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Isolation and characterization of the seasonal H1N1 influenza A virus (2014) from an Egyptian patient

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Abstract: *This study reports on an isolate of seasonal H1N1 influenza A virus from an Egyptian patient. Virus detection in the clinical sample was carried out via RT-PCR using specific primers targeting the M2-gene. The virus propagating in MDCK cells was only successful upon addition of TPCK trypsin to the inoculums and showed both mild pathogenicity and replicative capacity resulting in virus titer of 10 4.24 TCID 50 ml -1 after 4 days post infection. It was further confirmed to be influenza "A" virus by Western blotting using specific polyclonal antibody raised against the M1-viral protein antigen and delimited to be one of the swine influenza A subtypes using specific rapid antigen detection kit. Blast and phylogenic analysis of the obtained partial NP nucleotide sequence demonstrated that the closest previously reported viruses were human, avian and swine H1N1 viruses, whereas, the highest homology with the obtained PB2 partial sequence was with swine, avian and human H1N1 viruses, respectively. This finding eventually suggests that the seasonal swine influenza circulating in Egypt in 2014 is more likely a human H1N1 that includes sequences with potential homology to both swine and avian H1N1 viruses and reflect continuous reassortment among such viruses.*
Keywords: *Seasonal, Swine, Influenza, Egypt,2014,M2, PB2, NP, Phylogeny*

I. INTRODUCTION

The major problem concerning influenza viruses is the unlimited chances of reassortments resulting in changes of existing strains or the emergence of completely novel strains of unknown behavior. Swine influenza virus H1N1 represented a major concern over the past decade as a cause for devastating pandemics and endemics. The most recent swine influenza outbreak in April 2009 was initially reported in California followed by being reported in 120 other countries very shortly thereafter. In August 2009, 182,166 cases were confirmed to be infected with the novel swine influenza H1N1 virus strain resulting in 1799 deaths in 40 countries reaching a total of 12,220 deaths by the end of the same year [1]. Throughout the peak pandemic period WHO reports about Middle east and in particular Egypt were either extremely limited or even absent. WHO declared the end of the pandemic influenza in August 2010. Since then this virus has been circulating in humans as seasonal influenza virus. In 2014 the Egyptian Ministry of Health and Population (MOHP) reported an increase in seasonal swine influenza virus activity. Such activity caused mild to severe illness that ended up in some cases with death among a number of hospitalized patients of which a few were laboratory confirmed to be infected with a homologous virus to the influenza A (H1N1) pdm 2009 [2].

Pandemic 2009 swine H1N1 virus was a 'triple-reassortment' strain of a swine origin (S-OIV), containing genes from human, swine, and avian influenza A viruses (IAV) [3]. This reassorted S-OIV H1N1 strain was found to be circulating since 2005 with no fatal consequences [4], and was reported in previous studies before the pandemic starts [5,6]. Cases of patients suffered from the virus infection before pandemic events was completely defined in CDC reports as a routine national influenza surveillance [4]. Steady epidemiological studies on seasonal strains help in understanding the course of mutational changes and possibilities of the re-emergence of previous pandemic strains.

Tracking the changes of circulating seasonal strains and preparedness to possible sudden outbreaks emphasize the need for systematic frameworks for continuous monitoring and characterization of strains transmissibility, severity, antiviral resistance and vaccine escape [7]. Rapid diagnostic assays for detection of, and discrimination among, swine influenza strains have been established and adapted on widely conserved common features among different strains. Modifying and adapting such diagnostics is of equal great importance in the inter-pandemics, pandemics and urgent need periods.

In the present study we report on a seasonal influenza H1N1 virus from a clinical sample in Egypt. Which identity was characterized using a series of laboratory assays which can apply to monitor resurgence of future seasonal outbreaks.

II. MATERIALS AND METHODS

Nasal swab from an Egyptian patient suffering from flu symptoms was kindly provided from central public health laboratories of Egyptian MOHP. Work on human sample was approved by Medical Research Ethics Committee (Registration number 11133). Assays were performed under biosafety level 2 clean bench with enhanced decontamination practices.

2.1. Identification of the infecting virus from nasal sample medium

The medium in which the nasal swab was preserved was subjected to viral total RNA extraction using the GeneJET Viral DNA and RNA Purification Kit (*Thermo fisher scientific Inc,; MA, USA)* followed by cDNA synthesis using the M-MLV reverse transcriptase (Promega, Wisconsin, USA) and the previously published M segment-specific reverse primer [8]. The amplification of the partial M2 fragment was done in a DNA thermocycler (BiometraGmbH; Goettingen, Germany) using DreamTaq DNA Polymerase, 10mM dNTP mix (Both from *Thermo fisher scientific Inc; MA, USA*), a newly designed forward primer by ourselves namely HIA-MF 5'-AGATGCAGCGATTCAAGTG-3' and the same reverse primer used in the reverse transcription step at concentration 20 pmole/ reaction and annealing temperature of 58^{o}C. Both the master mix and cycling program were carried out according to the instructions of the manufacturer. Amplified DNA fragments were subjected for electrophoresis on 2% agarose gel in 1x TAE buffer and visualized by ethidium bromide staining [9].

