

Influenza

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Taxonomy

Influenza viruses belong to a family *Orthomyxoviridae*, family of **single-stranded, negative-sense RNA** viruses causing **influenza** and other **respiratory diseases**. Viruses are named according to their type, host organism, location, the number of isolate from that location, year of isolation and subtype, for example: A/shorebird/Delaware/224/2006(H13N9).

Viruses - ssRNA viruses - ssRNA negative-strand viruses - *Orthomyxoviridae*

- **Orthomyxoviridae**

- **Influenzavirus A**

Aquatic birds are the evolutionary hosts and the major reservoir of influenza A viruses. These **zoonotic** viruses exhibit great genetic diversity and infect the wide range of host species including humans. They caused several pandemics that resulted in unprecedented

- **Influenza A virus**

Antigenic variation occurs frequently between strains, allowing classification into subtypes and variants. Transmission is usually by aerosol (human and most non-aquatic hosts) or waterborne (ducks). Infected birds shed the virus in their saliva, nasal secretions, and feces. Subtype name is formed by combination of abbreviation of key glycoproteins and their types: **H[number]** - hemagglutinin's type and **N[number]** - neuraminidase's type. There are 15 distinct HAs (numbered 1-15) and nine NAs (1-9) that define the specific subtype of influenza A viruses. Only three subtypes (H1, H2, and H3) and two NA subtypes (N1, N2) have caused human influenza epidemics.

- **H9N2 Subtype**

Usually infects domestic birds but there have been some human infections reported.

- **H7N7 Subtype**

Produced an epidemic in 2003 which was highly pathogenic among domestic birds. Some infections in humans were reported.

- **H5N2 Subtype**

Highly pathogenic in chickens.

- **H5N1 Subtype**

Frequently referred to as the **bird flu virus**, is endemic in wild birds and very contagious among both domestic and wild birds. Was responsible for avian flu outbreaks in 1968 and 1997. In 1997 the strain passed from chickens to humans in Hong Kong, causing the death of 6 people (out of 18) and leading to the destruction of some millions chickens. In 2003-2004 the strain re-surfaced in poultry farms throughout Asia. This subtype has limited transmissibility from birds to humans, and unable to pass from human to human.

- **H3N8 Subtype**

Has frequently been found in horses. A stable lineage of A/H3N8 influenza virus emerged in dogs in the United States following a host switch event without reassortment from the equine A/H3N8.

- **H3N2 Subtype**

Was responsible for the Hong Kong flu pandemic of 1968.

- H2N2 Subtype

Was responsible for the Asian flu pandemic of 1957.

- H1N1 Subtype

- "Spanish flu"

Caused pandemic of 1918. Unusual features: extremely virulent, affected young adults, unusually high occurrence of post-flu encephalitis (**encephalitis lethargica**) that affected ~5 million people worldwide killing one third and leaving another third with severe disability.

- S-OIV A (2009)

Swine origin influenza virus A. Outbreak, which has become apparent in April, 2009 in Mexico, later, was declared a pandemic.

- Influenzavirus B

Viruses that cause a disease with a similar clinical presentation as influenza A virus but less severe. Also, no distinct antigenic subtypes of hemagglutinin and neuraminidase are recognized. Pandemics did not occur.

The influenza B virus was isolated in 1939 by Francis.

- **Influenza B virus**

Antigenic variation is less extensive than in type A viruses and consequently there is no basis for distinct subtypes or variants. Epidemics are less likely than with type A viruses. Previously only found in humans, Influenza B virus has been isolated from seals.

- **Influenzavirus C**

Viruses similar to types A and B but less common, more stable, more homogeneous, and lacking the neuraminidase protein. They have not been associated with epidemics but may cause mild upper respiratory disease. The influenza C virus was isolated in 1950 by Taylor.

- **Influenza C virus**

Type species of Influenzavirus C.

- **Isavirus**

Contains one species: Infectious salmon anemia virus.

- **Infectious salmon anemia virus**

Infectious salmon anemia (ISA) virus (ISAV), an economically important new pathogen in marine aquaculture. ISAV causes fatal systemic infections in marine-farmed Atlantic salmon and

asymptomatic infections in feral fish.

