Emergence of equine influenza virus H3Nx Florida clade 2 in Arabian racehorses in Egypt

Basem Mohamed Ahmed, Mahmoud Mohamed Bayoumi, Mohamed Ali Farrag, Mahmoud Aly Elgamal, Janet Mary Daly and Haitham Mohamed Amer*

Abstract
Background: Equine influenza is an important cause of respiratory disease in equids. The causative virus, EIV, is highly variable and can evolve by accumulation of mutations, particularly in the haemagglutinin (HA) gene. Currently, H3N8 is the sole subtype circulating worldwide with Florida clade 1 (FC1) is most prevalent in the Americas and FC2 in Asia and Europe. In Egypt, EIV was detected in two occasions: subtype H7N7 in 1989 and subtype H3N8 (FC1) in 2008. No data is available on the circulation pattern of EIV during the last decade despite frequent observation of suspected cases.

Methods: Twenty-two nasal swabs were collected from vaccinated and non-vaccinated horses showing respiratory signs suggestive of EIV infection in 2017–18. Three additional swabs were retrieved during a national race event in January 2018 from Arabian mares with high fever, gait stiffness and dry cough. Samples were screened by RT-qPCR and HA1 domain of the hemagglutinin gene was amplified and sequenced for sequence and phylogenetic analysis.

Results: RT-qPCR screening revealed that only the 3 samples from the race were positive with cycle thresholds ranging from 16 to 21 indicating high viral load. Isolation attempts in hen's eggs were unsuccessful. Sequence analysis of the HA1 domain gene has revealed two identical nucleotide sequences, while the third contained 3 synonymous mutations. Phylogenetic analysis clustered study sequences with recent FC2 sequences from Europe. Amino acid alignments revealed 14 and 13 amino acid differences in the study sequences compared to A/equine/Egypt/6066NANRU-V5VRI/08 (H3N8) and A/equine/Kentucky/1997 (H3N8), respectively, available as EIV vaccines in Egypt. Nine amino acids were different from A/equine/Richmond/1/2007 (H3N8), the recommended FC2 vaccine strain by the world organization of animal health expert surveillance panel (OIE-ESP), two of which were unique to the Egyptian sequences while the remaining 7 changes were shared with the FC2-144V subgroup detected in the United Kingdom from late 2015 to 2016.

Conclusions: The study represents the first reported detection of FC2-144V related EIV from Arabian mares in Egypt, and probably from the entire middle east region. The presented information about EIV epidemiology and spread may require reconsideration of the vaccine strains used in the national vaccination programs.

Keywords: Arabian racehorse, Egypt, Emergence, Equine influenza, Florida clade 2, HA1 subunit gene

Background
Equine influenza (EI) is a highly contagious upper respiratory disease affecting horses and other equid species [1, 2]. EI is characterized by anorexia, marked increase in body temperature, nasal discharges, and dry cough [3]. The disease is widespread internationally and is a major challenge for global equestrian activities [4]. EI is caused

*Correspondence: hamoamer@cu.edu.eg
1 Department of Virology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
Full list of author information is available at the end of the article

© The Author(s) 2022. Open Access. This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
by equine influenza virus (EIV), which is a member of the genus Alphainfluenzavirus, and family Orthomyxoviridae [5]. EIV is an enveloped virus with an octameric negative sense single-stranded RNA genome that encodes at least 10 structural and non-structural proteins. Two major surface glycoproteins; hemagglutinin (HA) and neuraminidase (NA), have been described as determinants of virus infectivity and antigenicity [3, 6].

