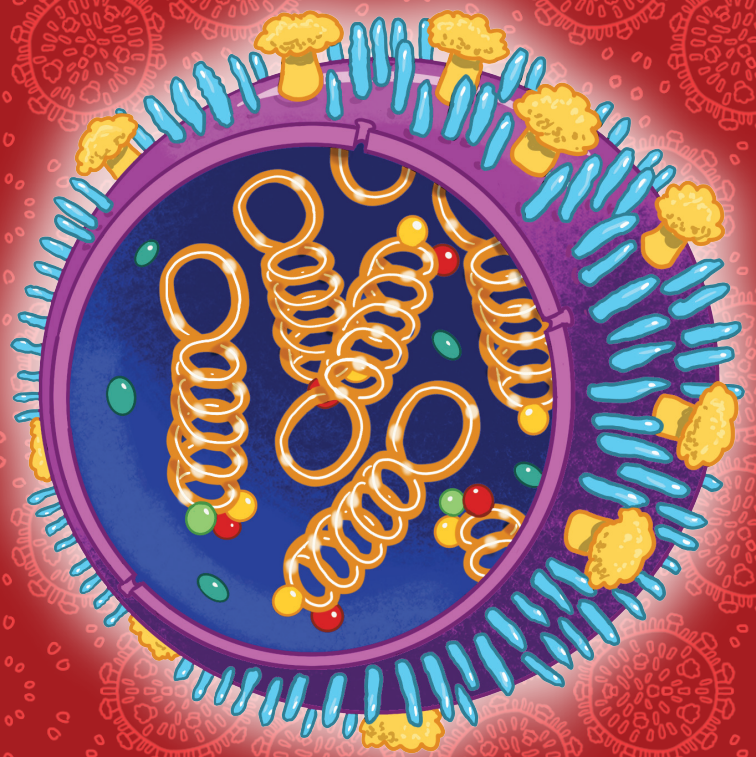
INFLUENZA VIRUS

A CHALLENGE TO THE IMMUNE SYSTEM AND TO MEDICINE IN THE LIGHT OF EVOLUTION



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INFLUENZA VIRUS:

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INFLUENZA IN HISTORY

The arrival of autumn each year brings new flu outbreaks[1], a "season" which lasts over autumn and winter. The seasonality of the disease can be explained by prevailing weather conditions of higher humidity and lower temperatures, which favour the spread of the virus that causes the disease, and the fact that we also become more susceptible to transmission of the virus by spending more time indoors in closed environments.

This disease is known as the flu or influenza. The word influenza — which is also the name of the virus that causes the disease —, reflects the seasonality of the disease and is derived from an Italian word meaning "influence". The disease was given this name because doctors in the Middle Ages believed the disease was caused by the "influence" of the stars[2].

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[1] Outbreak: a sudden increase of occurrences of a disease in a certain geographic region; a synonym of epidemic.

[2] The Portuguese word for the disease — gripe — is derived from the French grippe, which means "a tight hold", reflecting the sudden way in which initial symptoms manifest themselves.

Although there are flu outbreaks every year, some outbreaks or seasons involve higher numbers of cases and infected people than others. In some cases, global outbreaks known as pandemics occur, as was the case of the terrible pandemic of 1918 (also known as the "Spanish Flu") that killed more young adults than the First World War. Other examples include the pandemic of 1957-1958 (also known as the "Asian Flu"), the pandemic of 1968 (also known as the "Hong Kong" flu), and the more recent pandemic of 2009.

The 1918 pandemic was one of the most severe pandemics ever recorded. It is estimated that 500 million people were infected (1/3 of the world's population at the time) and that it killed at least 50 million[3] people between the spring of 1918 and the winter of 1919. The pandemic emerged at the end of the First World War. Spain, which had remained neutral during the war, was the only country where the media reported freely on the disease, leading it to become known as the "Spanish Flu", even though it did not originate in Spain. It is not yet known where the pandemic started: northern Europe, France, China and the USA all experienced outbreaks earlier than that of Spain. The 1918 pandemic was also unusual in that most fatalities occurred among young adults aged between 20 and 40, rather than

[3] In 2019, the population of Portugal was 10.2 million people, while that of the UK was 66.6 million.

among the more typically vulnerable segments of small children and the elderly. We know now that the pandemic was caused by an H1N1 strain and research is still underway to discover why this strain was so deadly.