2.2. Isolation and titration of the influenza A virus

After being confirmed to be infected with IAV by RT-PCR, the medium in which the swab was transferred was used to inoculate Madin Darby Canine Kidney cells (MDCK; American type culture collection no. CCL-34) grown on DMEM supplemented with 10% FBS (Both from Lonza Walkersville, Inc, Switzerland) as previously described [10]. The Infection medium used to dilute the inoculums was supplemented with 0.2% FBS and 2µg/ ml TPCK-trypin (SERVA; Heidelberg, Germany). The cells were microscopically checked daily for observing incidence of cytopathic effects due to influenza propagation. Virus $TCID_{50}$ was calculated following the method of Reed and Muench [11] in a 96-well microtiter plate seeded with $5x10^4$ cells count/well and each of the prepared virus dilution was used to infect 6 individual wells.

2.3. Serologic confirmatory assays

After being amplified on MDCK cells the type of the virus was further confirmed to be an IAV by both Western blotting analysis [12] using HRP-conjugated goat polyclonal anti-M1 antibody (Abcam Inc; Cambridge, MA, USA) and the CERTEST influenza A-swine flu rapid antigen detection card (*CerTest, BIOTEC, Spain*) according to instructions of the manufacturer.

2.4. Molecular characterization and phylogenic analysis of the influenza A virus

To further characterize the isolate at the molecular level, the cDNA encoding both the PB2 and NP fragments was amplified using previously published primers and standard PCR protocol [8]. The amplified fragments were subjected for agarose gel electrophoresis, visualized, and the gel slices carrying the fragments were excised and subjected for DNA elution using QIAquick Gel Extraction Kit (Qiagen; Hilden, Germany). The purified DNA was subjected for automated sequencing and the obtained sequences were aligned against previously published sequences on the GenBank using the nucleotide Megablast analysis tool available at http://www.ncbi.nlm.nih.gov/. Multiple alignment of the obtained sequences with chosen recently published ones from the GenBank database was carried out using Clustal Omega application, followed by building up a neighbor-joining phylogenic tree using the online based Clustalw2 application, both Clustal applications available at http://www.ebi.ac.uk.

III. RESULTS AND DISCUSSION

The emergence of the 2009 pandemic H1N1 stimulated the urgent need for fast and accurate detection systems. The list of diagnostic tools which can be utilized to diagnose influenza ranges from classic virology techniques to new emerging methods [13].

3.1. Identified influenza A virus from the nasal sample via RT-PCR targeting conserved M2-gene

Because the sequence encoding the M2 protein of various IAV strains was thought to be highly conserved over long periods of time, the N-terminal amino acid domains of these proteins were considered as the basis of a universal anti-IAV vaccine [14]. However, when we conducted multiple alignments on such domains of various IAV isolates from different localities in different years results clearly reflected that this conservation is not absolute on the nucleotide level, as scattered point mutations were recorded among various sequences. That's why we intended to place our designed Forward primer for amplifying the M2 partial sequence in the highly conserved M2 regions among various strains and years (see highlights on "Fig.1.A"). Using the M2 specific primers mentioned in the M & M section in performed RT-PCR on the extracted RNA from the nasal swab resulted in amplification of a 263 bp fragment "Fig.1.B" that fits to the expected size predicted based upon the *in silico* primer annealing position. This result confirmed that the flu symptoms the patient had were due to IAV infection and encouraged further characterization of the virus.

3.2. Isolation of the influenza virus on MDCK showing mild replication capacity:

To enrich the virus titer and to make sure that we have enough stock of the virus to carry out the rest of the characterization steps, the medium in which the swab was preserved was used to inoculate MDCK cells. The reason of using this cell line to further propagate the virus is its high susceptibility to infection with IAV that allows development of a distinguishable CPE upon infection in comparison to uninfected cells in a considerably short period which made it the most commonly used cell line to propagate such viruses *in vitro* [10].

In our hands inoculated MDCK cells with the medium in which nasal swab was preserved exhibited CPE within 72 hours post infection (hpi) which was evidenced as cell rounding and deformation with no cell detachments "Fig. 1.C". In our *in vitro* infection experiments, the virus could induce CPE only upon adding TPCK trypsin to the infection medium which can be explained by the need for such enzyme to, activate, cleave the virus HA domain which is a rate limiting step in IAV entry [15]. No further changes or increased CPE occur over increased time of incubation or medium re-fed.

The poor capacity of the virus to cause cell detachment although its ability to cause CPE stimulated our first suspicion that it can be a swine virus, which fits to previous observation by others that swine influenza viruses replicate poorly in MDCK cells [16]. Calculations of the $TCID_{50}$ at 4 days pi of MDCK cells indicated that the titer of the propagated virus was $10^{4.24}$ ml^{-1}. In a previous study on IAV, virus titer and pathogenicity were considered high when the $TCID_{50}$ value ranged between 10^{6}-10^{8} ml^{-1}, whereas, the titer and pathogenicity were considered low when the value ranged between 10^{1}-10^{3} ml^{-1} [17]. Since the calculated $TCID_{50}$ for our isolate, $10^{4.24}$ ml^{-1}, falls in the middle, this suggests that the herein described virus has got both mild pathogenicity and replicative capacity in MDCK cells.

3.3. Influenza virus type "A" serologic confirmation and delimitation to swine subtypes

The causative virus for the CPE was further confirmed to be "A", by western blotting analysis of lysates prepared from infected MDCK cells using a specific anti-IAV-M1-protein antibody [18] that does not cross react with the other types of influenza that reacted to a protein band at ~27 kDa corresponding to the M1 protein Fig. 2.A". An additional immunogenic peptide was visualized on the blot at a higher molecular weight of ~63 kDa which might either be a dimmer of the M1 or a piece of the whole virion associated to the M1 protein. Noteworthy, both peptides were absent in lysates prepared from uninfected MDCK cells. The identity of the causative IAV for the CPE in the cells was delimited to be one of the swine subtypes using a commercial rapid antigen detection kit specific to swine influenza viruses "Fig. 2.B" [19].

