, and partial sequencing of the HA and NA genes of HPAI H5N8 viruses using tissue specimens. The primary aim is to discern and characterize the circulating strains of HPAI H5N8 in the specified region during the defined timeframe. This meticulous analysis endeavors to unravel the evolutionary dynamics, transmission patterns, and potential factors influencing the prevalence of this virus. By shedding light on these intricate aspects, the study aspires to provide nuanced insights for the development of robust control and prevention strategies against HPAI H5N8, safeguarding not only avian populations but also mitigating potential risks to human health.

## Materials and Methods

The current study adhered to ethical guidelines and received an approval [ZU-IACUC/2/F/70/2024] from Zagazig University Institutional Animal Care and Use Committee [ZU-IACUC], Egypt, ensuring the humane treatment of animals involved in the research.

### *The farms' locations and descriptions*

The research was carried out in privately owned commercial laying hen facilities situated in the eastern region of

Delta, Egypt, specifically in Al-Sharkia province. The facility is oriented in the north–south direction, with an average altitude above sea level of approximately 132 m. The dimensions of the farms vary, with heights ranging from 2.9 to 3.2 m, lengths ranging from 85 to 120 m, and widths ranging from 12 to 16 m. Constructed with reinforced concrete foundations, the house floor is covered with a 15 cm thick layer of concrete. The poultry house features plastered walls and a ceiling made of double sandwich panels with thermal insulation foam. To streamline management tasks such as feeding, drinking, and egg collection, proper equipment arrangement is paramount. Laying hens are typically provided with feed through a mechanical automatic feeder system. Access to water is facilitated by adjustable nipple drinkers, an automatic feeding bunker, and a microclimate controller for environmental control, ensuring optimal conditions for the laying hens [11].

### *Laying hens and cage systems*

The farms housed a total of 25,000 to 50,000 Hy-line W-80 white chickens, primarily utilized for egg production. At the commencement of the study, these birds were 280 days old and had an average weight of 1.760 kilograms. In poultry layer farming for commercial egg production, the widespread use of cages is due to their efficiency and effective egg management. Typically, constructed from metal, these cages provide a controlled environment for each bird. In this specific study, the cages were organized in a configuration of 6 rows and 3 tiers, optimizing the utilization of available space. Such multitiered systems efficiently utilize the vertical space within poultry houses. Each tier was equipped with an egg collection belt and a feeding chain row located in front of the cages, complemented by a dropping belt beneath them. Continuous monitoring of air temperature (°C), relative humidity (RH %), and air velocity (AV m/s) was systematically conducted within each laying hen facility [12].

### *Sampling*

Sampling procedures targeted ten-layer chicken flocks that had received vaccinations with inactivated H5N2 and recombinant H5 on baculovirus. The sampling period spanned from November 2019 to March 2021, during which the laboratory received trachea, liver, spleen, and lung specimens from recently deceased layer chickens. These specimens were meticulously preserved on ice and subsequently stored at -80°C until further analysis (Figure 1).

### *Isolation of the virus from the collected specimens*

For the isolation of the virus from the collected specimens, organ specimens from each of the seven unique flocks were pooled. The supernatants of the prepared specimens (0.2 ml) were injected into the allantoic fluid of 10-day-old embryonated chicken eggs (ECEs) from commercial non-vaccinated chickens in a class II biosafety cabinet for the first and second passes. With daily observations revealing characteristic changes, the control embryos developed properly (Figure 2A), while the embryos exhibiting hemorrhages and congestion and died within 48 hours, confirming the presence of the virus (Figure 2B). Allantoic fluids were then tested for viral hemagglutination activity using the HA gene, following the protocol outlined by the World Organization for Animal Health [13]. Subsequently, the collected allantoic

fluid was stored at -70°C until further investigation.