The 1957-1958 pandemic was recorded for the first time in 1957 in East Asia and caused between one and two million deaths worldwide. This epidemic was caused by a H2N2 strain that emerged from a genomic mix of human and avian strains of the virus. The 1968 pandemic originated in Hong Kong and was caused by a new viral strain — H3N2 — which was a novel mix of viral genome segments, much like the previous pandemic. It is estimated that this pandemic caused a million deaths. Another pandemic arose in 2009, this time caused by a novel H1N1 strain that contained genetic material from human, avian and swine influenza strains, which, although not as deadly as the Spanish Flu, was highly contagious.

Some strains of influenza viruses infect avian species such as chickens and ducks, and mammals such as pigs, horses, cats, whales and seals. Such strains have characteristics that make them specific to these species, though over time they may undergo changes that allow them to infect new host species.

The influenza virus undergoes changes — or evolves — rapidly. These changes can occur through the accumulation of small genetic changes within a single strain or through rearrangements of genetic material between different strains, (possible because the genome of these viruses is segmented).

Current research seeks to understand why some strains are more harmful than others, looking for answers in the genome evolution of these viruses, among other areas.

THE INFLUENZA VIRUS

STRUCTURE AND ORGANISATION

Influenza, or the flu, is an acute respiratory disease that is generally benign, but which can have a significant impact on public health due to its morbidity, mortality and impact on health economics. This disease is caused by a virus from the Orthomyxoviridae family. There are four genera of influenza virus and at least three of them (Alphainfluenzavirus, Betainfluenzavirus and Gammainfluenzavirus) can cause flu in humans, though with varying frequency and severity.

In general terms, viruses are particles composed of genetic material (DNA or RNA) surrounded by a capsid (mostly composed of a protein). These particles are inert outside of living cells, but in some cases can remain in the environment for long periods of time, as also happens with the spores and seeds of some living species. When viruses enter the host cells, however, they behave like intracellular parasites, exploiting cellular machinery for the synthesis of new viral particles — the viral progeny or offspring.

Members of the Orthomyxoviridae family have nucleocapsids of helical symmetry and a genome of single-stranded negative RNA (the negative strand is the non-coding strand). The viral genome is divided into six to eight segments, with each one of the segments encoding one or two different viral proteins.

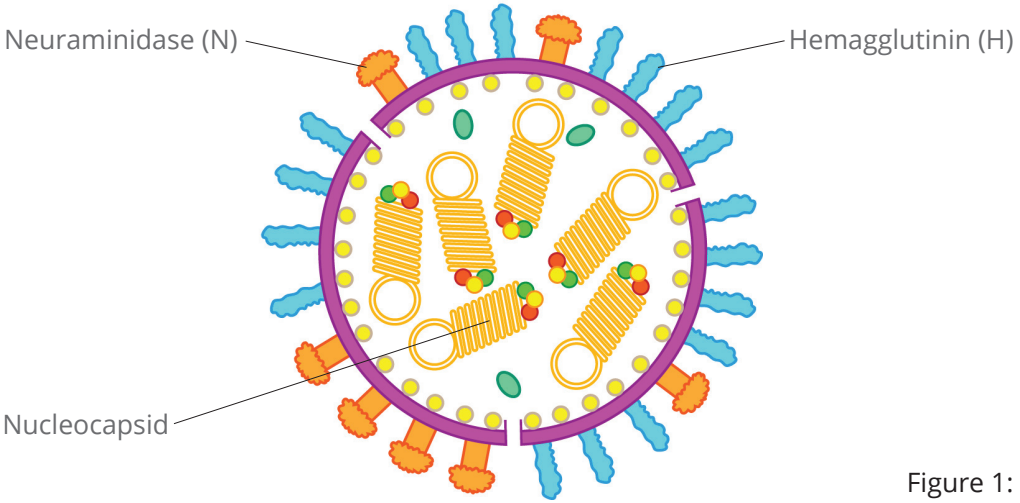

Figure 1:
structure of the influenza virus

Each RNA segment is packaged with viral proteins forming structurally independent nucleocapsid units (there are eight units in the case of Influenza type A). One of these viral proteins is the enzyme RNA-dependent RNA-polymerase, which is essential to the replication of the virus as it allows the synthesis of novel RNA molecules using the viral genome as a template. This function is unique to RNA virus genomes.

Viral particles are pleomorphic, i.e., they do not have a fixed structure, and can be spherical with a 100 nm diameter, or filamentous measuring 80 to 120 nm. They are covered by a lipid envelope that is derived from the infected host cell and is studded on its external surface by two glycoproteins of viral origin: the hemagglutinin and the neuraminidase. These glycoproteins form structures (spikes) that protrude around 10 nm from the surface of the particle and play an important role in the replicative cycle and pathogenesis of the virus. They function as important surface antigens, responsible for viral antigenic variation and host immunity.