EIV of the subtype H7N7 was first reported in various European countries in 1955–1956 [7]. A few years later, another subtype (H3N8) emerged in the US and became the predominant subtype worldwide [8]. Viruses of the H7N7 subtype were apparently outcompeted by the H3N8 subtype viruses and are not thought to circulate in the equine population today [9]. In contrast, H3N8 diverged in the 1980s into two lineages; American and Eurasian [10]. The American lineage later further diverged into three sub-lineages (Kentucky, South America, and Florida). The Florida sub-lineage continued to predominantly circulate globally to date [11]. In the last two decades, the Florida sub-lineage was further subdivided according to the HA gene sequence into two clades; Florida clade 1 (FC1) and Florida clade 2 (FC2) [12]. FC1 was mostly identified in the Americas, while FC2 was predominant in Asia and Europe. Intercontinental circulation was frequently documented as a result of international equine races and exhibitions [13, 14].

Egypt is an important centre for raising and selling pure-bred Arabian horses [15]. To the best of our knowledge, the earliest EIV study was conducted in 1983 in Egypt [16], but it is thought that EIV had an earlier impact on the equine population in Egypt, before subtype H7N7 was identified in horses and donkeys with respiratory disease in 1989 [17]. This was the last reported isolation of the H7N7 subtype from equids. During the winter of 2000, an EI epizootic affected large numbers of horses, donkeys, and mules in Upper Egypt. Serological evidence indicated that the circulating strain belonged to the H3N8 subtype, however no sequences were available for genetic analysis [18]. EIV was again isolated from the equine population in nine Egyptian governorates in 2008 [19]. Molecular characterization of partial HA gene sequences and phylogenetic analysis showed that the prevalent viruses were all members of FC1 [20]. A concurrent isolation of another FC1 EIV, A/equine/Egypt/6066NAMRU3-VSVRI/2008 (H3N8), was used to prepare an inactivated whole virus vaccine [15, 21]. No further data on circulating equine influenza were published during the last decade.

Vaccination is considered the most effective measure for controlling EIV worldwide [3, 22]. However, EI is still reported in both vaccinated and non-vaccinated equine populations [4, 14, 23, 24]. This may result from antigenic drift in the surface glycoproteins and subsequent emergence of variants, and frequent introduction and circulation of genetically diverse EI viruses due to equine trade [6]. Therefore, continuous surveillance of the circulating strains in endemic regions to inform the choice of vaccine strains is of utmost importance according to the recommendations – World Organisation for Animal Health expert surveillance panel (OIE-ESP) [25]. In this study, an H3 subtype of equine influenza in Florida clade 2 was identified for the first time in Egypt in three Arabian racehorses. The study reports FC2 introduction into Egyptian horse population a decade after FC1. Comparison of deduced amino acid sequences with the OIE recommended and available vaccine strains highlighted the urgency of updating the candidate(s) used in national vaccination programs.

Methods
Clinical specimens and virus strain
Nasal swab samples (n = 22) were collected from horses showing one or more of the following signs: high fever, harsh dry cough, serous to mucoid nasal discharge, and gait stiffness. The specimens were collected from 7 vaccinated and 15 non-vaccinated horses located in different governorates in Egypt during the period October 2017 to April 2018. Additionally, three swab samples were obtained from race Arabian mares showing severe respiratory symptoms in January 2018. The Egyptian Florida clade 1 (FC1) strain of EIV [A/equine/EG/VRLCU/08 (H3N8)] served as a positive control [20].

Reverse transcription quantitative PCR (RT-qPCR)
RNA extraction from the clinical specimens was performed using TRIzol reagent (Thermo Fisher Scientific, Carlsbad, CA) according to the manufacturer’s protocol. EIV H3N8 was detected using reverse transcription quantitative PCR (RT-qPCR) [26]. The reaction was prepared by mixing 12.5 µl QuantiFast multiplex RT-PCR 2 × master mix (Qiagen, Hilden, Germany), 0.4 µM of both primers qHA3F and qHA3R, 0.2 µM of qHA3 probe (Table 1), and 5 µl RNA in PCR grade water. Target amplification and detection was performed using the StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific) with the following conditions: 50 °C for 20 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s, and 60 °C for 30 s. Samples with Ct value equal to 40 were considered negative, while those ranging from 35 to 39 were considered suspicious and retested for confirmation.

