BIOINFORMATICS ANALYSIS TO ASSESS THE HOMOLOGY AMONG INFLUENZA A VIRUSES AND OTHER PATHOGENS

A. M. SHOKRY[1], SABAH M. HASSAN[2] AND FATMA M. I. BADAWY[2]

1. *Agricultural Genetic Engineering Research Institute, Agriculture Research Center, Giza, Egypt*
2. *Genetics Dept., Fac. of Agric., Ain Shams University, Cairo, Egypt*

The mechanisms by which influenza viruses cross species barriers to infect humans or other mammals, either causing dead-end infections or leading to subsequent human-to-human transmission, are unknown. Moreover, the properties of influenza viruses that have the greatest medical and public health relevance, such as human infectivity, transmissibility, and pathogenicity, appear to be complex and polygenic and are poorly understood (Morens *et al*., 2009). Influenza viruses are members of the Orthomyxoviridae family of RNA viruses and are grouped into types A, B, and C on the basis of their nucleoprotein (NP) and matrix protein characteristics. Type A influenza viruses are classified into subtypes based on two proteins on the surface of the virus, hemagglutinin (HA) and neuraminidase (NA) (Oliveira *et al*., 2003; Marjuki *et al*., 2007).

Every influenza A virus has a gene coding for 1 of 16 possible hemagglutinin (HA) surface proteins and another gene coding for 1 of 9 possible neuraminidase (NA) surface proteins. These two proteins (facilitating viral attachment and release) are critical for the infection of susceptible cells of a host (Portela and Digard, 2002). Of the 144 total combinatorial possibilities, only three HAs and two NAs, in only 3 combinations (H1N1, H2N2, and H3N2), have ever been found in truly human-adapted viruses (Morens *et al*., 2009). Influenza A viruses infect a large variety of mammals and birds, occasionally producing devastating pandemics in humans (Alexander and Brown, 2000). Epidemics frequently occur between pandemics as a result of gradual antigenic change in the prevalent virus; this phenomenon is termed antigenic drift (Laver *et al.,* 1990). Three notable (years: 1918, 1958 and 1968) severe pandemics have occurred during the 20th century: An H1N1 caused the 1918's "Spanish flu" pandemic, while an H3N2 was responsible for the 1968 "Hong Kong flu" pandemic (Taubenberger and Morens, 2006 a and b).

All avian influenza viruses are classified as type A. Only four avian influenza A viruses including H5N1, H7N3, H7N7 and H9N2 viruses have jumped host species to infect humans (Bao *et al.,* 2008). The H5N1 subtype, in particular, has been reported in 410 human cases and has caused 256 human deaths in 15 countries. In Egypt it has been reported in 57 human cases and has caused 23 human

deaths, as reported in World Health Organization website in the year 2009 (http://www. who.int/csr/disease/avian_ influenza/country/cases_table_2009_03_ 10/en/index.html).

The species barrier reflects, at least in part, the different receptor preferences of mammalian and avian viruses. Researchers have suggested that human tracheal epithelial cells lack receptors for the attachment of avian influenza viruses and that avian tracheal epithelial cells lack the appropriate receptors for human viruses (Rogers *et al.,* 1983). Pigs, however, possess receptors for both avian and mammalian viruses and are postulated to be the host in which influenza viruses of different origins can genetically reassort (Castrucci *et al.,* 1994; Kida *et al.,* 1994).

The genome of type A influenza is single-stranded, negative-sense RNA, that is their genomes cannot be translated into protein directly upon entering the host cell. It contains eight genome segments that encode 10 proteins (Huang *et al.,* 1990; Portela and Digard, 2002). The eight influenza A viral RNA segments encode 10 recognized gene products. These are PB1, PB2, and PA polymerases, HA, NP, NA, Ml and M2 proteins, and NS1 and NS2 proteins. PB2 polymerase is encoded by RNA segment 1, PB1 polymerase is encoded by RNA segment 2, PA polymerase is encoded by RNA segment 3, HA is encoded by RNA segment 4, NP is encoded by RNA segment 5, NA is encoded by RNA segment 6, M1 is encoded by RNA segment 7, the mRNA for M2 is also transcribed from RNA segment 7 and RNA segment 8 encodes the two nonstructural proteins NS1 and NS2 (Webster *et al.,* 1992).