There are at least three types of influenza viruses capable of causing disease in humans: influenza types A, B and C. Types A and B are the most common and are most closely associated with epidemics. Type B can exhibit some antigenic variation and is generally responsible for seasonal outbreaks, while type C is antigenically stable and only causes moderate disease in immunocompromised individuals.

Type A viruses can cause moderate to severe disease and can also be transmitted to other animals. They also have a lot of antigenic variation, are responsible for most flu cases and have always been at the origin of pandemics. They are subdivided into different subtypes, such as influenza A H1N1, H3N2, H5N1 or H7N9, names which indicate the different molecules of hemagglutinin (H) and neuraminidase (N) found at the surface of the viral particle.

Hemagglutinin is a glycoprotein anchored to the viral envelope. It is responsible for the specific recognition and binding to the sialic acid — a surface receptor present on the surface of the membranes of the epithelial cells of the respiratory or digestive tract (in some rare instances the flu virus can cause gastrointestinal symptoms). Hemagglutinin's main role is to bind the virus to the

host cell receptor. Its name is derived from the first tests undertaken to identify influenza viruses, which caused the red blood cells used in the tests to clump (or agglutinate) together. There are different variants numbered from H1 to H15, though not all are specific to human cells.

Neuraminidase is the other protein found on the external surface of the viral envelope and it too recognises the sialic acid of the host cell and degrades it, playing an important role in the release of new viral particles during the virus replication cycle. The different variants are numbered from N1 to N9.

VIRUS REPLICATION CYCLE

Viruses are particles that are generally much smaller than cells. The viral particle, or virion, enters a host cell to start the viral replication cycle, synthesising all the necessary molecules in order to generate new virions. It is thus said that viruses are obligate intracellular parasites.

The replicative cycle of the influenza virus starts with the attachment of the virion to the host cell. This process is mediated by hemagglutinin spikes on the surface of the viral particle, which bind to the sialic acid receptors on the surface of the host cell. After this specific attachment, the virus, due to its lipid nature, is internalised and the nucleocapsids (that contain RNA molecules) are released in the cytoplasm of the host cell.

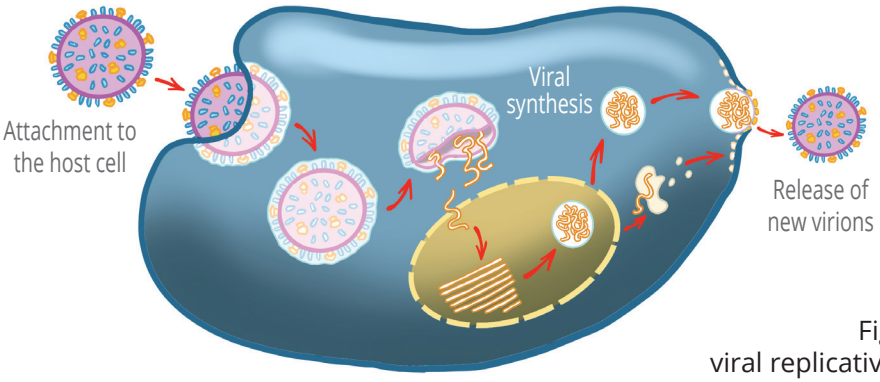

Figure 2:
viral replicative cycle

A central tenet of molecular biology is that genetic information in a biological system flows from DNA to RNA, and from RNA to proteins through the molecular mechanisms of transcription and translation (respectively). However, there are several exceptions to the direction of this flow of genetic information, such as the case of some viruses like the influenza virus. As the genetic material of the influenza virus is negative-sense RNA (the non-coding strand), the viral genetic material only serves as a template for the synthesis of the complementary coding sequence. The transcription of the viral messenger RNA, which serves as a template for the translation of the viral proteins, happens inside the host nucleus and is mediated by the RNA-dependent RNA-polymerase enzyme present in the virion associated with its nucleocapsids.

In order for replication and synthesis of novel negative-sense RNA strands to be encapsidated and to form new virions, it is still necessary for a positive-sense intermediate to be synthesised for each one of the segments of the genome. During this step there is a relatively high mutation rate.

These newly synthesized viral RNAs and virus proteins made from messenger RNA in the cell cytosol are exported to the cell membrane where new viral particles are assembled before being released.

THE FLU 

The flu is a respiratory infection that is widespread among the population during winter.