***Molecular detection.***

Molecular detection was carried out through qualitative reverse transcriptase polymerase chain reaction (RT–qPCR). Total RNA extraction was performed using the GeneJET Viral RNA Purification Kit (Thermo Fisher Scientific, Inc., USA). To produce cDNA, reverse transcription of RNA from each specimen was performed with a QuantiTect Reverse Transcription Kit (Qiagen, Germany). Conventional polymerase chain reaction (qPCR) was used to target the influenza A virus HA and NA genes, and specific primer sequences were used. The following primer sequences were used for the NA genes: forward, 5'-ATGGAGAAAATAGTGCTT-3'; reverse, 5'-TTTTCTGGAGCAATGAAGTTTCC-3'. The forward primer 5'-AGCAAAAGCAGGAGTTTA-3' and the reverse primer 5'-TTTGCCTTGATTTGCTTTGTAT-3' were used for the amplification of 541 bp from the hemagglutinin (HA) gene and 754 bp from the neuraminidase (NA) gene. Amplification was achieved using Dream TaqTM Green PCR Master Mix (2X) from Fermentas, Glen Burnie, MD. The amplified fragments were separated on 1.5% agarose gels using a 1-kbp DNA marker (Fermentas, Glen Burnie, MD) as a standard, visualized with an ultraviolet light transilluminator (Spectroline) (Figure 3), and refined using the QIAquick Gel Extraction Kit (Qiagen, Germany) as directed by the manufacturer before storage at -20°C until sequencing. Negative control groups were included to monitor potential contamination.

***Sequencing and phylogenetic analysis***

Sequencing and phylogenetic analysis were subsequently conducted on the purified and amplified HA and NA segments of the four selected isolates. The BigDye Terminator v3.1 cycle sequencing kit (Perkin-Elmer, CA) facilitated sequencing in both directions using amplification primers from the Applied Biosystems 3130 genetic analyzer (ABI, USA), and the resulting sequences were subjected to a BLAST search (http://www.ncbi.nlm.nih.gov/BLAST). Amino acid sequence identities and divergences were determined using MegAlign software (DNA STAR® Laser gene® version 7.2, USA). The phylogenetic tree was constructed with the maximum-likelihood method with the Kimura 2-parameter model in MEGA 6 software [14], and 1000 bootstrap iterations were performed using the ClustalW alignment algorithm. Comparative analysis involved matching Egypt's publicly available H5 vaccine sequences to the HA and NA nucleotide sequences of H5N8 viruses developed during this study, alongside H5N8 viruses from Asia, the Middle East, and Europe spanning from 2011 to 2022 (Figure 4). This extensive methodology ensures rigorous and comprehensive exploration of the isolated HPAI H5N8 virus strains, offering valuable insights into their genetic characteristics and evolutionary relationships.

**Results**

***Clinical Findings and Gross Lesions***

The observed clinical manifestations in the studied layer chickens infected with HPAI H5N8 were diverse and included lethargy, fragility, and substantial mortality rates ranging from 50% to 100%. Clinical signs included dark red to blue depressed areas of ischemic necrosis on the comb and wattle, nasal discharge,

dyspnea, coughing, sneezing, greenish diarrhea, ataxia, cyanosis, sharp egg drop, hyperemia, and hemorrhage on the shanks and feet (Figure 1A). Gross abnormalities in the affected flocks were characterized by generalized congestion in vital organs such as the lungs, heart, liver, pancreas, intestines, spleen, kidneys, and brain. The enlarged, swollen liver exhibited a multifocal white mottling pattern accompanied by petechial and ecchymotic hemorrhages in the pancreas and gut. Infected hens also displayed enlarged hemorrhagic ovarian follicles, indicative of the severity of the infection (Figure 1B &C).