3.4. phylogenic analysis of partial viral segments showing its relatedness to other avian and swine origin isolates

The serological results were further confirmed by sequencing of both the NP and PB2 fragments of the virus under investigation. BLAST analysis of the obtained partial sequences of the two viral segments against the GenBank clearly showed that the top hits with the NP sequence were mainly human H1N1 strains "Fig. 3.A", whereas those with the PB2 gene were swine followed by human H1N1 strains "Fig. 3.B". Phylogenic analysis of the obtained NP and PB2 sequences with chosen recently published H1N1 nucleotide sequences from human, avian and swine isolates showed that the most closely related nucleotide sequences to our herein reported partial NP nucleotide sequence were from human, avian and swine H1N1 viruses, respectively "Fig. 4.A", while the most closely related ones to our partial PB2 sequence are from swine, avian and human H1N1 viruses "Fig. 4.B".

IV. FIGURES

4.1. Legends of the figures

Fig. 1. (A) Multiple alignment of the M2-nucleotide sequences of different influenza A virus (IAV) strains. The highlighted nucleotide sequence shows the position of the conserved HIA-MF forward primer. (B) RT-PCR amplification of the partial M2-fragment from RNA extracted from the transport medium of the clinical sample

using specific primers. An expected amplicon size of 263 bp appeared in case of the cDNA prepared from the nasal swab RNA (lane I) while no products were visualized in the minus cDNA control prepared from uninfected transport medium (lane II). (C) Infection of MDCK cells with the transport medium of the clinical sample supplemented with 2µg/ml TPCK trypsin. CPE appeared 72 hpi as cell rounding and monolayer sheet deformation with no cell detachments (I) compared to uninfected MDCK cell control (II).

Fig. 2. (A) Detection of the M1-protein of the virus in lysates of infected MDCK cells by Western blotting using specific antibody for the IAV-M1 domain. An immunogenic band at, 27 kDa, the expected M1-protein M. Wt. with an addition band at ~63 kDa were seen in the lysate prepared from the infected lane cells (Lane I) which were absent in the lysates from the uninfected cells lysates (Lane II). (B) Detection of the propagating virus in the supernatant (I) of the infected cells using the commercial CERTEST influenza A-swine flu rapid detection card. The isolated virus supernatant test showing a red line in test position (T) that was not evidenced in case of the supernatant of the uninfected MDCK cells (II).

Fig. 3. Multiple alignment of the obtained partial sequences of the two viral genes NP and PB2 with the top ten hits resulted from their BLAST analysis against the GenBank individually. The top hits of NP sequence analysis were mainly human H1N1 strains (A) with approximately 94% homology, whereas those with the PB2 gene were swine followed by human H1N1 strains (B) with approximately 90% homology.

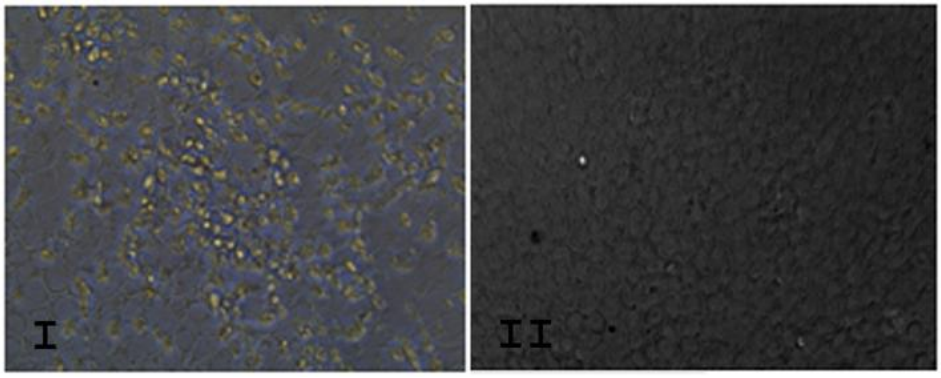
Fig. 4. Neighbor-joining phylogenic tree for the partial NP (A) and PB2 (B) nucleotide sequences obtained from the isolated virus in comparison to recently published sequences of H1N1 viruses. The analysis showed that the most closely related nucleotide sequences to our partial NP nucleotide sequence were from human, avian and swine H1N1 viruses (A), while the most closely related ones to our partial PB2 sequence are from swine, avian and human H1N1 viruses, respectively (B).

4.2. List of figures

Figure 1 (A)

(B)

(C)



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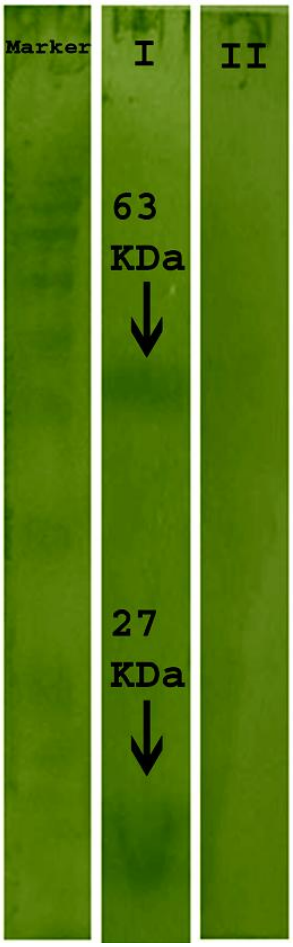