Influenza virus is very changeable. Mutations, including substitutions, deletions, and insertions, are one of the most important mechanisms for producing variation in influenza viruses. The lack of proofreading among RNA polymerases contributes to replication errors (Robert *et al.,* 2008). RNA recombination would be another mechanism leading to genetic variation. Recombination in RNA viruses occurs by two different methods; reassortment and template switching or copy-choice replication (Posada *et al.,* 2002). Recombination by the process of reassortment is limited to viruses with segmented genomes such as influenza and rotaviruses (Lai, 1992). Reassortment occurs when two or more strains infect the same cell and exchange genomic segments during viral replication. This mechanism has been well studied for influenza A and is postulated to account for the emergence of antigenically and genetically novel viruses that enable microbes to evade the immune response and persist in the host's body (Worobey and Holmes, 1999; Posada *et al.,* 2002). The second method, copy-choice replication, can be utilized by either segmented or unsegmented viruses. Copy-choice is a process whereby the viral RNA-dependent RNA polymerase jumps from one RNA template to the other during replication creating a chimeric recombinant that is an amalgamation of both parental strands (Worobey and Holmes, 1999).

In the past few years, there has been a worldwide effort to isolate and sequence the genomes of influenza A viruses, which has led to the depositing of more than 46,000 sequences in the Influenza Virus Resource of the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html). As of May 25, 2009, the NCBI database included sequences from more than 220 strains from the 2009 swine-origin human influenza A (H1N1) virus isolated at various sites around the world. Consequently, the origin and recent history of new strains can be inferred from study of the most similar deposited sequences. The percentage of matching nucleotides (the nucleotide identity) after nucleotide alignment, as determined with the use of the NCBI Basic Local Alignment Search Tool (BLAST) or other tools, is a common measure of similarity used by researchers in the field.

To gain insight into the biology of this devastating disease, this study was aimed to assess the relationships among influenza A viruses including human and avian influenza virus in addition to other pathogens using basic local alignment search tool (BLAST) and multiple sequence alignment (MSA) program for proteins.

MATERIALS AND METHODS

Basic local alignment search tool (BLAST)

The BLAST finds regions of local similarity between sequences. The program compares nucleotide or amino acid sequences to sequence databases and calculates the statistical significance of matches based on pair-wise alignment method. BLAST can be used to infer functional and evolutionary relationships between sequences. In addition it helps identify members of gene families (http://www.ncbi.nlm.nih.gov/BLAST).
The whole proteome of the influenza A virus [A/Chicken/Hong Kong/258/97 (H5N1)] isolate, which contains 10 proteins was downloaded from the national center for biotechnology information (NCBI) website (http://www.ncbi.nlm.nih.gov/). Then, the same whole proteome was used to search the RefSeq database for similar sequences.

RefSeq database project

The Reference sequence (RefSeq) database (http://www.ncbi.nlm.nih.gov/RefSeq) is a nonredundant collection of richly annotated DNA, RNA, and protein sequences from diverse taxa. The collection includes sequences from plasmids, organelles, viruses, archaea, bacteria, and eukaryotes. Each RefSeq represents a single, naturally occurring molecule from one organism. The goal is to provide a comprehensive, standard dataset that represents sequence information for a species. It should be noted that, RefSeq has been built using data from public archival databases only.

RefSeq biological sequences (also known as RefSeqs) are derived from GenBank (http://www.ncbi.nlm.nih.gov/Genbank) records but differ in that each

RefSeq is a synthesis of information, not an archived unit of primary research data. A RefSeq represents the consolidation of information by a particular group at a particular time. RefSeqs are available without restriction and can be retrieved in several different ways such as: searching NCBI's databases including Nucleotide, Protein, Gene, and Map Viewer [http://www.ncbi.nlm.nih.gov/mapview]; searching with a sequence via BLAST (http://www.ncbi.nlm. nih.gov/BLAST); doing an FTP download [ftp.ncbi.nih. gov/gene]; or through links from other NCBI resources including Gene, Map Viewer, and PubMed.

AlignX and ClastalW

ClustalW (Higgins and Sharp, 1988; Larkin *et al.,* 2007) is a general purpose multiple sequence alignment (MSA) program for DNA or proteins. It produces biologically meaningful multiple sequence alignments of divergent sequences. It calculates the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be shown. Evolutionary relationships can be shown *via* viewing Cladograms or Phylograms.