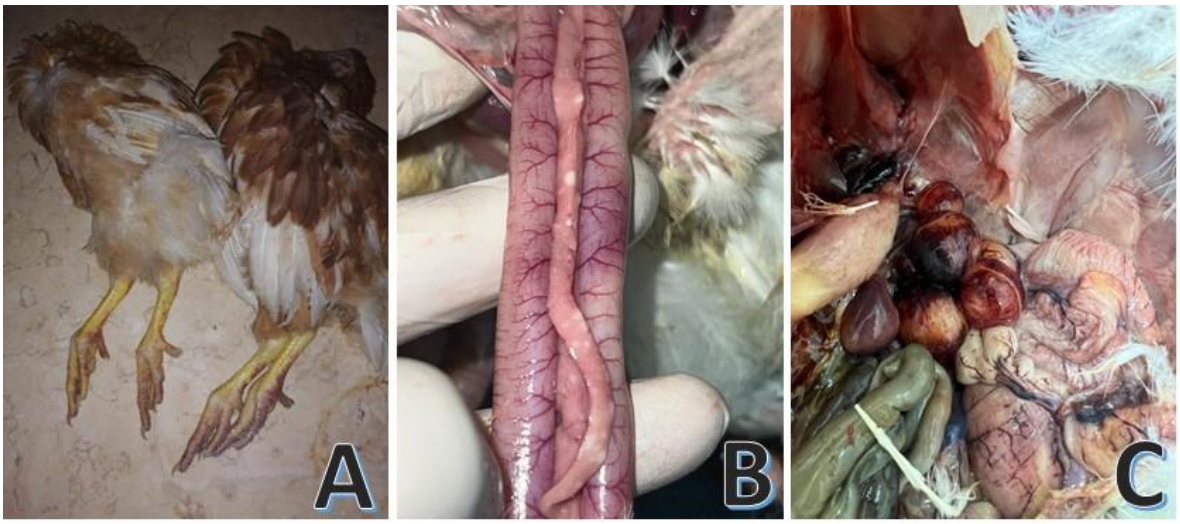


**Figure 1.** (1A) Clinical signs of HPAIV H5N8 infected chickens showed (1) dark red to blue depressed areas of ischemic necrosis of comb and wattle of infected chicken, (2) hyperemic and hemorrhagic shank and feet of infected layer chicken, and (3) cyanosis and congestion of the comb and wattle of chicken. (1B) Post-mortem changes of HPAIV H5N8 infected chickens showed petechial and ecchymotic hemorrhage in pancreas. (C) Post-mortem changes of HPAIV H5N8 infected chickens showed enlarged hemorrhagic ovarian follicles.

***Positive AIV Flock Distribution***

In conjunction with the distinct fluency bands detected by RT–qPCR, the geographical distribution of positive AIV flocks revealed three positive chicken flocks in El Salhaya and Abo Kabeir and two positive layer flocks in Minya Al Qamh.

Upon sample injection into the allantoic fluid of 10-day-old ECEs, the control negative embryo exhibited normal development (Figure 2A). However, figure 2B shows that embryos that died within 48 hours displayed hemorrhages and congestion, in stark contrast to the normal development of the control negative embryo.


Figure 2. Embryonated chicken eggs (10 days age) after 48 hours of injection (A) the control negative embryo showed normal development. (B) the inoculated Egyptian H5N8 virus with hemorrhages and congestion after second blind passage

### *AIV HA and NA Gene Sequencing and Phylogenetic Analysis*

Out of ten immunized flocks from different districts, five tested positives for HPAI/A/H5N8 according to quantitative PCR to identify the HA and NA genes (Figure 3). The virus was successfully recovered in embryonated chicken eggs (ECEs) through allantoic sacs. Sequencing and phylogenetic analysis of the amplified NA and HA segments revealed that the isolates (A/H5/layer/Sharqia/2021) belonged to clade 2.3.4.4b, with no significant genetic differences observed when compared to Egyptian isolates from 2017 to 2021. The isolates clustered closely with Russian-like reassorted H5N8 viruses of clade 2.3.4.4, extending their association to isolates from Russia, Iran, and Iraq, including both domestic and wild birds.

Phylogenetic analysis of (A) the partial NA gene and (B) the complete Egyptian H5N8 virus in comparison to other viruses in clade B, aligning them with viruses isolated from wild birds in Iraq, Nigeria, Russia, and India. Notably, the HA gene-based phylogenetic tree highlighted the presence of the specific amino acid motif "PLREKRRKR/GLF" at HA cleavage sites characteristic of the HPAIV H5N8 clade 2.3.4.4b. The examined isolates exhibited a high degree of similarity (97.8% to 98.2%) with other Egyptian isolates (2017–2021) and were part of a broader cluster comprising recent H5N8 strains from Asia, Europe, and Africa. The nucleotide identity percentage displayed a relatively constrained range between the field isolates and local vaccine strains, ranging from 88.7% to 92.4% (Figure 4).