When an infected person coughs or sneezes, millions of viral particles are released into the environment in the form of droplets and aerosols that can spread in the air and land on surfaces. Poorly ventilated environments promote the persistence of these particles in the air and lead to other people inhaling them. Transmission can also happen through contact with contaminated surfaces.

Frequent hand washing and ventilation of potentially contaminated environments are thus very important measures against transmission.

When the viral particles reach the mucous layer of the upper respiratory tract, they start the replicative cycle and begin to disseminate, allowing some virions to reach the lungs. The first symptoms of the disease show within four days of the viral infection (incubation period). However, the severity of the disease can vary from person to person and according to each immune system.

The infection and inflammation induced by the virus lead to changes in the functioning of the epithelium of the respiratory system, which allows opportunistic secondary infections that can be potentially fatal, such as bacterial pneumonia in the lungs caused by *Streptococcus pneumoniae, Staphlyococcus aureus* or *Haemophilus influenzae*.

While antibiotic therapy is only justified in cases of secondary bacterial infection, according to the 2013 Eurobarometer, forty per cent of Europeans wrongly believe that antibiotics are effective against the flu and colds.

The flu is frequently mistaken for a cold due to both diseases sharing some of their most common symptoms, such as stuffy nose, sneezing, sore throat and cough. However, these are two different diseases, caused by infections of the respiratory tract by different viruses.

A cold is an infection of the upper respiratory tract, known as nasopharyngitis or rhrinopharyngitis, and can progress into an ear infection. The flu, on the other hand, is characterised by its sudden onset of symptoms of general illness, high fever accompanied by headaches, muscle or body aches, and dry cough. It is a disease that can affect both the upper and lower respiratory tracts and can lead to pneumonia and gastrointestinal symptoms.

THE EVOLUTION OF THE INFLUENZA VIRUS 

(ECOLOGY)

The influenza virus provides a good model for studying evolutionary mechanisms. Genetic variability is the “raw material” of evolution. The influenza virus evolves rapidly and has a high level of genetic diversity. Through successive generations, influenza viruses diversify their genomes by two different mechanisms: antigenic drift and antigenic shift (by reassortment of segments of the genome). New variants can enable the virus to escape the action of the immune system, thus acquiring an adaptive advantage. These new variants can then persist and disseminate among populations of susceptible hosts.

ANTIGENIC DRIFT

Antigenic drift refers to the point mutations that occur and accumulate in the genes that encode the viral proteins, particularly in the surface proteins of the virus — those which are recognised by antibodies.

These mutations occur during the replication of the virus' genetic material. Each time the genetic material is replicated through the action of the viral RNA-dependent RNA-polymerase, mistakes in the synthesis can occur and give rise to mutations. While the DNA-polymerase, the enzyme that synthesises DNA, has a proofreading ability (it detects mistakes during the synthesis and corrects them), the viral RNA-dependent RNA-polymerase does not have this proofreading ability. As a result, many more mistakes occur during the replication of the genetic material of the influenza virus than during the replication of our own genetic material, for example.

Mutations are random and can occur in any location of the genome. The consequences of mutations can be negative (such as a protein losing its functionality), neutral (if it does not affect the protein, as in the case of synonymous substitutions), or positive (if

it bestows some kind of advantage). If these mutations occur within the genes that encode the surface proteins, they can enable the virus to escape the host's immunity.

ANTIGENIC SHIFT — REASSORTMENT OF THE INFLUENZA VIRUS GENOME

A molecular mechanism that contributes to the rapid evolution of the influenza virus is the reorganisation of its genome through the reassortment of its segments. The genome of the type A influenza virus is divided into eight segments, with each generally encoding for one or two viral proteins/functions. If a susceptible host cell is exposed and infected simultaneously with two or more different variants of the influenza virus, several segments of the genomes can be replicated simultaneously. During this process different nucleocapsids from genetically distinct viral strains will be formed simultaneously. When the novel virions are released, however, different combinations of nucleocapsids can be encapsidated in each virion. Each virion is formed by the eight different nucleocapsids that constitute the complete viral genome, though each can be of a different origin if a reorganisation of the genome has occurred.