- **Thogotovirus**

Tick-borne viruses occasionally infecting humans. Dhori and Thogoto viruses were formerly thought to be members of *Bunyaviridae*. Thogoto virus is the type species.

- **Dhori virus**

Mice infected with Dhori virus (DHOV) develop a fulminant, systemic, and uniformly fatal illness that has many of the clinical and pathologic findings seen in H5N1 influenza A virus infection.

PMID: 18385368

- **Thogoto virus**

Thogoto virus is the type species.

Brief facts

- First documented influenza pandemic began in Sicily in 1510, spread to Italy, and then to the rest of Europe.
- The incubation period of influenza A virus is from 1 to 5 days. The onset of clinical symptoms such as fever, myalgia, and a headache, is sudden and powerful. Tracheobronchitis, sore throat, unproductive cough, and rhinorrhea follow. Secondary infections can cause complications. In severe cases, patients can suffer from pulmonary, central nervous system, and other complications.
- Viral influenza is most severe in pregnant women, the very young, the elderly, and immunosuppressed individuals.
- More people have died from influenza than from any other infectious disease:

because of high virulence of influenza A viruses, usually very large segments of the population are affected, which leads to a staggering number of deaths even at a mortality rate as low as 1%. Three pandemics occurred in the past century: the Spanish influenza pandemic in 1918-1919, which claimed an estimated 50 million lives, and the Asian (1957) and Hong Kong (1968-1969) pandemics that resulted in 1-2 million deaths each.

- The virus has unique capacity for genetic variation that is based on 2 features: (1) the surface proteins are able to mutate up to 50% of their amino acid sequence simultaneously preserving their functionality; (2) the viral genome consists of eight independent RNA segments, which can almost randomly re-assort in organisms infected with more than one virus subtype, thus giving birth to hybrid genotypes.
- The severity of epidemic or pandemic is proportional to the susceptibility of the human population, which in turn positively correlates with "novelty" of the viral composition, especially with a degree of change in the surface proteins, the H and N antigens. The greater the change, the lower the **herd immunity** of the entire population.
- The influenza vaccine is still produced by methods developed in 1930-50s by growing virus in embryonated chicken eggs. Each dose of flu vaccine requires the use of 1.2 live eggs, or about 600 million embryonated eggs to produce 500 millions dose of vaccine for 6.77 billion people. Seasonal vaccines are trivalent containing 15 µg each of two influenza A (H1N1 and H3N2) and one B strain.
- Ferrets are susceptible to most strains of influenza that infect humans without adaptation, including both types A and B. It causes similar symptoms (runny nose, sneezing, conjunctivitis, etc.) and runs a similar time course. It is why ferrets are the ideal small animal model for influenza research and a lot of viral transmission studies were done using these animals.

Anatomy of influenza virus

The genome of influenza A virus (~13 kb) has eight segments: six code for internal proteins and two for the surface proteins. Each segment contains a coding region that encodes one or two proteins, as well as short 5' and 3' flanking sequences.

- Polymerase complex

- Basic polymerase 2 (PB2)

Segment 1. PB2 controls the recognition of host-

cell RNA; 2,277 nucleotides in the protein-coding region.

- **Basic polymerase 1 (PB1)**

Segment 2. PB1 catalyses nucleotide addition, and also, in a different reading frame (ORF), encodes a small apoptotic mitochondrial protein - PB1-F2. PB1-F2 protein has been shown to specifically target and destroy alveolar macrophages. The novel 2009/A/H1N1 has truncated PB1-F2, which should weaken its virulence. The segment 2 consists of 2,271 nucleotides.

- **Acidic protein (AP)**

Segment 3 (2,148 nucleotides). The AP might possess transcriptase protease activity.