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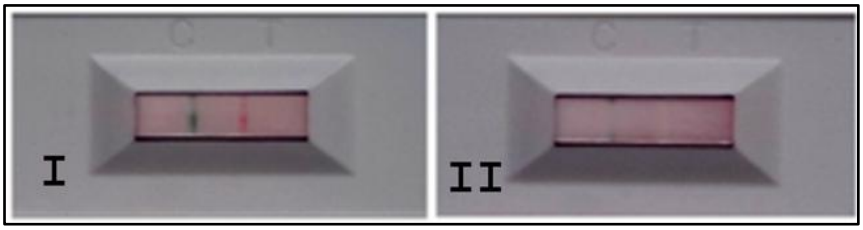

Figure 3. A

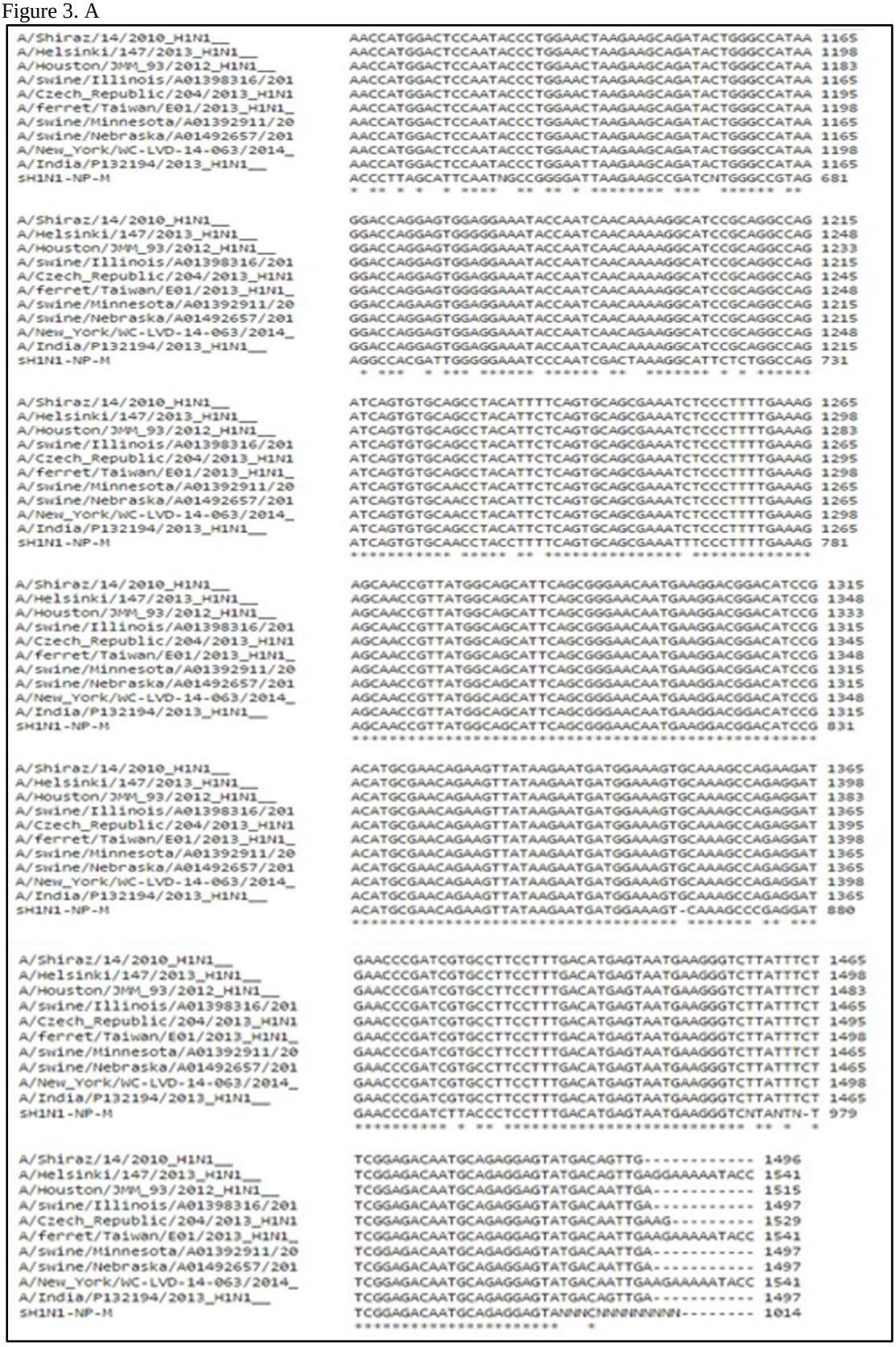
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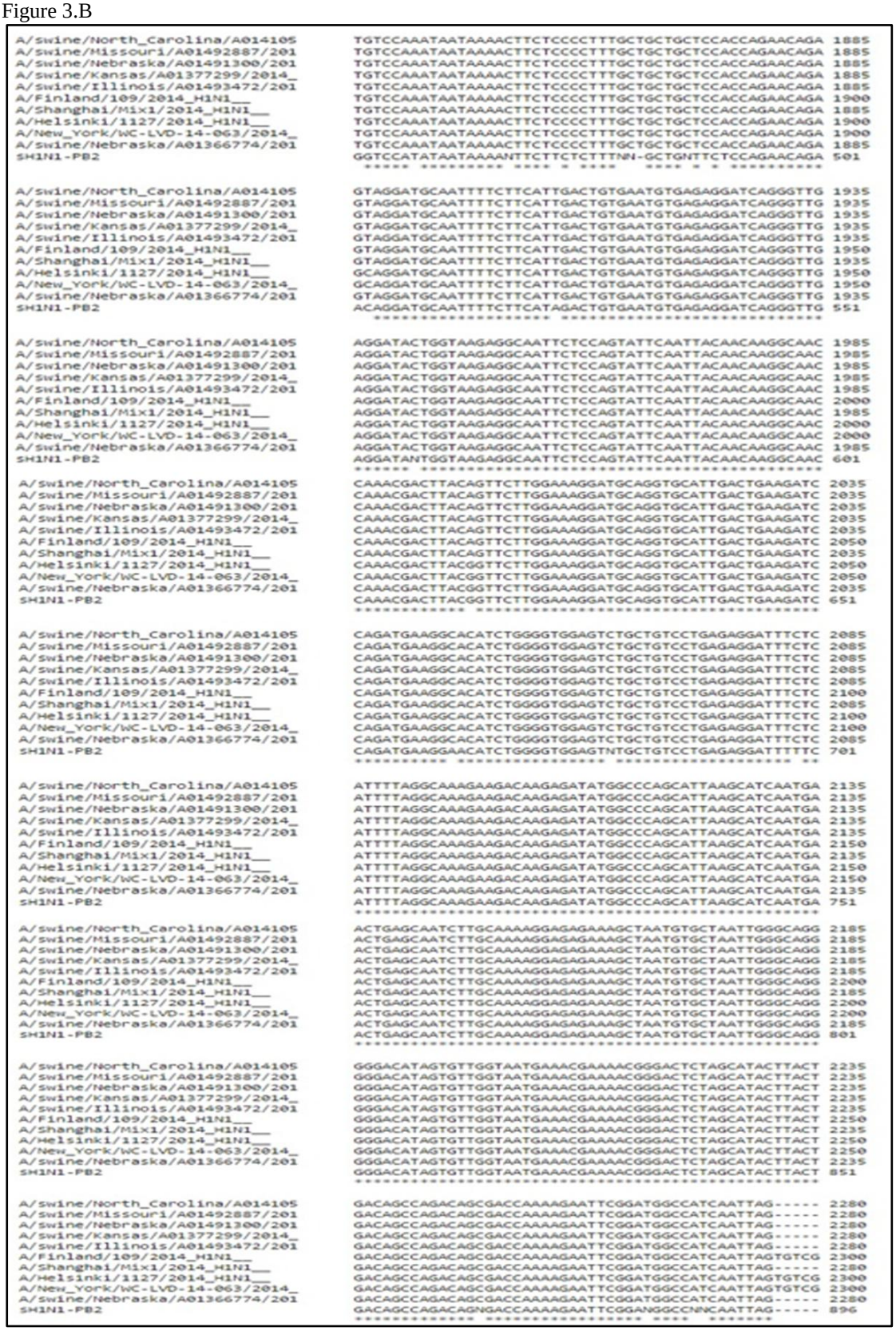
Figure 3.B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A/New_York/WC-LVD-14-063/2014_ TGTCCAAATAATAAAACTTCTCCCCTTTGCTGCTGCTCCACCAGAACAGA 1900
A/swine/Nebraska/A01366774/201 TGTCCAAATAATAAAACTTCTCCCCTTTGCTGCTGCTCCACCAGAACAGA 1885
SH1N1-PB2 GGTCCATATAATAAAANTTCTTCTCTTTNN-GCTGNTTCTCCAGAACAGA 501
***** ********* **** * **** **** * * **********
A/swine/North_Carolina/A014105 GTAGGATGCAATTTTCTTCATTGACTGTGAATGTGAGAGGATCAGGGTTG 1935
A/swine/Missouri/A01492887/201 GTAGGATGCAATTTTCTTCATTGACTGTGAATGTGAGAGGATCAGGGTTG 1935
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A/swine/Kansas/A01377299/2014_ GTAGGATGCAATTTTCTTCATTGACTGTGAATGTGAGAGGATCAGGGTTG 1935
A/swine/Illinois/A01493472/201 GTAGGATGCAATTTTCTTCATTGACTGTGAATGTGAGAGGATCAGGGTTG 1935
A/Finland/109/2014_H1N1__ GTAGGATGCAATTTTCTTCATTGACTGTGAATGTGAGAGGATCAGGGTTG 1950
A/Shanghai/Mix1/2014_H1N1__ GTAGGATGCAATTTTCTTCATTGACTGTGAATGTGAGAGGATCAGGGTTG 1935
A/Helsinki/1127/2014_H1N1__ GCAGGATGCAATTTTCTTCATTGACTGTGAATGTGAGAGGATCAGGGTTG 1950
A/New_York/WC-LVD-14-063/2014_ GCAGGATGCAATTTTCTTCATTGACTGTGAATGTGAGAGGATCAGGGTTG 1950
A/swine/Nebraska/A01366774/201 GTAGGATGCAATTTTCTTCATTGACTGTGAATGTGAGAGGATCAGGGTTG 1935
SH1N1-PB2 ACAGGATGCAATTTTCTTCATAGACTGTGAATGTGAGAGGATCAGGGTTG 551