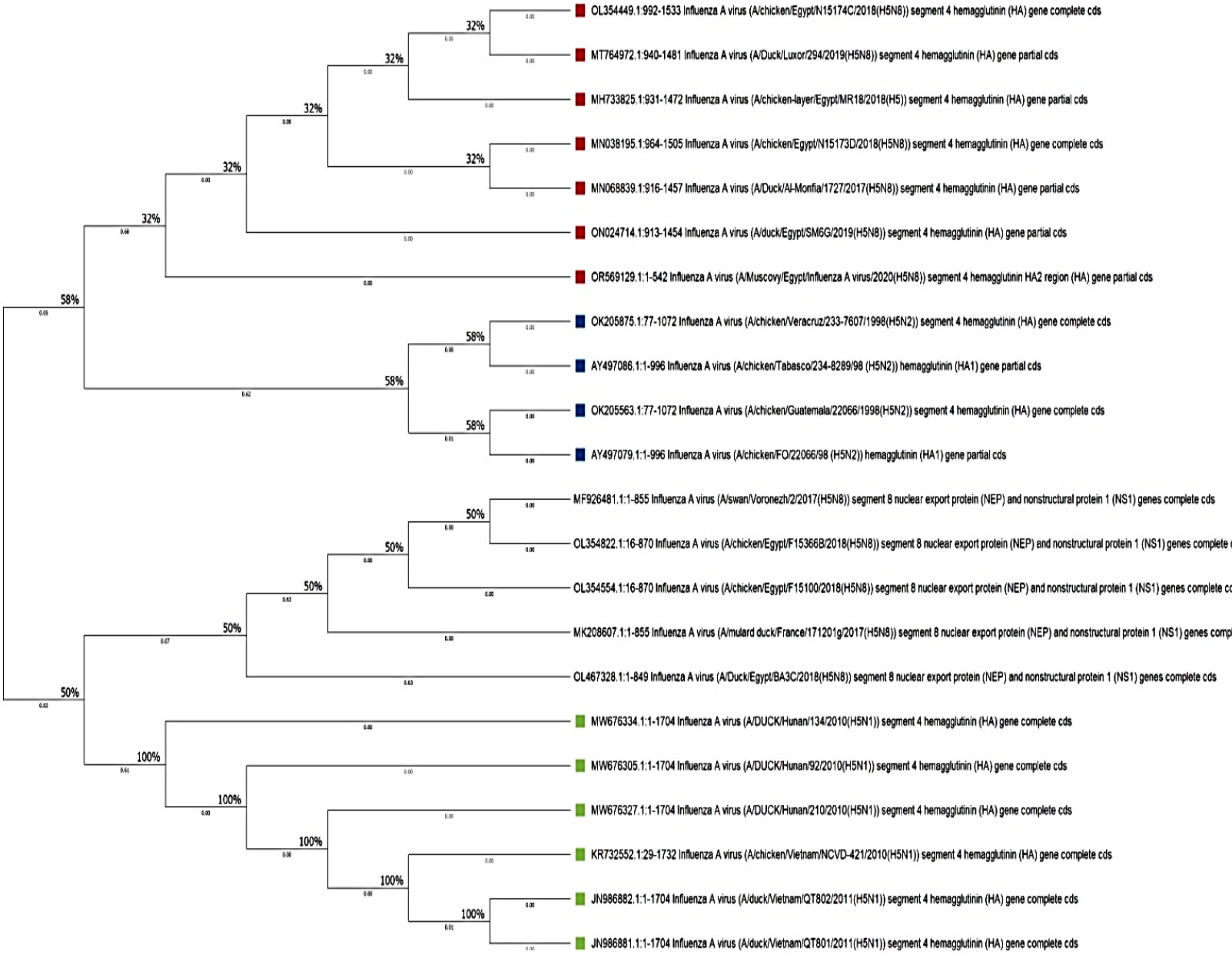
OL354449.1:992-1533 Influenza A virus (A/chicken/Egypt/N15174C/2018(H5N8)) segment 4 hemagglutinin (HA) gene complete cds
MT764972.1:940-1481 Influenza A virus (A/Duck/Luxor/294/2019(H5N8)) segment 4 hemagglutinin (HA) gene partial cds
MH733825.1:931-1472 Influenza A virus (A/chicken-layer/Egypt/MR18/2018(H5)) segment 4 hemagglutinin (HA) gene partial cds
MN038195.1:964-1505 Influenza A virus (A/chicken/Egypt/N15173D/2018(H5N8)) segment 4 hemagglutinin (HA) gene complete cds
MN068839.1:916-1457 Influenza A virus (A/Duck/Al-Monfia/1727/2017(H5N8)) segment 4 hemagglutinin (HA) gene partial cds
ON024714.1:913-1454 Influenza A virus (A/duck/Egypt/SM6G/2019(H5N8)) segment 4 hemagglutinin (HA) gene partial cds
OR569129.1:1-542 Influenza A virus (A/Muscovy/Egypt/Influenza A virus/2020(H5N8)) segment 4 hemagglutinin HA2 region (HA) gene partial cds
OK205875.1:77-1072 Influenza A virus (A/chicken/Veracruz/233-7607/1998(H5N2)) segment 4 hemagglutinin (HA) gene complete cds
AY497086.1:1-996 Influenza A virus (A/chicken/Tabasco/234-8289/98 (H5N2)) hemagglutinin (HA1) gene partial cds
OK205563.1:77-1072 Influenza A virus (A/chicken/Guatemala/22066/1998(H5N2)) segment 4 hemagglutinin (HA) gene complete cds
AY497079.1:1-996 Influenza A virus (A/chicken/FO/22066/98 (H5N2)) hemagglutinin (HA1) gene partial cds
MF926481.1:1-855 Influenza A virus (A/swan/Voronezh/2/2017(H5N8)) segment 8 nuclear export protein (NEP) and nonstructural protein 1 (NS1) genes complete cds
OL354822.1:16-870 Influenza A virus (A/chicken/Egypt/F15366B/2018(H5N8)) segment 8 nuclear export protein (NEP) and nonstructural protein 1 (NS1) genes complete
OL354554.1:16-870 Influenza A virus (A/chicken/Egypt/F15100/2018(H5N8)) segment 8 nuclear export protein (NEP) and nonstructural protein 1 (NS1) genes complete co
MK208607.1:1-855 Influenza A virus (A/mulard duck/France/171201g/2017(H5N8)) segment 8 nuclear export protein (NEP) and nonstructural protein 1 (NS1) genes compl
OL467328.1:1-849 Influenza A virus (A/Duck/Egypt/BA3C/2018(H5N8)) segment 8 nuclear export protein (NEP) and nonstructural protein 1 (NS1) genes complete cds
MW676334.1:1-1704 Influenza A virus (A/DUCK/Hunan/134/2010(H5N1)) segment 4 hemagglutinin (HA) gene complete cds
MW676305.1:1-1704 Influenza A virus (A/DUCK/Hunan/92/2010(H5N1)) segment 4 hemagglutinin (HA) gene complete cds
MW676327.1:1-1704 Influenza A virus (A/DUCK/Hunan/210/2010(H5N1)) segment 4 hemagglutinin (HA) gene complete cds
KR732552.1:29-1732 Influenza A virus (A/chicken/Vietnam/NCVD-421/2010(H5N1)) segment 4 hemagglutinin (HA) gene complete cds
JN986882.1:1-1704 Influenza A virus (A/duck/Vietnam/QT802/2011(H5N1)) segment 4 hemagglutinin (HA) gene complete cds
JN986881.1:1-1704 Influenza A virus (A/duck/Vietnam/QT801/2011(H5N1)) segment 4 hemagglutinin (HA) gene complete cds