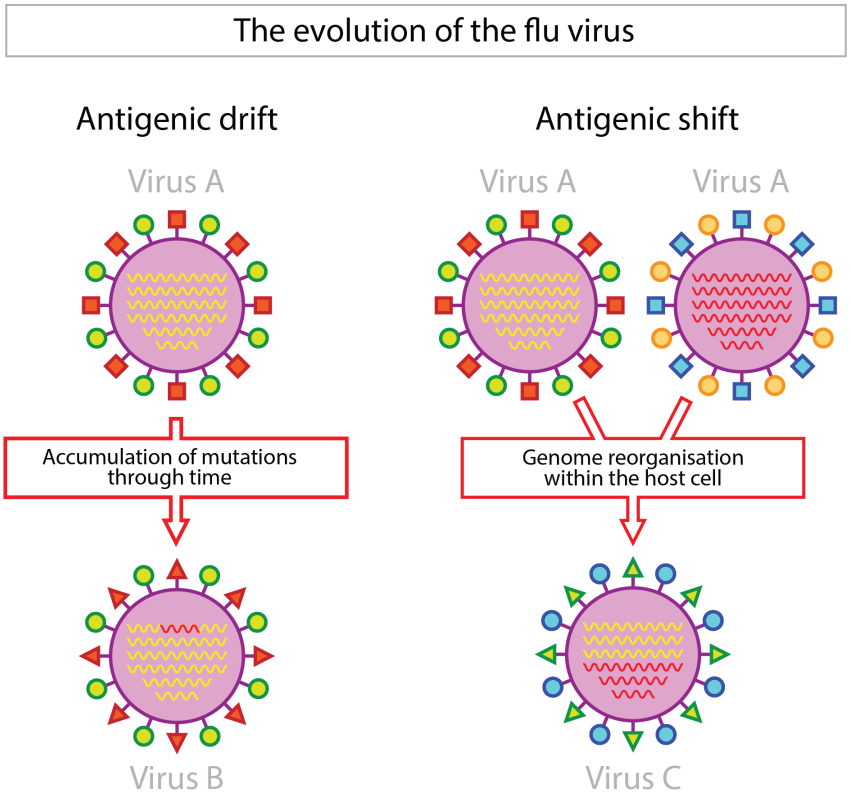

Figure 3:
The two mechanisms by which the genome of the influenza virus can evolve

This mechanism of genomic reorganisation occurs as a result of the fact that some animals can be simultaneously infected with different strains of the flu virus. Pigs, for example, are susceptible to both human and avian viral variants. As such, when the epithelial cells of the respiratory tract of pigs are simultaneously infected by different variants of the virus, novel virions with a combination of genetic material from pig, human and avian influenza virus can be formed.

This rearrangement of genomic RNA from different variants of the influenza virus can lead to the formation of novel combinations of the H and N proteins. If these new viral proteins are different enough from those in circulation, and there is still no immune protection to them, this new variant can rapidly spread in a human population susceptible to this new viral form. Moreover, the rearrangement can also result in a more virulent strain. Such reorganisations of viral genomes gave rise to the 1918 pandemic (the “Spanish flu”), the 1957-1958 pandemic (the “Asian flu”), the 1968 pandemic (the “Hong-Kong flu”), and the 2009 flu.

EPIDEMIOLOGY AND THE FLU VACCINE 

Flu outbreaks typically occur during autumn and winter. In Portugal, the peak of the flu season usually occurs in January/ February. As the virus maintains its viability in humid conditions (due to a need for the lipid viral envelope to be kept intact for infection to occur), the high humidity and lower temperatures of winter favour the propagation of the virus. Moreover, our behaviour during winter favours transmission, as more people spend more time indoors in enclosed spaces.

A study undertaken in USA has shown that the duration of flu outbreaks in cities seems to also depend on the characteristics of the cities themselves: in bigger cities with higher population density, a factor that promotes the transmission, flu seasons are generally longer and less dependent upon weather conditions; in smaller cities, on the other hand, outbreaks are generally shorter and more dependent upon weather conditions.

Flu outbreaks are monitored worldwide, with circulating strains and their effects on populations identified, allowing for appropriate warnings and vaccines to be developed every year. This monitoring happens all year round, given that the flu season during the southern hemisphere winter happens during the northern summer.

VACCINES

The World Health Organization (WHO) recommends that certain groups (care workers and people most at risk of developing serious complications from the influenza infection) be vaccinated against the flu every year before the season begins.

For instance, every autumn, the Portuguese health authority (Direção-Geral da Saúde, or General Directorate of Health) recommends a schedule for vaccinations and those groups considered to be most vulnerable. As the “peak” of the flu season usually takes place during the months of January and February, it is recommended that vaccination takes place before this time, potentially much earlier.

As with other vaccines, the flu vaccine is a preparation of substances derived from or chemically identical to a specific infectious agent.[4] When administered, a vaccine will induce an immune response specific to that infectious agent as if the person had been infected by it (that is, it is "immunogenic"), but without causing the associated disease because it does not contain an infectious agent with the ability to reproduce itself and cause the disease. As such, this immune response protects against the disease. In the event the vaccinated person gets infected, their immune system can respond rapidly and effectively.