****************** ****************************
A/swine/North_Carolina/A014105 AGGATACTGGTAAGAGGCAATTCTCCAGTATTCAATTACAACAAGGCAAC 1985
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A/Helsinki/1127/2014_H1N1__ AGGATACTGGTAAGAGGCAATTCTCCAGTATTCAATTACAACAAGGCAAC 2000
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A/swine/Nebraska/A01366774/201 AGGATACTGGTAAGAGGCAATTCTCCAGTATTCAATTACAACAAGGCAAC 1985
SH1N1-PB2 AGGATANTGGTAAGAGGCAATTCTCCAGTATTCAATTACAACAAGGCAAC 601
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A/swine/Kansas/A01377299/2014_ CAAACGACTTACAGTTCTTGGAAAGGATGCAGGTGCATTGACTGAAGATC 2035
A/swine/Illinois/A01493472/201 CAAACGACTTACAGTTCTTGGAAAGGATGCAGGTGCATTGACTGAAGATC 2035
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A/swine/Illinois/A01493472/201 CAGATGAAGGCACATCTGGGGTGGAGTCTGCTGTCCTGAGAGGATTTCTC 2085
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A/New_York/WC-LVD-14-063/2014_ ACTGAGCAATCTTGCAAAAGGAGAGAAAAGCTAATGTGCTAATTGGGGCAGG 2200
A/swine/Nebraska/A01366774/201 ACTGAGCAATCTTGCAAAAGGAGAGAAAAGCTAATGTGCTAATTGGGCAGG 2185
SH1N1-PB2 ACTGAGCAATCTTGCAAAAGGAGAGAAAAGCTAATGTGCTAATTGGGCAGG 801
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A/swine/Illinois/A01493472/201 GGGACATAGTGTTGGTAATGAAACGAAAACGGGACTCTAGCATACTTACT 2235
A/Finland/109/2014_H1N1__ GGGACATAGTGTTGGTAATGAAACGAAAACGGGACTCTAGCATACTTACT 2250
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A/Helsinki/1127/2014_H1N1__ GGGACATAGTGTTGGTAATGAAACGAAAACGGGACTCTAGCATACTTACT 2250