- **Surface envelope glycoproteins**

- **Hemagglutinin (HA)**

Segment 4 (1,698 nt). Denoted as HA (or H) is surface glycoprotein that is responsible for viral binding and entry into host epithelial cells. HA contains two domains (HA1 and HA2) and several important **epitopes** - parts of the viral protein that are recognized by either cellular or humoral arms of the immune system, and that undergo constant changes for virus to be able to breach host's immune defenses. H is the principal component of any influenza vaccine. For virus to be infectious, the HA must undergo post-translational cleavage into two peptides, **HA1** and **HA2**, by a trypsin-like protease. Modifications in cleavage mechanism and/or site can lead to drastic changes in the virus

pathogenicity. For example, influenza A viruses subtypes H5 and H7, usually asymptomatic in wild waterfowl, may become highly pathogenic once introduced into domestic poultry. Acquisition of extra amino acids at the cleavage site transforms the virus from one in which cleavage activation of the HA is restricted to the respiratory and intestinal tract, where there are trypsin-like proteases, into one that can be activated by cell-associated proteases that are found throughout the body, resulting in so-called viral **pantropism**.

- **Neurominidase (NA)**

Segment 5 (1,407 nucleotides). The NA constitutes 20-25% of the surface of the viral particle. The NA is an enzyme named for its ability to cleave neuraminic or sialic acid from complex carbohydrates such as mucin. NA is responsible for the release of influenza particles from the infected cells, and thus, is important as a spreading factor, and is targeted by the current antiviral drugs **oseltamivir (Tamiflu)** and **zanamivir (Relenza)**. The NA antigen is second most important component of anti-flu vaccine.

- **Nucleoprotein (NP)**

Segment 5 (1,494 nucleotides). This protein binds to the viral RNA.

- **Matrix proteins**

Segment 7 codes two proteins that share a short overlapping region.

- **Matrix protein M1 (M1)**

A span of 756 nucleotides encodes the main component of viral capsid.

- Matrix protein M2 (M2)

A span of 291 nucleotides encodes an integral membrane protein that functions as an ion channel.

- Non-structural proteins

Segment 8 (smallest) codes two proteins that share an overlapping region.

- Non-structural protein 1 (NS1)

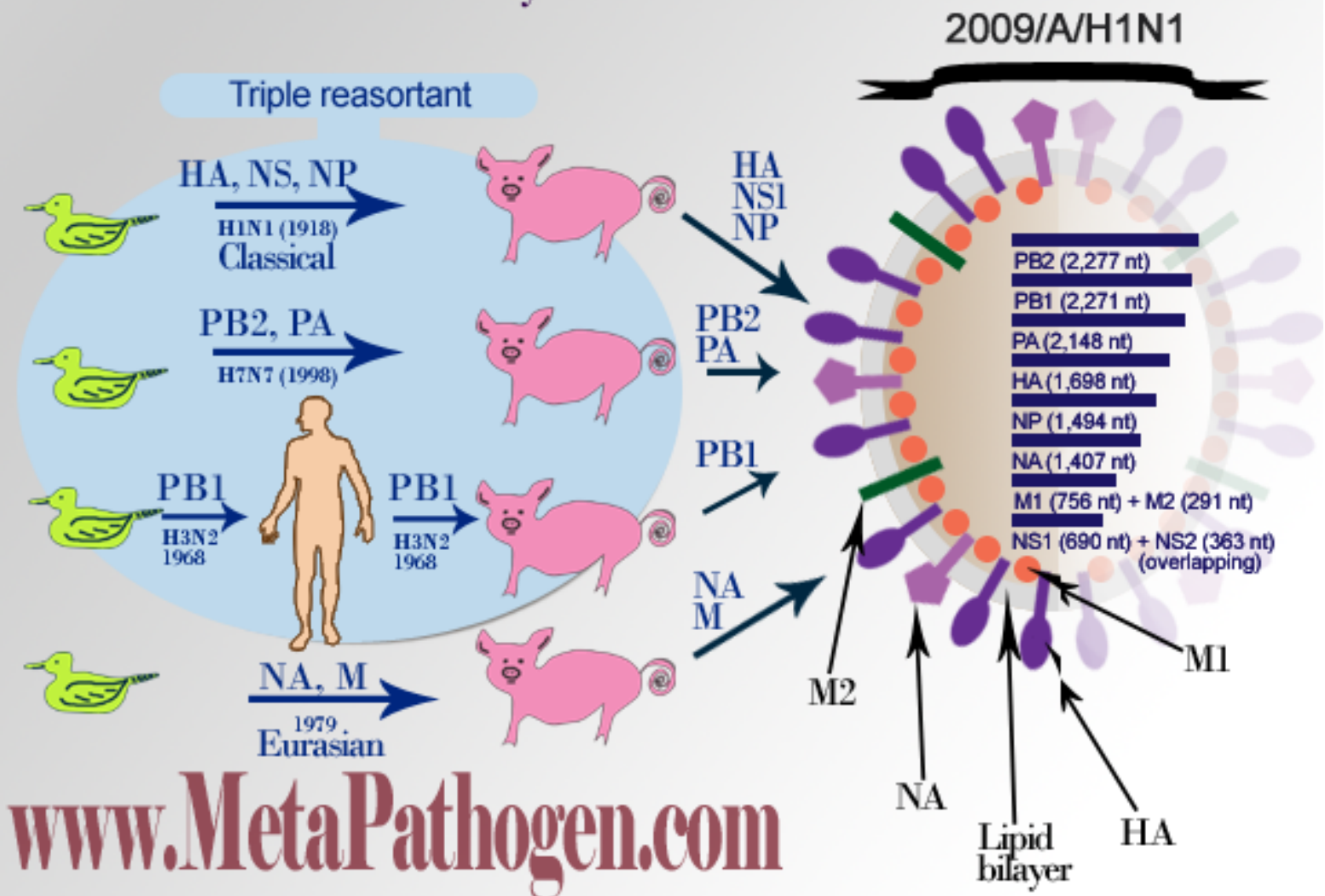
A span of 690 nucleotides encodes the NS1, which affects cellular RNA transport, splicing and translation. The novel 2009/A/H1N1 virus has a truncated version of this protein.