Nucleotide sequencing
Almost the complete HA1 gene domain sequence of RT-qPCR positive samples was PCR-amplified using HA3DF and H31R primers (Table 1). The reactions were
performed in GeneAmp 9700 thermal cycler using the SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity DNA Polymerase (Thermo Fisher Scientific) according to the kit’s manual. The amplicons were retrieved from agarose gel using QIAquick Gel Extraction Kit (Qiagen, GMBH) and were sequenced on both strands at Macrogen (Seol, South Korea). Raw sequence data were edited and assembled using BioEdit program, version 7.0.9.1 (Ibis Biosciences, Carlsbad, CA) and EditSeq tool of Lasergene software, version 3.18 (DNAStar, Madison, WI). The assembled sequences were deposited in GenBank with the accession numbers: MK089850 (A/horse/Egypt/BasM-FCL2/2018(H3N8)), MK089827 (A/horse/Egypt/BasB-FCL2/2018(H3N8)), and MK089810 (A/horse/Egypt/BasZ-FCL2/2018(H3N8)).

Sequence and phylogenetic analysis
Sequences of the Egyptian strains were aligned with their corresponding counterparts of 58 global strains retrieved from NCBI GenBank and GISAID (Additional file 1: Table S2). The international strains were selected to represent the different EIV lineages on a spatial and temporal basis including vaccine strains recommended by OIE-ESP or available in Egypt. Multiple sequence alignment was performed using Clustal W algorithm of the BioEdit program version 7.0.5.3 [28] to identify sequence diversity, allocate mutation sites and predict the amino acid changes. N-linked glycosylation sites of selected EIV strains were predicted using Net-N-glyc 1.0 (https://services.healthtech.dtu.dk/service.php?NetNGlyc-1.0) [29]. The phylogenetic tree was constructed using maximum likelihood method of MEGA 11.0.10 [30] with 1000 bootstrap test replicates. The obtained phylogram was further enhanced by InkScape 1.1 software.

Ethical statement
Guidelines for sample collection and animal use in research were followed according to the Institutional Animal Care and Use Committee, Cairo University (Approval code: CU-II-F-19–20).

### Results

#### Prevalence of EIV in the study group
All clinical specimens collected from different Egyptian governorates (vaccinated and non-vaccinated) over a period of 7 months during the winter season of 2017/2018 were negative for EIV H3N8 using RT-qPCR. In contrast, the three samples that were obtained from Arabian horses on a single racing occasion showed positive results with Ct values ranging from 16 to 21. All positive samples were confirmed by sequencing of a major portion of the HA1 domain of EIV H3N8.

#### Phylogenetic clustering of EIV strains
The mature HA1 subunit excluding signal peptide (975nt) was used for sequence and phylogenetic analysis of the Egyptian strains identified in this study. The phylogenetic tree indicated distinct clustering of EIV strains into pre-divergent, Euro-Asian, Kentucky, and Florida lineages (Fig. 1). The latter was further separated into two sub-lineages FC1 and FC2. FC2 showed further subdivisions into Asian subgroup, sequences with the 179V substitution (FC2-179V) subgroup, and sequences with the 144V substitution (FC2-144V) subgroup. The three current Egyptian viruses were defined as members of FC2-144V subgroup in proximity to strains identified in UK and France between 2011 and 2016 (Fig. 1).

#### Molecular characteristics and genetic diversity
Multiple sequence alignment has shown that the three Egyptian sequences were quite similar except for the strain Egypt-BasM-FCL2-2018, which had three synonymous substitutions at nucleotides 447, 468, and 897. No sequence gaps or duplications were demonstrated in any of the three sequences. In total, 35 (3.6%) point mutations were described for the Egyptian virus sequences. One of the two identical sequences was removed from further analyses. Compared to Kentucky/5/2002 sequence, there were nine amino acid residue changes in the Egyptian virus sequences: N3T, N7G, I9N, L103P, I112V, V144A, K192T, I267V, and I300V (Table 2, Fig. 2).