A/New_York/WC-LVD-14-063/2014_ GGGACATAGTGTTGGTAATGAAACGAAAACGGGACTCTAGCATACTTACT 2250
A/swine/Nebraska/A01366774/201 GGGACATAGTGTTGGTAATGAAACGAAAACGGGACTCTAGCATACTTACT 2235
SH1N1-PB2 GGGACATAGTGTTGGTAATGAAACGAAAACGGGACTCTAGCATACTTACT 851
****
A/swine/North_Carolina/A014105 GACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAG----- 2280
A/swine/Missouri/A01492887/201 GACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAG----- 2280
A/swine/Nebraska/A01491300/201 GACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAG----- 2280
A/swine/Kansas/A01377299/2014_ GACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAG----- 2280
A/swine/Illinois/A01493472/201 GACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAG----- 2280
A/Finland/109/2014_H1N1__ GACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAGTGTCG 2300
A/Shanghai/Mix1/2014_H1N1__ GACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAG----- 2280
A/Helsinki/1127/2014_H1N1__ GACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAGTGTCG 2300
A/New_York/WC-LVD-14-063/2014_ GACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAGTGTCG 2300
A/swine/Nebraska/A01366774/201 GACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAG----- 2280
SH1N1-PB2 GACAGCCAGACAGNGACCAAAAGAATTCGGANGGCCNNCAATTAG----- 896
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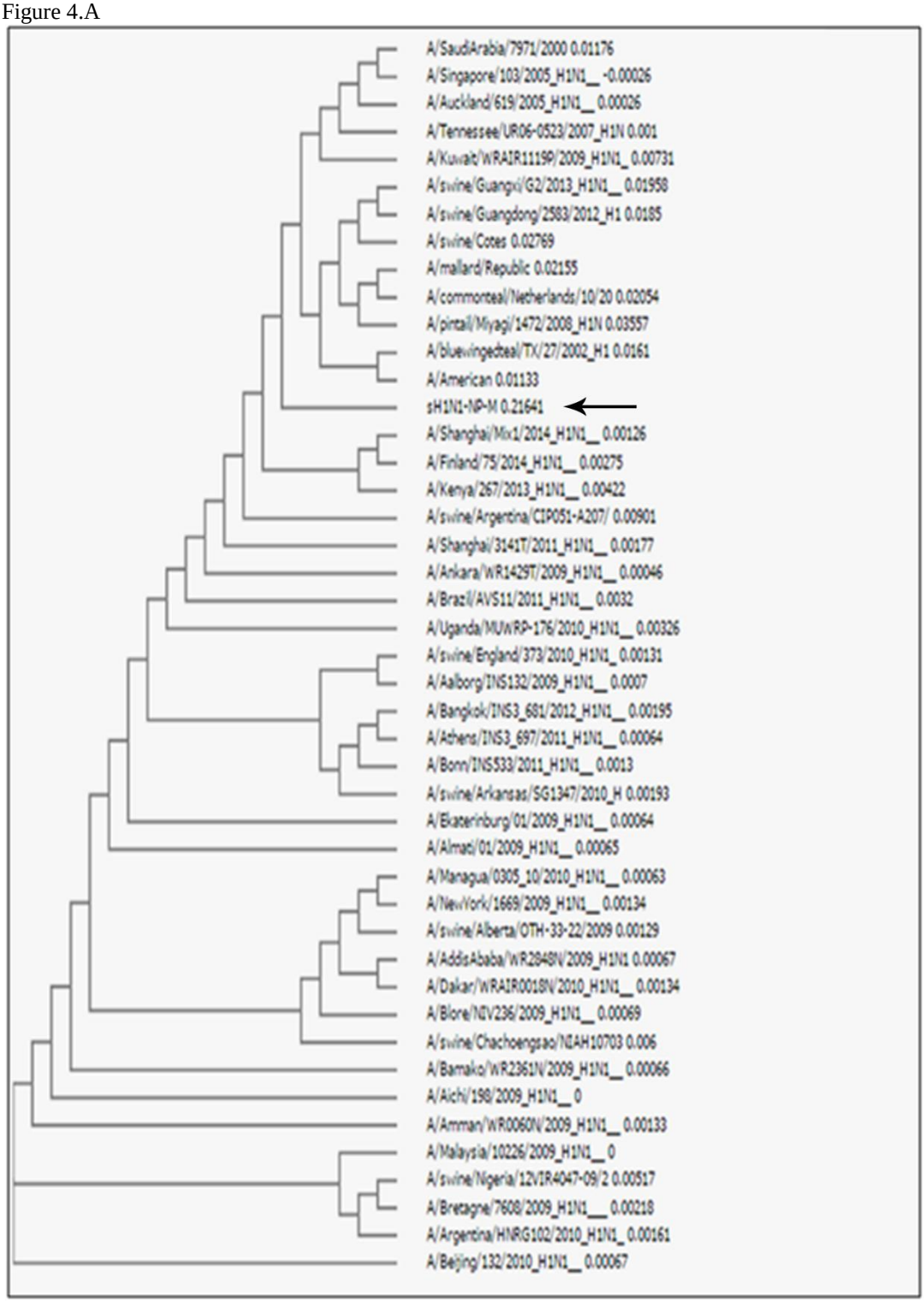
Figure 4.A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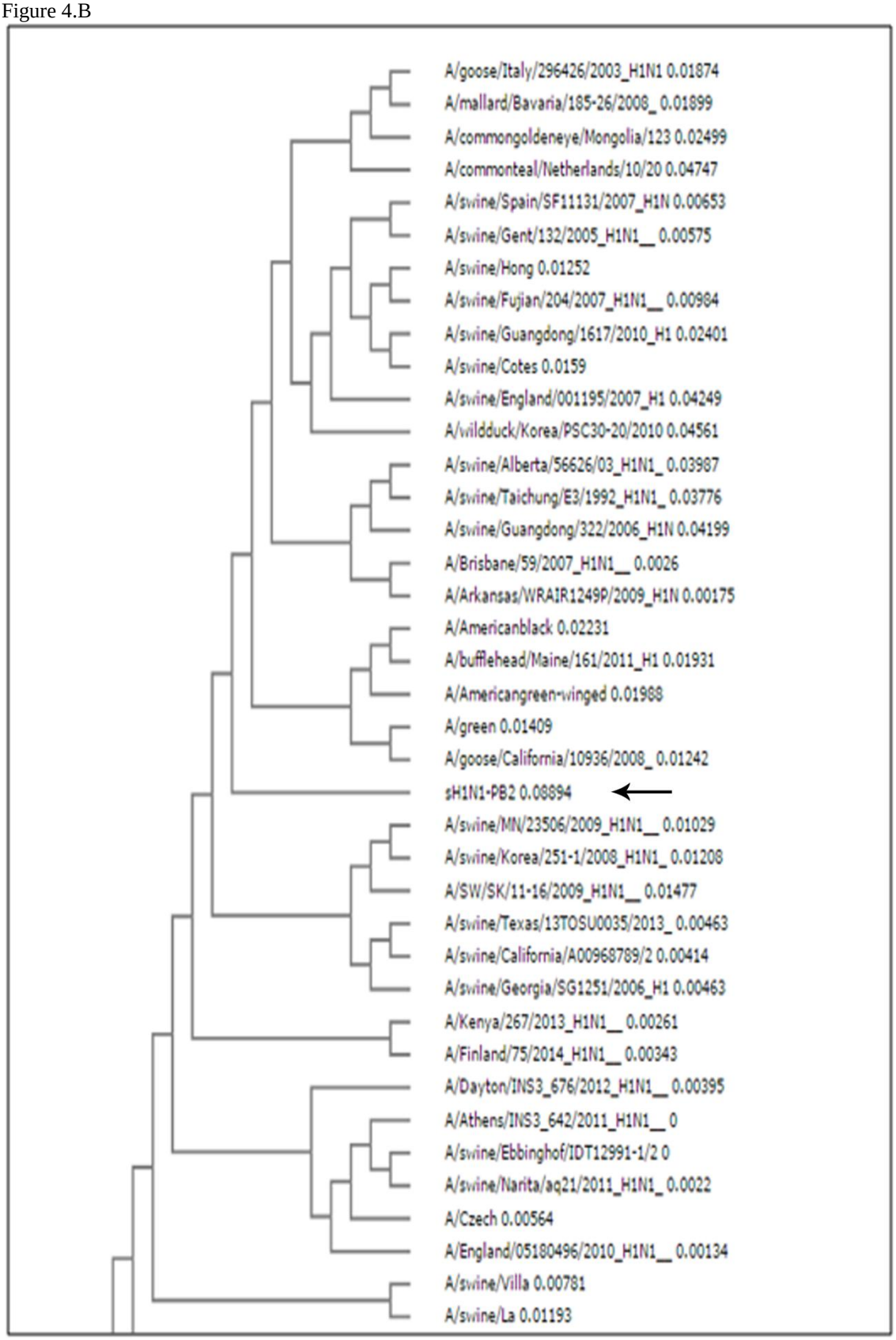