AlignX Module: rapid multiple sequence alignment with minimal preparation. AlignX uses a modified ClustalW algorithm to generate multiple sequence alignments of either protein or nucleic acid sequences for similarity comparisons and for annotation. The power of AlignX is that it maintains annotated features within the alignment for easy visualization and localization of regions of interest.

RESULTS AND DISCUSSION

Phylogenetic relationships among influenza viruses

The *Orthomyxoviridae* is a family of RNA viruses with a single-stranded segmented RNA ranging from six to eight fragments with three major genera, *Influenzavirus*, *Isavirus*, and *Thogotovirus*. The first genus includes some of the most important human viral pathogens. Influenza viruses A (eight RNA segments), B (eight RNA segments), and C (seven RNA segments) are responsible worldwide for most respiratory disease epidemics and are associated with thousands of deaths annually (Murphy and Webster, 1996).

The whole proteome (10 proteins) of the influenza A virus [A/Chicken/Hong Kong/258/97 (H5N1)] isolate was downloaded from (http://www.ncbi.nlm.nih.gov/) and used to search the RefSeq database for similar sequences. Each segment showed high similarity with several types of influenza viruses. Of the three virus types, A and B viruses are much more similar to each other in protein homology than to C viruses.

The general structural features and genome organization of influenza A, B, and C viruses suggest that they share a common ancestry distinct from other negative-strand RNA viruses (Desselberger *et al.,* 1980). The virus cannot proofread its RNA for errors resulted in high mutation

load (Robert *et al.,* 2008). These accumulated mutations selectively permit influenza to partially evade a host's immune system, so that, new strains and lineages belong to the influenza A, B, and C viruses could be produced (Wong *et al.*, 2006).

Phylogenetic relationships between influenza A viruses and Salmonella typhi

In addition to the observed similarities among the different types of influenza viruses, BLAST search of six influenza A virus [A/Chicken/Hong Kong/258/97 (H5N1)] segments surprisingly showed high significant similarity (>90%) with 12 sequences belonging to *Salmonella typhi*. Four of the similar *Salmonella typhi* protein sequences similar to viral polymerase subunits, PB2 (produced by viral genome segment No.1) and PA (produced by viral genome segment No.3), two *Salmonella* protein sequences for each polymerase subunit were downloaded to build four datasets each set contains protein sequences similar to one protein sequence of the four *Salmonella typhi* protein sequences. Multiple sequence alignment (MSA) was performed with AlignX to find the best MSA and to construct phylogenetic tree within each dataset (Figures 1-4).

The MSA and phylogenetic relationship results revealed that, the Zp03359657 and Zp03374490 hypothetical proteins belonging to *Salmonella typhi* shared similarities with polymerase subunit PB2 produced by the viral segment No.1. Moreover the Zp03359711 and Zp03348148 hypothetical proteins shared similarities with PA polymerase subunit produced by the viral segment No.3.

The *Salmonella typhi* Zp03359657 (84 amino acid) MSA and phylogenetic tree (Fig. 1) showed strong relationship with viral PB2 polymerase subunit protein belonging to different influenza A viruses [A/Goose/Guangdong/1/96(H5N1), A/Puerto Rico/8/34(H1N1), A/Hong Kong/1073/99(H9N2), A/Korea/426/68(H2N2) and A/New York/392/2004(H3N2)], with identities (98%, 96%, 96%, 93% and 91%), respectively. While the lowest identity was obtained between the *Salmonella typhi* Zp03359657 and the PB2 polymerase subunit protein belongs influenza B viruses (38%).

The MSA and phylogenetic tree of *Salmonella typhi* Zp03374490 (37 amino acid) revealed 100% identity with viral PB2 polymerase subunit of five different influenza A viruses including human and avian viruses [A/Goose/Guangdong/1/96(H5N1), A/HongKong/1073/99(H9N2), A/Korea/426/68(H2N2), A/New York/392/2004(H3N2), and A/Puerto Rico/8/34(H1N1)]. The lowest identity (54%) was obtained with influenza B viruses (Fig. 2).