### Table 1 Oligonucleotides used in the study

| Name     | Sequence (5’–3’)   | Position* | References     |
|----------|--------------------|-----------|----------------|
| qHA3F    | TCACATGGACAGGTCCTCACA | 448–469   | Lu et al. [26] |
| qHA3R    | GGCTGATCCCCTTTGCAG  | 485–506   |                |
| qHA3 probe | FAM-AACGGAAGAAGGGAGC-BHQ1 | 471–487   |                |
| HA3DF    | CACCATGAMGCAACCCATTTGATACACT | 499–708   | Ahmed et al. [27] |
| H31R     | CGGTTATGCTCCAAAGATCC | 1039–1056 |                |

The term ‘up’ indicates upstream of HA gene start codon
FAM: Fluorescein, BHQ-1: Blackhole quencher
*Nucleotide positions relative to hemagglutinin gene (HA) sequence
Discussion

Influenza is the most important respiratory disease in equines worldwide. It gained its importance from the direct economic impact on equine trade and international equine events and shows. Mild EI cases may go unnoticed but complicated cases can be fatal. EIV H3N8 has shown ability to infect vaccinated horses causing low to moderate losses [14, 33, 34], however great losses are often linked to virus introduction into naïve equine populations. For instance, introduction of EIV into the Australian equine population has resulted in infection of about 70,000 horses [35]. Furthermore, recent emergence of EIV FCL1 into Western and Central African countries led to more than 66,000 deaths in horses and donkeys [36, 37]. Proper vaccination against EIV in terms of timing, frequency, and relevance of vaccine antigen, stimulates a cumulative antibody response in animals to control virus spread and reduce disease outcomes. It also provides partial protection against newly introduced strains [33]. Conversely, improper vaccination and/or poor vaccination coverage may result in new disease outbreaks, particularly with the introduction of novel strains.

The equine population in Egypt and its health status is mostly underestimated. Unofficial reports from the Egyptian ministry of agriculture estimated the equine population as approximately one million in 2017 and three million in 2021; among which horses may represent 10–25%. Data on the prevalence and distribution of EIV in Egypt over 40 years are limited to a total of four reports [16–18, 20]. The most comprehensive study was conducted in 2008, when a countrywide outbreak affected all equine species in Egypt. EIV H3N8 FCL1 was detected and isolated as the causative pathogen [20], a concurrently isolated strain (Egypt/6066NAMRU-VSVRI/2008) was used to prepare a whole inactivated virus vaccine [15] and the Fluvac innovator® 4 (Zoetis-US) was also registered for use in Egypt. Since then, no data on the circulation pattern of the virus were retrieved although sporadic cases are frequently observed in vaccinated and non-vaccinated animals nationwide.

In this study, we aimed to investigate whether EIV H3N8 was still circulating as a cause of respiratory disease in the equine population in Egypt. Therefore, 22 samples were collected from vaccinated and non-vaccinated horses showing respiratory signs suggestive for EI
Fig. 1 (See legend on previous page.)
between August 2017 and April 2018 from different localities in Egypt. Samples were screened by RT-qPCR and none of them was positive. The sensitivity of the assay [26] and the successful amplification of positive controls suggest negative results were either due to improper timing of sampling or absence of the target pathogen in these particular samples. Unfortunately, no sera were available to evaluate antibody responses and possible exposure of non-vaccinated horses.

Fortunately, within the course of this study, nasal swab samples from three Arabian mares suffering from fever and gait stiffness were submitted to our lab for EIV diagnosis. These mares were unvaccinated and participating in a national horse race, which was later cancelled upon confirmation of EIV infection. Samples were screened using RT-qPCR and all of them were positive for EIV H3N8 with low Ct values [16–21] suggestive of high virus load. Attempts for virus isolation in embryonated chicken eggs were unsuccessful using single, pooled, stock, or diluted samples (Data not shown). Such failure is sometimes expected due to difficult adaptation of some EIV strains in chicken embryos [3].