- Non-structural protein 2 (NS2)

A span of 363 nucleotides encodes a minor component with currently unknown function.

The origin of 2009A/H1N1 components

General anatomy of Influenza A virus



Types of genetic variations in influenza viruses

- **Antigenic drift** constitutes minor incremental changes in viral surface proteins (antigens). In the course of continual evasion of host's immune responses, viruses accumulate mutations in the HA and NA surface glycoproteins thus modifying their antigenic makeup. The antigenic drifts within single species usually result in mild outbreaks. However, antigenic drift that lead to adaptation of zoonotic virus to the human host, might result in a major pandemic.
- **Re-assortment** (also can be referred as an **antigenic shift**) is a form of recombination in which two or more influenza viruses, of same or of different subtypes, co-infect a single cell and exchange their RNA segments to form genetically novel viruses. Antigenic shifts frequently result in pandemics. The

novelty of 2009/A/S-OIV constitutes a major **antigenic shift**. Pigs have been hypothesized to serve as a mixing vessel in which avian and human influenza A viruses reassort. Swine are very susceptible to infection with avian flu viruses, which further facilitated by proximity to natural water reservoirs inhabited by wild fowl. This has been attributed to the fact that swine tracheal epithelium expresses both α 2,3- and α -N-acetylneuraminic acid-galactose linked receptors. The former, found in waterfowl enteric tract, bind avian influenza A viruses, and the latter, located in the human respiratory tract, bind human host enabled influenza A viruses, thus facilitating occurrence of co-infections and making re-assortment possible.

- To cause a pandemic among humans the zoonotic influenza virus should undergo genetic modifications that would lead to a **host switch**. The host switch means that the virus not only can pass from birds or animals to humans, but also acquires human-to-human transmissibility. The host switch can occur as a result of incremental changes in the process of **adaptation** to the foreign host (antigenic drift) as well as a result of accidental re-assortment between two or more viral subtypes (antigenic shift). For example, the 1918 virus may have arisen wholly from an avian influenza virus by adaptive mutations, whereas, 1957 and 1968 pandemic viruses resulted from re-assortments.