Salmonella typhi Zp03359711 (50 amino acid) hypothetical protein MSA and phylogenetic tree (Fig. 3) showed 100% identity with viral PA polymerase subunit of [A/Goose/Guangdong/1/96(H5N1 and A/Korea/426/68(H2N2))] and high similarity (97%, 97% and 95%) with [A/Hong Kong/1073/99(H9N2), A/New York/392/

2004(H3N2) and A/Puerto Rico/8/34 (H1N1)] PA polymerase subunit, respectively.

Figure (4) illustrated that, the MSA and phylogenetic tree of Zp03348148 (36 amino acid) hypothetical protein showed high similarity (97%, 94%, 94%, 94% and 91%) with viral PA polymerase subunit of [A/Goose/Guangdong/1/96(H5N1), A/Korea/426/68(H2N2), A/New York/ 392/2004(H3N2), A/Puerto Rico/8/34 (H1N1) and A/Hong Kong/1073/99 (H9N2)], respectively. On the other hand Zp03348148 revealed low similarity (41%) with PA polymerase subunit of influenza B viruse.

The mentioned results (Figures 1-4) revealed that, *Salmonella typhi* is the organism who had acquired partial viral sequences, because the complete gene sequences are known for different types of influenza viruses. The complete gene and protein sequences could not be found in *Salmonella typhi*, only short sequences (hypothetical proteins).

Influenza A virus PB1 polymerase subunit protein didn't show any similarity with any of *Salmonella typhi* sequences. On the other hand no similarities were found between the under investigation *Salmonella typhi* sequences and other *Salmonella* strains.

Salmonella enterica typhi (referred to as *Salmonella typhi*) is the causative agent of worldwide, typhoid fever that affects roughly 17 million people annually, causing nearly 600,000 deaths. *Salmonella enterica* is a major cause of gastroenteritis in humans. These facultative intracellular pathogen infections are originated in food-producing animals (cattle pig and poultry species), that are infected with *S. enterica* (Humphrey, 2004; Mastroeni *et al.*, 2009). Members of the public are less likely to know that domestic pets, birds, rodents and cold blooded animals including tropical fish and reptiles, also harbor *S. enterica* (Ward, 2000).

It is important to mention that, the term viteria (bacteria-related sequences) was chosen to describe stealth-adapted viruses that had acquired bacterial genes. The term vifungus was also introduced since some of the novel sequences in the stealth-adapted virus culture were of apparent fungal origin (Martin, 2005).

The highly similar sequences between different influenza A viruses and *Salmonella typhi* could be explained by the possibility that, influenza A virus had acquired the capacity to recombine with *Salmonella typhi* genetic sequences *via* nonhomologous recombination and presumably had overcome the normal barrier restricting eukaryotic virus growth in prokaryotes. This argument was strengthened by the fact that, both avian influenza A virus and *Salmonella typhi* are intestinal and intracellular pathogens, that can infect human, pigs and avian species (Matrosovich *et al.*, 2004; Mastroeni *et al.*, 2009). Furthermore bacterial super infections after viral infections have been studied extensively in human and animals (Beadling and Slifka, 2004) meaning that, both

of them can be found in the same cell at the same time. The consequences of non-homologos recombination between viral RNA segments and *Salmonella typhi* could be resulted in highly pathogenic strains of *Salmonella typhi* and influenza A viruses.

Many recombinant RNA virus strains provide ample indication that recombination can generate beneficial new variation. In some viruses this new variation is achieved by borrowing genetic material from their hosts. Influenza A virus has been observed to recombine with cellular RNA, resulting in increased pathogenicity for the hybrid viruses (Khatchikian *et al*., 1989). Recombination between virus and host genetic material evidently occurs in plant viruses as well as illustrated by a luteovirus isolate with 5-terminal sequence derived from a chloroplast exon (Mayo and Jolly, 1991) and closteroviruses which have acquired host cellular protein-coding genes (Dolja *et al*., 1994) which are nonessential for replication and virion production (Peremyslov *et al*., 1998).

Horizontal gene transfer is the transfer of genetic material between cells or genomes belonging to unrelated species, by processes other than usual reproduction. In the usual process of reproduction, genes are transferred vertically from parent to offspring; and such a process can occur only within a species or between closely related species. Horizontal gene transfer, where a significant proportion of the coding sequence is contributed by external sources, might give rise to extremely dynamic genomes, which brings impact on the ecological and pathogenic characters of the recipient organisms. The results of this study likely will encourage scientists in several fields to rethink their approach to the study of host-virus systems, which are believed to play a key evolutionary role by facilitating the transfer of genes between species.