**Figure 4.** Phylogenetic tree based on a partial nucleotide sequence of HA gene. The tree was constructed using the Neighbor-joining method in MEGA11. Egyptian H5N8 clade 2.3.4.4 remarked by red square. The robustness of individual nodes of the tree was assessed using 1000 replications of bootstrap re-sampling of the originally aligned nucleotide sequences. Bootstrap values ≥50% are shown above the branches. The viruses isolated in this study are marked with red square. On other hand, H5 with clade 2.3.2 which used as a recombinant on baculo virus remarked by green square, and h5n2 used in inactivated vaccine remarked by blue square

## Discussion

The Egyptian poultry industry has a lucrative investment opportunity, given the substantial domestic market available for poultry meat and egg consumption [15]. This sector exhibits significant growth potential, fueled by population increase and a rise in per capita consumption [16]. Improving the economy of the country further contributes to favorable conditions for investment in the poultry industry [17].

The incursion of highly pathogenic avian influenza (HPAI/A/H5N8) viruses into Egypt in 2016, introduced by wild birds, marked the onset of a significant threat to the domestic poultry industry, resulting in substantial economic losses [18]. The previously isolated Egyptian H5N8 viruses belong to the Russian reassorted strain clade 2.3.4.4b, underscoring the global movement of avian influenza viruses and their propensity for reassortment [19]. This discussion delves into the genetic properties of the H5N8 HPAI subtype in layer chickens spontaneously infected on Al-Sharkia farms between 2019 and 2021, examining the implications for ongoing control and surveillance efforts.