The majority of the HA1 domain of all positive samples was amplified and sequenced. Sequence and phylogenetic analysis have indicated that the three viruses belong to FC2-144V subgroup, the clade that has never been reported in Egypt or in the middle east region before, compared to FC2 isolates from Algeria 2011 [38] and Turkey 2013 [39]. The sequence of two viruses was identical whereas the third contained 3 synonymous nucleotide changes. This variation may suggest the FC2 virus had been circulating in Egypt prior to detection as the horse race event was national where all participating horses came from Cairo and other provinces in Egypt. Furthermore, the high similarity between the Egyptian virus sequences and others identified in the UK between 2015 and 2016 [14] suggest an earlier introduction of the virus.

Divergence of the Florida sub-lineage was first identified in 2003, when two distant outbreaks were caused by virus strains of significant HA1 sequence heterogeneity; A/equine/South Africa/03 the prototype of FC1 and A/equine/Newmarket/5/03 the prototype of FC2 [40]. Originally, FC1 was exclusively dominant in equids of the US [3], while both clades co-circulated in other parts of the world with apparent dominance of FC2 in Europe [40, 41]. FC2 introduction into the US was reported in 2014 through a diseased mare imported from Germany [42]. It is not expected that FC2 arrived in Egypt similarly as importation of horses is not a common occurrence. We assume that one or more of the Egyptian Arabian horses participated in an international event or show 2015 or later and contracted the infection before spreading the virus upon return home. This is supported by the V267I substitution which is shared between the study sequences and sequences from UK November 2015 [14] and 2016 [43] (data not shown). Proper vaccination of these horses might suppress the clinical disease or mild clinical signs may have been misdiagnosed as being a result of travel stress [44].

The limited spread and late observation of FC2 in Egypt can be explained by the fact that the Arabian horses represent only 10% of the total horse population in Egypt (1% of total equids) and are mostly owned by government or private studs with high care, vaccination, and very little contact with other horses.

HA is the major determinant of pathogenicity and antigenicity in influenza viruses. It is composed of two domains; HA1 which forms the globular head and HA2

---

**Table 2** Amino acid differences between study sequences, available vaccine strains in Egypt, recommended vaccine strains by OIE-ESP, Florida clade 2 prototype sequence and recent FC2 detected from middle eastern countries (2011–2013) and from UK (2015–2016)

| Sequence | 3 | 7 | 9 | 72 | 78 | 103 | 112 | 138 | 144 | 159 | 179 | 192 | 223 | 267 | 291 | 300 | 326 |
|----------|---|---|---|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Kentucky/5/2002 | N | G | N | G | V | P | V | A | A | N | I | T | V | V | D | V | K |
| Ohio/1/2003 | . | . | A | . | . | S | . | . | . | . | . | . | . | . | . | . |
| South Africa/4/2003 | . | . | A | . | . | S | . | . | . | . | . | . | . | . | . | . |
| Richmond/1/2007 | . | N | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| Egypt/6066NAMRU3-VSVRI/2008 | . | D | . | A | S | S | . | . | . | . | . | . | . | . | . | . | . |
| Kentucky/1/1997 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| Tiaret/1/2011 | . | N | . | . | L | I | . | . | . | . | . | . | . | . | . | . | . |
| Ankara/1/2013 | . | N | . | . | L | I | . | . | . | . | . | . | . | . | . | . | . |
| Lanarkshire/2/2015 | . | N | A | . | L | I | V | . | K | . | . | . | . | . | . | . | . |
| East Sussex/1/2015 | . | N | . | . | L | I | V | . | K | . | . | . | . | . | . | . | . |
| Gloucestershire/3/16 | . | N | . | . | L | I | V | . | K | . | . | . | . | . | . | . | . |
| Egypt/BusB-FC2/2018 | T | N | I | . | L | I | V | . | K | . | . | . | . | . | . | . | . |
| Egypt/BusM-FC2/2018 | T | N | I | . | L | I | V | . | K | . | . | . | . | . | . | . | . |
| Egypt/BusZ-FC2/2018 | T | N | I | . | L | I | V | . | K | . | . | . | . | . | . | . | . |
Fig. 2 Putative N-glycosylation sites in selected Florida sub-lineage, clade 1, and 2 sequences indicated in transparent orange boxes. Dashed clear boxes indicate antigenic sites in the HA1 of EIV.
that forms the stalk [45–47]. The HA1 domain carries in
its structure the receptor-binding domain (RBD) and five
major antigenic sites (A–E), hence it represents the prin-
cipal inducer of strain specific immunity against EIV.
Trivial changes in the RBD or in one of the antigenic
sites can cause vaccination failure [12, 47, 48]. On the
deduced amino acid level, study HA1 sequences were
identical to each other with 14 amino acid differences
from the Egyptian FC1 vaccine strain, 13 amino acid
differences from Kentucky/1/97 vaccine strain and 9
amino acid differences from Richmond/1/2007, the OIE-
ESP recommended FC2 vaccine strain [32]. Most of the
variations observed were not linked to any of the anti-
genic sites in the HA1 protein [11] except for A144V and
T192K in the antigenic sites A and B respectively (Fig. 2).
It was previously shown that 2 amino acid substitutions
in the H3 HA1 surface were enough for antigenic drift
and vaccine update [49] but it is accepted that at least 4
amino acid changes in no-less-than two antigenic sites
are required to update the strains in the vaccine [50].
All three Egyptian sequences shared the amino acid
variant A144V specific for FC2-144V subgroup isolated
from Europe 2011–2015 [14] (Fig. 1). Two unique amino
acid substitutions in the Egyptian HA1 sequences (T3N
and I9N) were not located in an antigenic site.