SUMMARY

Influenza A virus causes annual epidemics and every 10 to 50 years, at unpredictable intervals, causes major pandemics. It is able to generate a high degree of genetic diversity by the high mutation rate, the ability of gene segments to reassort, recombination and the huge pool of influenza viruses in birds and mammals explain their changing behavior and the difficulty in developing a permanent, long-lasting, and effective vaccine. The whole proteome of the influenza A virus [A/Chicken/Hong Kong/258/97(H5N1)] was used to search refseq database for similar sequences. Each segment showed high similarity to several types of influenza viruses. In addition to the observed similarity among the different types of influenza viruses, BLAST search showed high similarity between different influenza A virus [A/Chicken/HongKong/258/97 (H5N1)] proteins and twelve sequences belonging to *Salmonella typhi*. Only four from the twelve *Salmonella typhi* sequences were chosen for further analysis.

BLAST search and multiple sequence alignment for these four sequences had identified high significant homology to PB2 and PA polymerase subunits (two *Salmonella* sequences for each polymerase subunit). These findings highlight the dynamic interface between bacterial and viral genomes and the potential of this interaction in the emergence and spread for novel and more virulent viral and bacterial pathogens.

REFERENCES

Alexander, D. J. and I. H. Brown (2000). Recent zoonoses caused by influenza A viruses. Rev Sci Tech., 19: 197-225.

Bao, Y., P. Bolotov, D. Dernovoy, B. Kiryutin, L. Zaslavsky, T. Tatusova, J. Ostell and D. Lipman (2008). The influenza virus resource at the national center for biotechnology informatio J. Virol., 82: 596-601.

Beadling, C. and M. K. Slifka (2004). How do viral infections predispose patients to bacterial infections? Curr. Opin. Infect. Dis., 17: 185-191.

Castrucci, M. R., L. Campitelli, A. Ruggieri, G. Barigazzi, L. Sidoli, R. Daniels, *et al.,* (1994). Antigenic and sequence analysis of H3 influenza virus haemagglutinins from pigs in Italy. J. Gen. Virol., 75: 371-379.

Desselberger, U., V. R. Racaniello, J. J. Zazra and P. Palese (1980). The 3'- and 5'-terminal sequences of influenza A, B and C virus RNA segments are highly conserved and show partial inverted complementarity. Gene, 8: 315-328.

Dolja, V. V., A. V. Karasev and E. V. Koonin (1994). Molecular biology and evolution of closteroviruses : sophisticated buildup of large RNA genomes. Annual Review of Phytopathology, 32: 261-285.

Higgins, D. G. and P. M. Sharp (1988). CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. Gene, 73: 237-244.

Huang, T. S., P. Palese and M. Krystal (1990). Determination of influenza virus proteins required for genome replication. J. Virol., 64: 5669-5673.

Humphrey, T. (2004). Salmonella,stress responses and food safety. Nat. Rev. Microbiol., 2: 504-509.

Khatchikian, D., M. Orlich and R. Rott (1989). Increased viral pathogenicity after insertion of a 28S ribosomal-RNA sequence into the hemagglutinin gene of an influenza virus. Nature, 340: 156-157.

Kida, H., T. Ito, J. Yasuda, Y. Shimizu, C. Itakura, K. F. Shortridge, *et al.,*

(1994). Potential for transmission of avian influenza viruses to pigs. J. Gen. Virol., 75: 2183-2188.

Lai, M. M. (1992). RNA recombination in animal and plant viruses. Microbiological Reviews, 56: 61-79.

Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. Mcwilliam, F. Valentin, I. M. Wllace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibsom and D. G. Higgins (2007). ClustalW and ClustalX version 2.0. Bioinformatics Applications Note, 23: 2947-2948.

Laver, W. G., G. M. Air, R. G. Webster and S. J. Smith-Gill (1990). Epitopes on protein antigens: misconceptions and realities. Cell, 61: 553-556.

Marjuki, H., H. Yen, J. Franks, R. G. Webster, S. Pleschka and E. Hoffmann (2007). Higher polymerase activity of a human influenza virus enhances activation of the hemagglutinin-induced Raf/MEK/ERK signal cascade. J. Virol., 4: 134-153.