A/Kuwait/WRAIR1119P/2009_H1N1_ 0.00731
A/swine/Guangxi/G2/2013_H1N1__ 0.01958
A/swine/Guangdong/2583/2012_H1 0.0185
A/swine/Cotes 0.02769
A/mallard/Republic 0.02155
A/commonteal/Netherlands/10/20 0.02054
A/pintail/Miyagi/1472/2008_H1N 0.03557
A/bluewingedteal/TX/27/2002_H1 0.0161
A/American 0.01133
sH1N1-NP-M 0.21641
A/Shanghai/Mix1/2014_H1N1__ 0.00126
A/Finland/75/2014_H1N1__ 0.00275
A/Kenya/267/2013_H1N1__ 0.00422
A/swine/Argentina/CIP051-A207/ 0.00901
A/Shanghai/3141T/2011_H1N1__ 0.00177
A/Ankara/WR1429T/2009_H1N1__ 0.00046
A/Brazil/AVS11/2011_H1N1__ 0.0032
A/Uganda/MUWRP-176/2010_H1N1__ 0.00326
A/swine/England/373/2010_H1N1_ 0.00131
A/Aalborg/INS132/2009_H1N1__ 0.0007
A/Bangkok/INS3_681/2012_H1N1__ 0.00195
A/Athens/INS3_697/2011_H1N1__ 0.00064
A/Bonn/INS533/2011_H1N1__ 0.0013
A/swine/Arkansas/SG1347/2010_H 0.00193
A/Ekaterinburg/01/2009_H1N1__ 0.00064
A/Almaty/01/2009_H1N1__ 0.00065
A/Managua/0305_10/2010_H1N1__ 0.00063
A/NewYork/1669/2009_H1N1__ 0.00134
A/swine/Alberta/OTH-33-22/2009 0.00129
A/AddisAbaba/WR2848N/2009_H1N1 0.00067
A/Dakar/WRAIR0018N/2010_H1N1__ 0.00134
A/Blore/NIV236/2009_H1N1__ 0.00069
A/swine/Chachoengsao/NIAH10703 0.006
A/Bamako/WR2361N/2009_H1N1__ 0.00066
A/Aichi/198/2009_H1N1__ 0
A/Amman/WR0060N/2009_H1N1__ 0.00133
A/Malaysia/10226/2009_H1N1__ 0
A/swine/Nigeria/12VIR4047-09/2 0.00517
A/Bretagne/7608/2009_H1N1___ 0.00218
A/Argentina/HNRG102/2010_H1N1_ 0.00161
A/Beijing/132/2010_H1N1__ 0.00067