Conclusions
This report is the first that describes detection of EIV
H3N8 in Egypt 10 years after of the outbreak caused by
FC1 in 2008 [20]. Unexpectedly, the identified strains in
this study were all members of FC2-144V subgroup,
which was not reported in Egypt or in the middle east
region before. Sequence analysis and circumstances of
the outbreak suggest that the Egyptian FC2 was intro-
duced at least 2 years prior to detection, most likely
through horses that traveled abroad, and became infected
during horse races or shows. The partially isolated nature
of pedigree Egyptian Arabian horses and the proper care
provided to these high-value horses, the high antigenic
similarity between both Florida clades and the under-
detection/under-estimation of EIV in Egypt are all fac-
tors that may explain why detection of FC2 was delayed
for this period. This proposed silent circulation may have
resulted in the Egyptian FC2 acquiring shared unique
amino acid changes and some nucleotide variations
between them. Therefore, routine surveillance of EIV in
equids should be followed to identify the circulation
pattern of EIV in Egypt and to identify mutant strains
as early as possible. The current vaccine containing only
the 2008 FC1 virus strain requires an update by inclusion
of both Florida clades to enable superior protection and
proper disease control.

Acknowledgements
Authors are thankful to Mr. Ahmed Ahdy (NRC, Egypt) for providing samples
from the race event.

Author contributions
BMA and HMA developed the concept and designed the experiments. MMB
and MAE performed the experiments. BMA, MAF, and MMB prepared the original draft. GMD and HMA
revised the manuscript. All authors approved the final manuscript.

Funding
Open access funding provided by The Science, Technology & Innovation
Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank
(EKB). This work is funded by science, technology, and innovation funding
agency (STIFA), Egypt, Grant Number STF-25338.

Availability of data and materials
All data generated or analysed during this study are included in this published
article (and its supplementary information files).

Declarations
Ethics approval and consent to participate
Guidelines for sample collection and animal use in research were followed
according to the Institutional Animal Care and Use Committee, Cairo Univer-
sity (Approval code: CU-II-F-19–20).

Consent for publication
Not applicable.

Competing interests
Authors declare that they have no competing interests. STIFA as a funding
innovator has no impact on study design, results, or interpretations of the results.