While contracting some diseases once is enough for us to be protected from them for life, catching the flu one year does not prevent us from catching it again the following year. This is because the flu virus undergoes genetic changes (mutations and rearrangements) as the circulating strains change from one year to the next. For this reason, the flu vaccine has a different composition every year and is designed to protect us for that specific year.

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[4] Specific antigens and production methods used vary according to each vaccine.

The vaccines administered in Portugal follow the recommendations of the WHO for the influenza season (Autumn-Winter) of the Northern hemisphere[5]: in February each year, the WHO issues information about the variants of influenza virus predicted to be in circulation in the following northern winter, and manufacturers begin production of the appropriate vaccine. As the process of manufacturing, regulatory approval and licencing takes 6 to 7 months, the vaccine only becomes available in September-October.

WHO recommendations are based on circulating influenza viruses and knowledge of their population dynamics. For example, in 2018-2019, the trivalent vaccine for the northern hemisphere contained inactivated viruses (or surface antigens) from the following strains[6,7]:

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[5] The recommendations from the WHO for the Northern hemisphere and for the Southern hemisphere are issued in separate e according to its own calendar.

[6] The nomenclature of influenza virus strains follows international conventions that indicate the type of antigen (A, B, or C), the original host in case this was not human, the geographic region, the number of the strain and the year it was isolated. In the case of A type viruses, the hemagglutinin and neuraminidase are also indicated (e.g. H1N1)

[7] The recommendations for each year can be found on the WHO website (www.who.int). Information about vaccine composition is also found in the vaccine package leaflet.

- One viral strain A (H1N1) pdm09 identical to A/Michigan/45/2015;
- One viral strain A (H3N2) identical to A/Singapore/INFIMH-16-0019/2016;
- One viral strain B (B/Victoria/2/87 lineage) identical to B/Colorado/06/2017

The quadrivalent vaccine, in addition to the strains present in the trivalent vaccine, would also include:

- One viral strain B (Yamagata/16/88 lineage) identical to B/Phuket/3073/2013.

Different production methods are used for different vaccines. While several methods have been approved for the production of the flu vaccine, the most common is the egg method, which involves propagating the viruses in embryonated chicken eggs before their subsequent purification and inactivation. The process begins with the choice of which target strains to include in the vaccine. The fertilised chicken eggs are inoculated with two viral strains — one target strain and another strain that is adapted to the chicken eggs and that is harmless to humans. The viruses will replicate inside the eggs and a rearrangement between the genome segments of the two strains will occur. The viruses that contain the H and N segments of the target

strain and the rest of the genome (the remaining six segments) from the strain that is able to replicate effectively inside the chicken cells are then selected. This new strain will be mass produced in fertilised chicken eggs and later selected and inactivated. This is followed by further purification and test phases. While this is a reliable method, it is slow and thus inadequate in the event of high demand or a pandemic. Other components in the final composition of the vaccine include stabilisers to maintain the stability of the solution during storage and adjuvants, which improve the immune response.

The capacity of a vaccine to protect from the flu depends on a match between the strains included in the vaccine (based on predictions made months earlier) and the strains circulating in the community, as well as the characteristics of the individual receiving the vaccine, such as their age and health status.

Research is currently underway to develop faster production methods and “universal “vaccines” that could offer protection from all influenza strains.

THE GAME

AN EVOLVING VIRUS

The objective of this game is to promote an understanding of the evolution of the influenza virus genome through the redistribution of its genomic segments and genetic mutations.

This game aims to explore the seasonal generation of new variants through rearrangements that naturally occur in the genome of the influenza virus as a result of the interaction between different infected animals.

Each team represents a different host animal and will be exposed during the game to different variants of the virus and thus potentially become infected. The virus will also undergo changes during the game. The game ends when a new strain with the ability to infect human hosts emerges.

This activity can be used at different teaching levels from middle school and secondary school (Key stage 3 to Key stage 5 in UK) to higher education, to explore science topics such as evolution, microorganisms, health and disease, development of medicines, individual and community health, among others.

It can be used to promote critical thinking and the use of the scientific method in developing and testing hypotheses.

INTRODUCTION

The influenza virus is the agent that causes the flu. This virus not only infects humans, but also a great number of other vertebrates such as birds, pigs and horses.