The majority of samples analyzed in this investigation were derived from vaccinated domestic layer chickens, reflecting the severity of the outbreak despite immunization efforts. The observed clinical signs, including greenish diarrhea, ataxia, cyanosis, coughing, sneezing, and nasal discharge, point toward the virulence and adaptability of the H5N8 virus in domestic poultry. The affected birds exhibited a wide age range from 5 to 40 weeks, highlighting the vulnerability of various age groups to HPAI H5N8. Moreover, the manifestations of hyperemic feet and shanks, ataxia, congestion of the comb and wattle, and a sudden decrease in egg production underscore the systemic impact of the virus on layer chickens.

Five out of the ten examined flocks tested positive for a geographically distributed strain of the virus, as confirmed by the hemagglutination assay and AIV through virus isolation into embryonated chicken eggs (ECEs) using allantoic sacs. This finding emphasizes the continued circulation and adaptation of HPAI H5N8 within the domestic poultry population. The positivity of both geographically distributed and AIV strains points toward the robustness and prevalence of the virus in the studied region. Additionally, five of the ten immunized flocks from different cities in Sharqia tested positive for HPAI/A/H5N8 according to RT–PCR analysis of the HA and NA genes, further validating the

widespread presence of the virus despite vaccination efforts.

A crucial aspect of genetic characterization lies in the identification of specific amino acid patterns at the HPAIV H5N8 HA cleavage sites, notably "PLREKRRKR/GLF." This motif, elucidated in previous studies by Kandeil *et al.* [9] and Yehia *et al.* [19], is indicative of the high pathogenicity of avian influenza viruses. The amino acid sequence of the protease determines the cleavage site of the HA protein, and the presence of multiple basic amino acids signifies a predisposition toward high pathogenicity in avian influenza viruses [9, 20].

Phylogenetic analysis of the partial HA and NA genes of the sequenced viruses revealed their association with group B, Russian-like reassortant H5N8 viruses of clade 2.3.4.4 (Figure 4), consistent with previous findings [9, 19]. The interconnection between Egyptian HPAI H5N8 viruses and newly discovered reassorted viruses underscores the dynamic nature of avian influenza evolution and the constant introduction of novel genetic variants into the local poultry population.

Egypt is identified as an endemic hotspot for H5N8 in the Middle East and was found to play a crucial role in the global epidemiology of the H5N8 clade 2.3.4.4b [20, 21]. The country serves as a migration flyway for millions of wild birds annually, acting as a critical stopover on their journey between the Palearctic and Afrotropical areas. The extensive movement of wild birds introduces the constant risk of new introductions and reassortments of avian influenza viruses, contributing to the persistence and adaptability of HPAI H5N8 in the region.

Furthermore, the clustering of both the HA and NA genes in the present study with the A/chicken/Sergiev/Posad/1/17/HA gene discovered in chickens in Russia suggested common ancestry. This genetic linkage highlights the transboundary nature of avian influenza viruses, emphasizing the need for international cooperation in surveillance, prevention, and control strategies to mitigate the global spread of such viruses.

The current investigation provides critical insights into the genetic properties of HPAI H5N8 in layer chickens on Al-Sharkia farms between 2019 and 2021. The widespread prevalence of the virus, its association with reassorted strains, and its identification of specific amino acid patterns underscore the complexity and adaptability of avian influenza viruses. The role of Egypt as an endemic hotspot and migration flyway necessitates continuous surveillance. During this study the inactivated vaccines were H5N2 with clade 2.2.1 and Bacula recombinant vaccine H5 with clade 2.3.2, on other hand, the isolated strain in Egypt H5N8 with clade 2.3.4.4 (Figure 4), timely vaccination strategies, and international collaboration to effectively manage and control the ongoing threat posed by HPAI H5N8. Future studies should focus on understanding host–virus interactions and their impact on human health, and refining control measures to safeguard both avian populations and public health.

## Conclusions

This study highlights the identification of H5N8 avian influenza viruses in vaccinated flocks in Egypt between 2019 and 2021, revealing genetic clustering with Middle Eastern counterparts in the Russian-like reassorted H5N8 clade 2.3.4.4b. The genetic mismatch between

prevalent vaccines and the examined isolates underscores the urgency of reassessing vaccination strategies. It is crucial to enhance vaccine formulations, align them with circulating virus genetics, and optimize vaccination schedules. Simultaneously, strengthening biosecurity measures within poultry farms is imperative to curb virus introduction and spread. Integrated approaches, including genetic surveillance, adaptive vaccine development, and heightened biosecurity, are pivotal for fortifying Egypt's preparedness against the ongoing threat of H5N8 and other avian influenza strains in the poultry industry.