S-OIV A (H1N1) Swine origin influenza virus A

- **Re-assortment (novel gene combination) in 2009/A/H1N1**

Phylogenetic analysis of sequences of all genes of A/California/04/2009, the virus isolated from the recent outbreak in USA, showed that its genome represents a quadruple re-assortment of two swine strains, one human strain, and one avian strain of influenza A. The largest proportion of genes in this novel virus comes from swine influenza A viruses: 30.6% from North American swine influenza ("Classical influenza") and 17.5% from Eurasian swine influenza A. As it was mentioned above influenza has 8 genes: six code for internal proteins and two for the surface proteins (HA and NA). Before now all of the triple reassortant viruses contained the same internal genes, which were referred as **triple reassortant internal gene (TRIG)** cassette. Presence and stability of TRIG was considered as a fitness feature of virulent influenza A viruses. The novel H1N1 virus has a key difference from the triple reassortants in North American pigs: two of its genes trace back to Eurasian swine: one codes for NA, which is routinely replaced in TRIG-holding viruses, but the other codes for matrix, and internal protein. So the new virus violates TRIG.

- **Antigenic shift in 2009/A/H1N1 genes**

The sequence of H of 2009/A/H1N1 (and, correspondingly, the antigen in the current vaccine) is 27.2% different from the human H1N1 virus circulating in 2008, 18% different from the 1918 pandemic influenza virus, and 12% different from swine flu outbreak in Fort Dix, NJ in 1976. All five of the known antigenic sites on the protein are unique, and no human herd immunity is to be expected among human population of 6.77 billion.

N antigen in 2009/A/H1N1 influenza is also significantly novel, differing by 18.2% the 2008 H1N1 virus.

- **Symptoms**

Symptoms common with other flu viruses: fever (94%), cough (92%), and sore throat (66%). Not typical manifestations: high occurrence of diarrhea (25%) and vomiting (25%).

- **Affected population**

The vaccination against recent seasonal influenza H1N1 does not elicit a protective antibody response to the novel influenza A (H1N1) virus. There are indications that about one third of those aged >60 years may have had preexisting cross-reactive antibodies, which may explain why only 5% of S-OIV A (H1N1) patients were older than 52 years. Also the virus strikes predominantly younger people: 60% were younger than 18 years old.

- **Severity and transmissibility**

Clinical severity appears less than seen in 1918 but comparable with that in 1957. **Reproduction number (R₀)** is defined as the average number of individuals infected by a primary infected individual. Epidemiological analyses estimated S-OIV R₀ to be between 1.4 and 1.6. The values are considerably lower than that for the 1918-1919 pandemic strain (mean R₀ = ~ 2 (1.4 - 2.8)) and are comparable with seasonal strains of influenza (mean R₀ = ~ 1.3 (0.9 to 2.1)).

Therapy and prevention

- **Treatment and prevention with anti-viral agents**

Currently, two classes of anti-viral medications that target NA and M proteins are available for prophylaxis and treatment of influenza viruses:

amantadine/rimantadine and **osteltamivir/zanamivir (Tamiflu and Relenza, respectively)**. Novel influenza 2009/A/H1N1 is resistant to the former but sensitive to the latter. Based on experience with other flu viruses, treatment should be most effective if given no later than 2 days within of symptoms onset. The current seasonal H1N1 virus is resistant to Tamiflu.

This fact raises concerns that the two strains can re-assemble in individuals that carry both of them and give rise to multidrug resistant strain.

- **Prevention by vaccination**

Although cell-mediated immunity plays an important role in recovery from infection, specific immunity to influenza is principally **humoral** (antibody-based). Antibodies directed against HA protect from infection, whereas anti-NA antibodies limit the spread of the infection. Degree of immunity largely is strain-dependent. Immunity developed by host for one strain of circulating subtype is much weaker for the strain that is evolved next year. This necessitates an annual review of vaccine formulations. Individuals who previously were exposed to the current subtype become partially immune to its emerging replacement, and only one dose of new vaccine is usually sufficient to protect them from seasonal epidemic. In the event of pandemic when no immunity exists in most or all of the human population successful immunization usually requires two vaccine doses against the pandemic virus strain in addition to annual routine single-dose vaccine against seasonal flu epidemic.

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