On the surface of each viral particle are the antigenic glycoproteins hemagglutinin (H) and neuraminidase (N). There is a great variety of H proteins (H1, H2, H3...) and of N proteins (N1, N2, N3...), but each viral particle has only one type of H and one type of N. This combination is used for its identification (for example H3N2 or H1N1). The combination of H and N also determines the specificity of one virus to the host cells: for example, the H3N2 strain

generally infects pigs, but it can also infect humans, while the H1N1 strain generally only infects humans.

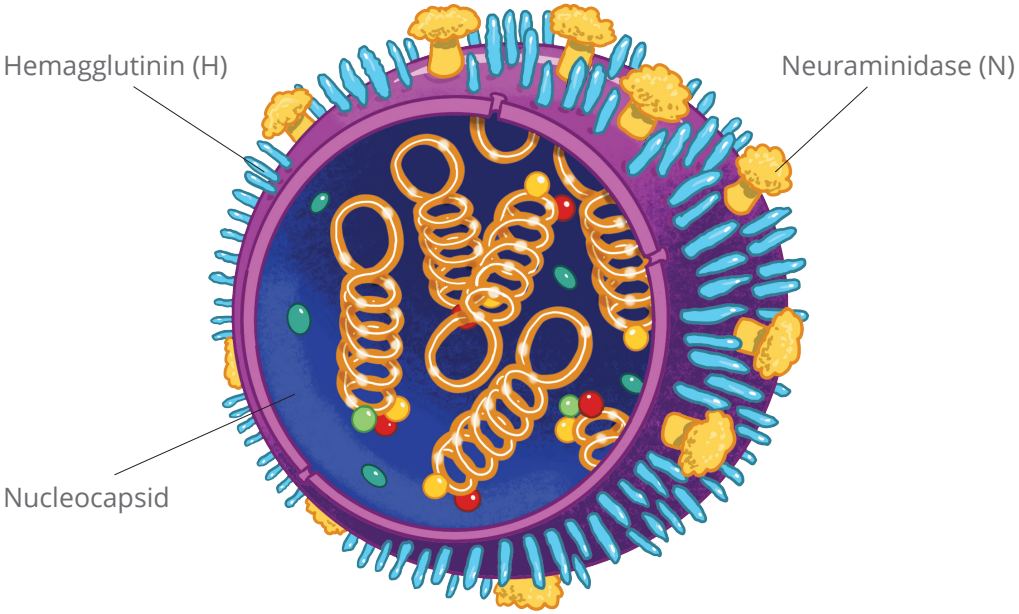


Figure 4: Three-dimensional structure of the influenza virus.

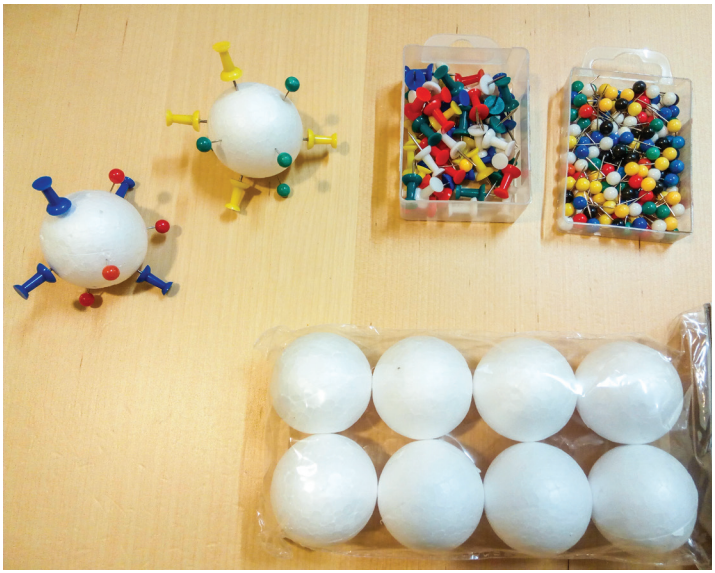
The genome of the influenza virus is segmented and encodes the information to synthetise viral proteins, including H and N. When the virus infects a cell, new viruses are formed and these can infect other cells. When a host is simultaneously infected with two different variants of the influenza virus, however, the progeny of these viruses can contain a mixture of the genomes of the two original viruses and a new antigenic variant arises.