## Conflict of Interest

The authors have no conflict to declare.

## References

[1] Kaplan, B.S. and Webby, R. (2013): The avian and mammalian host range of highly pathogenic avian H5N1 influenza. Virus Res, 178(1): 3-11.

[2] Wu, Y.; Wu, Y.; Tefsen, B.; Shi, Y. and Gao, G.F. (2014): Bat-derived influenza-like viruses H17N10 and H18N11. Trends Microbiol, 22(4): 183-91.

[3] Neumann, G.; Shinya, K. and Kawaoka, Y. (2007): Molecular pathogenesis of H5N1 influenza virus infections. Antivir Ther, 12(4_part_2): 617-26.

[4] Lee, Y.-J.; Kang, H.-M.; Lee, E.-K.; Song, B.-M.; Jeong, J.; Kwon, Y.-K.; Kim, H.-R.; Lee, K.-J.; Hong, M.-S. and Jang, I. (2014): Novel reassortant influenza A (H5N8) viruses, South Korea, 2014. Emerg Infect Dis, 20(6): 1087.

[5] Bouwstra, R.; Heutink, R.; Bossers, A.; Harders, F.; Koch, G. and Elbers, A. (2015): Full-genome sequence of influenza A (H5N8) virus in poultry linked to sequences of strains from Asia, the Netherlands, 2014. Emerg Infect Dis, 21(5): 872.

[6] OIE,Two. (2018): Update on avian influenza in animals (types H5 and H7).

[7] Alexander, D.J. (2007): An overview of the epidemiology of avian influenza. Vaccine, 25(30): 5637-44.

[8] Salaheldin, A.H.; Elbestawy, A.R.; Abdelkader, A.M.; Sultan, H.A.; Ibrahim, A.A.; Abd El-Hamid, H.S. and Abdelwhab, E.M. (2022): Isolation of genetically diverse H5N8 avian influenza viruses in poultry in Egypt, 2019–2021. Viruses, 14(7): 1431.

[9] Kandeil, A.; Kayed, A.; Moatasim, Y.; Webby, R.J.; McKenzie, P.P.; Kayali, G. and Ali, M.A. (2017): Genetic characterization of highly pathogenic avian influenza A H5N8 viruses isolated from wild birds in Egypt. The Journal of general virology, 98(7): 1573.

[10] Pantin-Jackwood, M.J.; Costa-Hurtado, M.; Bertran, K.; DeJesus, E.; Smith, D. and Swayne, D.E. (2017): Infectivity, transmission and pathogenicity of H5 highly pathogenic avian influenza clade 2.3. 4.4 (H5N8 and H5N2) United States index viruses in Pekin ducks and Chinese geese. Veterinary Research Communications, 48: 1-14.

[11] Kamal, M.; Khalaf, M.; Ahmed, Z.A.; Fathy, M.; El-Miniawy, H.M.; Mahmoud, A.; Osman, A. and Zaki, E.M. (2023): Effect of water organic load and total ammonia nitrogen on broilers' humoral immune response against Newcastle disease virus vaccination in Egypt. Int J Vet Sci, 12(1): 107-113.

[12] Kamal, M.A.; Alhotan, R.A.; Al Sulaiman, A.R.; Hussein, E.O.; Galik, B. and Saleh, A.A. (2024): From source to house: unraveling the seasonal effect of water distribution system on drinking water quality of poultry farms under

Egyptian environmental condition. Environ Sci Pollut Res, 1-12.

[13] OIE. (2019): In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2019. OIE: Paris, France.

[14] Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A. and Kumar, S. (2013): MEGA6: molecular evolutionary genetics analysis version 6.0. Molec Biol Evol, 30(12): 2725-2729.

[15] Saleh, A.A.; Hafez, A.; Amber, K.; Abdelhady, A.Y.; Salem, H.M.; Fathy, M.; Kamal, M.; Alagawany, M. and Alzawqari, M.H. (2023): Drug-independent control strategy of clostridial infection in broiler chickens using anti-toxin environmentally friendly multienzymes. Sci Rep, 13(1): 5614.

[16] Salama, T.M.; Kamal, M.A.; Abdelfatah, S.H.; Salem, H.M. and Mohamed, F.F. (2024): Ameliorative effect of a novel enzymatic detoxifier against natural field levels of mycotoxins in the broiler chicken diet. Journal of Adv Vet Res, 14(1): 228-34.