Figure 4.B


A/goose/Italy/296426/2003_H1N1 0.01874
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A/commongoldeneye/Mongolia/123 0.02499
A/commonteal/Netherlands/10/20 0.04747
A/swine/Spain/SF11131/2007_H1N 0.00653
A/swine/Gent/132/2005_H1N1__ 0.00575
A/swine/Hong 0.01252
A/swine/Fujian/204/2007_H1N1__ 0.00984
A/swine/Guangdong/1617/2010_H1 0.02401
A/swine/Cotes 0.0159
A/swine/England/001195/2007_H1 0.04249
A/wildduck/Korea/PSC30-20/2010 0.04561
A/swine/Alberta/56626/03_H1N1_ 0.03987
A/swine/Taichung/E3/1992_H1N1_ 0.03776
A/swine/Guangdong/322/2006_H1N 0.04199
A/Brisbane/59/2007_H1N1__ 0.0026
A/Arkansas/WRAIR1249P/2009_H1N 0.00175
A/Americanblack 0.02231
A/bufflehead/Maine/161/2011_H1 0.01931
A/Americangreen-winged 0.01988
A/green 0.01409
A/goose/California/10936/2008_ 0.01242
sH1N1-PB2 0.08894
A/swine/MN/23506/2009_H1N1__ 0.01029
A/swine/Korea/251-1/2008_H1N1_ 0.01208
A/SW/SK/11-16/2009_H1N1__ 0.01477
A/swine/Texas/13TOSU0035/2013_ 0.00463
A/swine/California/A00968789/2 0.00414
A/swine/Georgia/SG1251/2006_H1 0.00463
A/Kenya/267/2013_H1N1__ 0.00261
A/Finland/75/2014_H1N1__ 0.00343
A/Dayton/INS3_676/2012_H1N1__ 0.00395
A/Athens/INS3_642/2011_H1N1__ 0
A/swine/Ebbinghof/IDT12991-1/2 0
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A/Czech 0.00564
A/England/05180496/2010_H1N1__ 0.00134
A/swine/Villa 0.00781
A/swine/La 0.01193

V. CONCLUSION

The obtained nasal swab was from a patient suffering from IAV infection. The causative virus for this infection was mildly replicating in MDCK cells. Both serological and molecular experiments further confirmed that the propagating virus in the cell is an IAV. Analysis of both the NP and PB2 obtained partial sequences reflected that the herein reported seasonal IAV is a human isolate with potential homologies to both swine and avian H1N1 viruses which reveals continuous reasortment among influenza strains circulating among humans.

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