Author details
1 Department of Virology, Faculty of Veterinary Medicine, Cairo University,
Giza 12211, Egypt. 2Division of Biomedical and Life Sciences, Faculty of Health
and Medicine, Lancaster University, Lancaster LA1 4YG, UK. 3Department
of Botany and Microbiology, College of Science, King Saud University,
Riyadh 11451, Saudi Arabia. 4One Virology, Wolfson Centre for Global Virus
Research, School of Veterinary Medicine and Science, University of Notting-
ham, Sutton Bonington Campus, Leicestershire LE12 5RD, UK.

Abbreviations
EIV: Equine influenza virus; FC: Florida clade; HA: Haemag-
glutinin; NA: Neuraminidase; OIE-ESP: World Organisation for Animal Health
expert surveillance panel; RT-qPCR: Reverse transcriptase quantitative PCR.

Supplementary Information
The online version contains supplementary material available at https://doi.
org/10.1186/s12985-022-01917-9.

Additional file 1. Supplementary Figure 1: comparison with the
deduced amino acid sequence of the OIE-ESP recommended vaccine
strain against Florida clade2. Supplementary Figure 2: comparison with the
deduced amino acid sequence of the whole inactivated EI vaccine strain available in Egypt (Egypt/6066NAMRU-VSVRI/2008).

Additional file 2. Supplementary Table 1: Accession codes for EIV
HA1 sequences included in phylogenetic analysis figures 1. The bold
sequences indicate the GISAID obtained sequences representing 2015-
2016 EI outbreak in Scotland and England. Supplementary Table 2:
Accession codes for sequences used in table 2 and figure 2 alignments.
GISAID accession numbers are highlighted in bold.

Ethics approval and consent to participate
Guidelines for sample collection and animal use in research were followed
according to the Institutional Animal Care and Use Committee, Cairo University (Approval code: CU-II-F-19–20).

Consent for publication
Not applicable.

Competing interests
Authors declare that they have no competing interests. STIFA as a funding
innovator has no impact on study design, results, or interpretations of the results.

Author details
1 Department of Virology, Faculty of Veterinary Medicine, Cairo University,
Giza 12211, Egypt. 2Division of Biomedical and Life Sciences, Faculty of Health
and Medicine, Lancaster University, Lancaster LA1 4YG, UK. 3Department
of Botany and Microbiology, College of Science, King Saud University,
Riyadh 11451, Saudi Arabia. 4One Virology, Wolfson Centre for Global Virus
Research, School of Veterinary Medicine and Science, University of Notting-
ham, Sutton Bonington Campus, Leicestershire LE12 5RD, UK.
References

1. Barrandeguy ME, Carossino M. Infectious diseases in donkeys and mules: an overview and update. J Equine Vet Sci. 2018;65:98–105.

2. Rickards KJ, Thiennemann AK. Respiratory Disorders of the Donkey. Vet Clin North Am Equine Pract. 2019;35(3):361–73.

3. Singh RK, Dhama K, Karthik K, Munjal A, Khurana SK, et al. A comprehensive review on equine influenza virus: etiology, epidemiology, pathobiology, advances in developing diagnostics, vaccines, and control strategies. Front Microbiol. 2018;9:1–26.

4. Fougerolle S, Legrand L, Lecouturier F, Saleille C, Paillot R, Hans A, et al. Genetic evolution of equine influenza virus strains (H3N8) isolated in France from 1967 to 2015 and the implications of several potential pathogenic factors. Virolology. 2017;505(Feburary):210–7.

5. Orthomyxoviridae - Negative Sense RNA Viruses - Negative Sense RNA Viruses (2011) - ICTV. Available from: https://talk.ictvonline.org/ictv-reports/ictv_9th_report/negative-sense-rna-viruses-2011/w/negrna_viruses/orthomyxoviridae/5

6. Gahan J, Garvey M, Asmah Abd Samad R, Cullinane A. Whole genome sequencing of the first H3N8 equine influenza virus identified in Malaysia. Pathogens. 2019;8(2):62.