[17] Kamal, M.A.; Khalaf, M.A.; Ahmed, Z.A.M. and El Jakee, J. (2019): Evaluation of the efficacy of commonly used disinfectants against isolated chlorine-resistant strains from drinking water used in Egyptian cattle farms. Vet world, 12(12).

[18] Selim, A.A.; Erfan, A.M.; Hagag, N.; Zanaty, A.; Samir, A.-H.; Samy, M.; Abdelhalim, A.; Arafa, A.-S.A.; Soliman, M.A. and Shaheen, M. (2017): Highly pathogenic avian influenza virus (H5N8) clade 2.3. 4.4 infection in migratory birds, Egypt. Emerg Infect Dis, 23(6): 1048.

[19] Yehia, N.; Naguib, M.M.; Li, R.; Hagag, N.; El-Husseiny, M.; Mosaad, Z.; Nour, A.; Rabea, N.; Hasan, W.M. and Hassan, M.K. (2018): Multiple introductions of reassorted highly pathogenic avian influenza viruses (H5N8) clade 2.3. 4.4 b causing outbreaks in wild birds and poultry in Egypt. Infect, Genetics Evol, 58: 56-65.

[20] Yehia, N.; Hassan, W.M.; Sedeek, A. and Elhusseiny, M.H. (2020): Genetic variability of avian influenza virus subtype H5N8 in Egypt in 2017 and 2018. Arch Virol, 165: 1357-66.

[21] Kilpatrick, A.M.; Chmura, A.A.; Gibbons, D.W.; Fleischer, R.C.; Marra, P.P. and Daszak, P. (2006): Predicting the global spread of H5N1 avian influenza. Proceedings of the National Academy of Sciences, 103(51): 19368-73.

**الملخص العربى**

## عزل وتشخيص فيروس أنفلونزا الطيور H5N8 شديد الضراوة من الدجاج البياض التجاري في محافظة الشرقية بمصر خلال 2019-2021

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هدفت هذه الدراسة الى توصيف فيروس انفلونزا الطيور شديد الضراوة H5N8 لما يأثرة على صناعة الدواجن فى مصر و قد تمت متابعة الاصابات فى قطعان البياض التجارية المحصنة فى محافظة الشرقية بين عامي 2019 و2021. وبسبب مشاكل الجهاز التنفسي والانخفاض المفاجئ والحاد في إنتاج البيض، تم تقييم عشر من المزارع الخمسة عشر المحصنة لهذا الغرض من الدراسة، مع معدل وفيات يتراوح بين 15% إلى 50%.. باستخدام تفاعل البلمرة المتسلسل العكسي وعزل الفيروس في بيض الدجاج الجنيني (ECEs) بتمرير الفيروس مرتين، وكانت نتيجة اختبار (AIV) إيجابية في العشرقطعان التي تم فحصها. بالنسبة للعزلات التي اجتازت اختبار التراص الدموي، تم تضخيم جينات الراصة الدموية (HA) والنورامينيداز (NA) جزئيًا باستخدام تفاعل البلمرة المتسلسل النوعي. تم بعد ذلك إخضاع خمس عزلات تمثل خمسة قطعان متعددة الطبقات للتحليل التسلسلي والتطوري. تظهر النتائج التي توصلنا إليها أنه تم التعرف على فيروسAIV/HPAI H5N8 انفلونزا الطيور شديد

الضراوة من خلال التسلسل والتحليل التطوري لجينومات HA و NAفي خمس قطعان. شاركت الأربعة HA و NA 99.8% و98.1% من تسلسلات النيوكليوتيدات الخاصة بهم مع فيروسات H5N8 التي تم اكتشافها سابقًا في الصين وإيران والعراق، على التوالي. علاوة على ذلك، كشفت التحليلات التطورية التي تركز على جينات HA و NAأن فيروسات H5N8 المصرية تنتمي إلى المجموعة B مع فيروسات H5N8 الشبيهة بالروسية من الفرع 2.3.4.4. خلاصة القول، أن الطيور التي تلقت التحصينات معرضة لخطر شديد من فيروس .HPAI H5N8 ولذلك فإن اكتشاف فيروس HPAI H5N8 في الطيور الداجنة أمر ضروري للمراقبة السنوية والرصد الجزيئي المتقطع وتقييم فعالية التحصين.