Martin, W. J. (2005). Alternative cellular energy pigments from bacteria of stealth virus infected individuals. Experimental and Molecular Pathology, 78: 215-217.

Mastroeni, P., A. Grant, O. Restif and D. Maskell (2009). A dynamic view of the spread and intracellular distribution of *Salmonella enterica*. Nat. Rev. Microbiol., 7: 73-80.

Matrosovich, M. N., T. Y. Matrosovich, T. Gray, *et al.*, (2004). Human and avian influenza (AI) viruses target different cell types in cultures of human airway epithelium. Proc. Natl. Acad. Sci., 101: 4620-4624.

Mayo, M. A. and C. A. Jolly (1991). The 5'-terminal sequence of potato leafroll virus RNA: evidence of recombination between virus and host RNA. J. General Virol. 72: 2591-2595.

Morens, D. M., K. Jeffery, Taubenberger, and S. F. Anthony (2009). The persistent legacy of the 1918 influenza virus. J. New England of Medicine, 361: 225-229.

Murphy, F. A. and R. G. Webster (1996). Orthomyxoviruses. Fields BN, Knipe DM, Howley PM, eds. Virology. Second edition. New York: Raven Press, 1091-1152.

Oliveira, E. C., L. Burton and L. C. Gene (2003). Influenza in the intensive care unit journal of intensive care medicine, 18: 80-91.

Peremyslov, V. V., Y. Hagiwara and V. V. Dolja (1998). Genes required for replication of the 15.5-kilobase RNA genome of a plant closterovirus. J. Virol., 72: 5870-5876.

Portela, A. and P. Digard (2002). The influenza virus nucleoprotein: a multifunctional RNA-binding protein pivotal to virus replication. J Gen. Virol., 83: 723-734.

Posada, D., A. C. Keith and C. H. Edward (2002). Recombination in evolutionary genomics. Annu. Rev. Genet., 36: 75-97.

Robert, B., A. Gardner, A. Rambaut and O. G. Pybus (2008). Pacing a small cage: mutation and RNA viruses. Trends in Ecology and Evolution, 23: 188-193.

Rogers, G. N. and J. C. Paulson (1983). Receptor determinants of human and animal influenza virus isolates: differences in receptor specificity of the H3 hemagglutinin based on species of origin. Virology., 127: 361-73.

Taubenberger, J. K. and D. M. Morens (2006a). Influenza revisited. Emerg Infect Dis., 12: 1-2.

Taubenberger, J. K. and D. M. Morens (2006b). 1918 Influenza: the mother of all pandemics. Emerg Infect Dis., 12: 15-22.

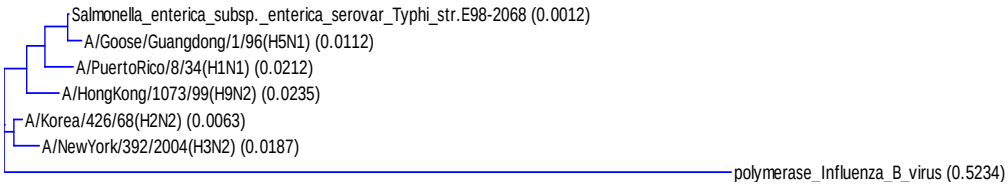
Ward, L. (2000). *Salmonella perils* of pet reptiles. Commun. Dis. and Public Health, 3: 1-2.

Webster, r. G., W. J. Bean O. T. Gorman, T. M. Chambers and Y. Kawaoka (2002). Evolution and ecology of influenza a viruses. Microbiological Reviews, 56: 152-179.

Wong S. S. Y., M. R. C. Path and K. Yuen (2006). Avian influenza virus infections in humans, Chest, 129: 156-168.

Worobey, M. and E. C. Holmes (1999). Evolutionary aspects of recombination in RNA viruses. J. Gen. Virol., 80: 2535-2543.