7. Sovinova O, Turnova B, Pouska F, Nemec J. Isolation of a virus causing respiratory disease in horses. Acta Virol. 1958;2(1):52–61.

8. Waddell GH, Teigland MB, Sigel MM. A new influenza virus associated with respiratory disease in horses. Acta Virol. 1961;4(6):1063–74. https://doi.org/10.1016/0001-7072(61)90077-9.

9. Yamanaka T, Nemoto M, Bannai H, Tsujimura K, Kondo T, Matsumura T, et al. Assessment of antigenic difference of equine influenza virus strains by challenge study in horses. Influenza Other Resp Viruses. 2016;10(6):336–9.

10. Kalad M, Ebied E, Madkour N, Warda S, Saleh N, El-Kabbany MMA, et al. Antigenic and genetic evolution of equine H3N8 influenza A viruses. J Gen Virol. 1996;77(4):661–71.

11. Lai ACK, Chambers TM, Holland RE, Morley PS, Haines DM, Townsend HGG, et al. Diverged evolution of recent equine-2 influenza (H3N8) viruses in the Western Hemisphere. Arch Virol. 2001;146(6):1083–7. https://doi.org/10.1075/0001-7072.1701.06.

12. Shittu I, Meseko CA, Sulaiman LP, Inuwa B, Mustapha M, Zakaria PS, et al. Fatal multiple outbreaks of equine influenza H3N8 in Nigeria, 2019: The first introduction of Florida clade 1 to West Africa. 2020;248:108820.

13. Hughes J, Allen RC, Baguelin M, Hampson K, Baille GI, Elton D, et al. Transmission of equine influenza virus during an outbreak is characterized by frequent mixed infections and loose transmission bottlenecks. PLoS Pathog. 2012;8(12):1–16.

14. Rash A, Morton R, Woodward A, Maes O, McCauley J, Bryant N, et al. Evolution and divergence of H3N8 equine influenza viruses circulating in the united kingdom from 2013 to 2015. Pathogens. 2017;6(1):6.

15. Abdel-Rehim MHA, Hussein M. An epizootic of equine influenza virus in Upper Egypt in 2000. Rev Sci Tech. 2004;23(3):921–30.

16. Sabit N, Estell K, Mapes S, Wadamian C. Detection of clade 2 equine influenza virus in an adult horse recently imported to the USA. Equine Vet Educ. 2014;26(9):453–5. https://doi.org/10.1111/eqe.12159.

17. Gärtner H. Studies on equine influenza virus. Giza: Cairo University, 1983.

18. Sack A, Cullinane A, Daramragchaa U, Chuluunbaatar M, Gonchigoo B, Gray GC. Equine influenza virus—disease threat. Emerg Infect Dis. 2019;25(6):1185–91.

19. Sack A, Cullinane A, Daramragchaa U, Chuluunbaatar M, Gonchigoo B, Gray GC. Equine influenza virus—disease threat. Emerg Infect Dis. 2019;25(6):1185–91.

20. Ahmed BM, Hussein HA, El-Sanoussi AA. Immunogenicity of DNA plasmid expressing HA1 of (A/eqine/Egypt/vlr6u/2008 (H3N8) equine-1 influenza virus in wistar rat model. Int J Virol. 2014;10(1).
48. Nemoto M, Ohta M, Yamanaka T, Kambayashi Y, Bannai H, Tsujimura K, et al. Antigenic differences between equine influenza virus vaccine strains and Florida sublineage clade 1 strains isolated in Europe in 2019. Vet J. 2021;272.
49. Jin H, Zhou H, Liu H, Chan W, Adhikary L, Mahmood K, et al. Two residues in the hemagglutinin of A/Fujian/411/02-like influenza viruses are responsible for antigenic drift from A/Panama/2007/99. Virology. 2005;336(1):113–9.
50. Wilson IA, Cox NJ. Structural basis of immune recognition of influenza virus hemagglutinin. Annu Rev Immunol. 1990;8:737–71.

**Publisher’s Note**
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.