A



B

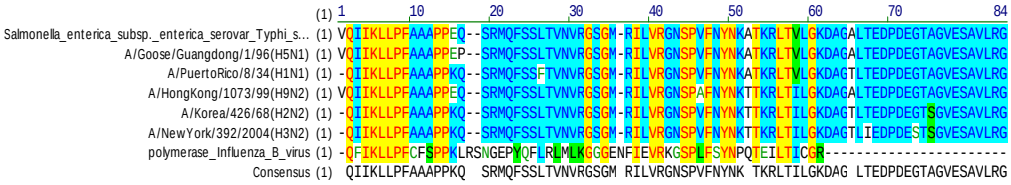
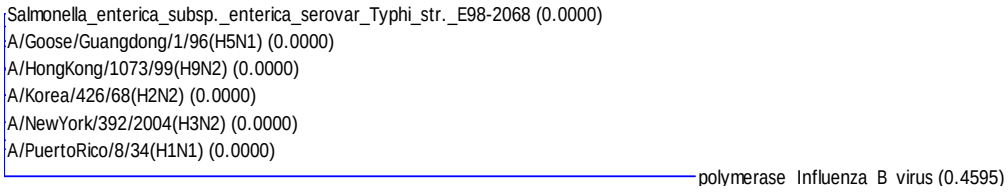


Fig. (1): Phylogenetic tree (A) and multiple sequence alignments (B) for the relationships between *Salmonella typhi* fragment (Zp03359657) and different PB2 segments from different influenza viruses, consensus sequence represents the most common sequence.

A



B

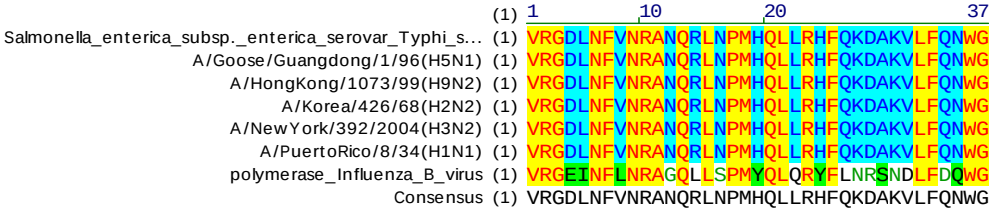


Fig. (2): Phylogenetic tree (A) and multiple sequence alignments (B) for the relationships between *Salmonella typhi* fragment (Zp03374490) and different PB2 segments from different influenza viruses, consensus sequence represents the most common sequence.

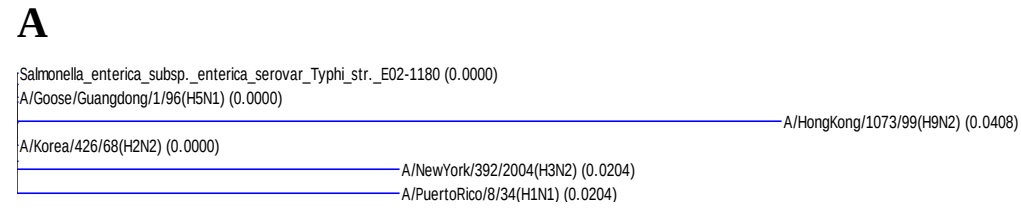


Fig. (3): Phylogenetic tree (A) and multiple sequence alignments (B) for the relationships between *Salmonella typhi* fragment (Zp03359711) and different PA segments from different influenza viruses, consensus sequence represents the most common sequence.

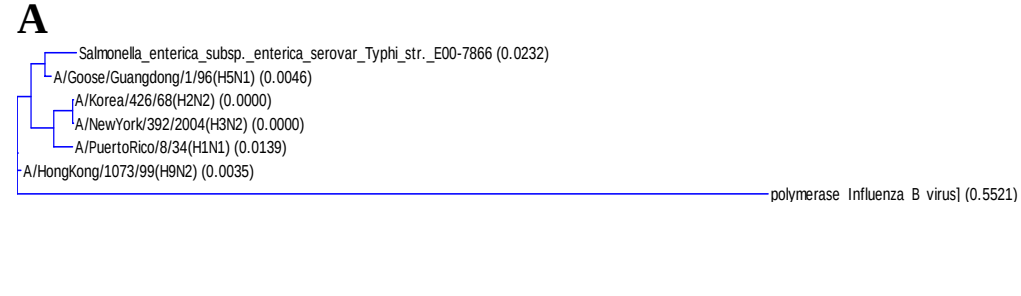


Fig. (4): Phylogenetic tree (A) and multiple sequence alignments (B) for the relationships between *Salmonella typhi* fragment (Zp03348148) and different PA segments from different influenza viruses, consensus sequence represents the most common sequence.