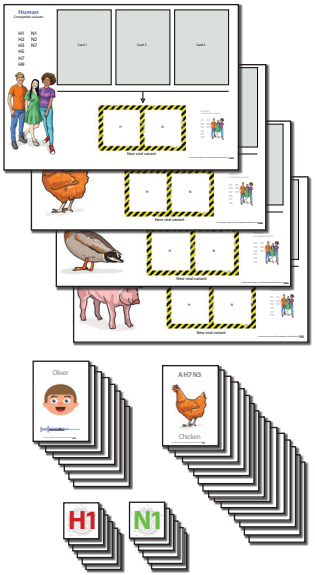
New mutations can arise in each new generation of viruses. New variants may have the ability to infect novel hosts.

MATERIAL

- A board representing the host (one per team);
- A cardboard box or bag representing the host cell for each team;
- our styrofoam balls, ping pong balls, or similar, for each team;
- Coloured sewing pins, push pins or stickers to represent the surface proteins H. Each colour will represent a given type (for example blue for H1, red for H2, and so on);
- Coloured sewing pins, push pins or stickers (different from those previously used for H) to represent the surface proteins N. Each colour will represent a given type (for example blue for N1, red for N2, and so on);

- A deck of cards of infected animals (in each card is indicated the combination of the antigens hemagglutinin (H) and neuraminidase (N) of the virus that infects that particular animal);
- A deck of cards with a card for each one the genes that encode the different H and N;
- Optional: A dice (to simulate the occurrence of mutations).





HOW TO PLAY

To start:

1. What host animal are you?

One member of the team takes a board. The board represents the potential host to the influenza virus. The board includes a list of variants of the H and N proteins that allow the virus to infect this host.

2. You have been in contact with an infected animal. Will you get sick?

Taking turns, each team collects a card from the deck of cards of the animals infected with a virus. This step represents the contact of the host with an animal infected with the influenza virus, thus exposing the team's host animal to the virus. This host will only get infected if exposed to a virus with compatible H and N.

a) The host is infected if exposed to a virus that has compatible H and N.

If this occurs, the card should be placed on the corresponding space on the board and a small model of the corresponding virus be built with the balls and pins. Four pins representing H and others

representing N (symbolising the genetic material) are also inserted inside the box representing a respiratory tract cell.

b) The host is not infected if H and N are not compatible with the host cells.
If this occurs, the game continues, and it is the turn of the next team.

On each turn, a new card is collected from the virus deck and the process repeats.

3. Will there be a new viral variant in circulation?
When there are two viruses (pins or other objects that correspond to the H and N) inside a box, the box is shaken and a set of H and N is taken out without looking. This set is placed on the board in the place "new variant" and a model of this new viral particle is built. A genetic reorganisation has occurred.
The host is exposed to this new virus and may or may not become infected.

4. The first team to generate a new viral variant that is capable of infecting a human wins.

Optional variants of the game:

1) Quicker version: the team that wins is the team that generates a new variant that can infect a host that is different from the original host.

2) To add the effect of mutations: in each team's turn, one of the players from the team must throw a dice. If an odd number is rolled, the virus has suffered a mutation that makes it more virulent. To infect a new host, it is necessary for the virus to undergo at least one mutation.

3) To add the effect of vaccination: for this option it is necessary to know the constitution of the vaccine for the current year. When a new virus is generated, a card from the hosts deck is taken. Some of these individuals have been vaccinated and some have not. In the event that the individual to be infected is vaccinated and the new viral variant that was generated during the game that is capable of infecting humans is one of the strains that is present in the vaccine of this year, the virus will not infect (and thus the team will not win).

The game continues until a strain that can infect vaccinated humans is generated.

4) On each turn, the player imagines a situation in which he or she could come into contact with the infected animal (for example, by going to a park).

REFERENCES

Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases (NCIRD). Transmission of Influenza Viruses from Animals to People. http://www.cdc.gov/flu/about/viruses/transmission.htm

Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases (NCIRD). Past Pandemics. https://www.cdc.gov/flu/pandemic-resources/basics/past-pandemics.html

Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases (NCIRD). Preevent Seasonal Flu. https://www.cdc.gov/flu/prevent/index.html

Eurobarometer Special Surveys: Antimicrobial resistance report (2013) https://europa.eu/eurobarometer/surveys/detail/1101

Jawetz, Melnick, & Adelberg's Medical Microbiology (22nd Edition) by George F. Brooks (Editor), Stephen A. Morse (Editor), Janet S. Butel (Editor), Ernest Jawetz. (2001) ISBN0-07-112066-1

Donna M. Tscherne, Adolfo García-Sastre. Virulence determinants of pandemic influenza viroses. J Clin Invest.121(1):6–13 (2011)

Haibo Wu, Xiuming Peng, et al. Genetic and molecular characterization of H9N2 and H5 avian influenza viruses from live poultry markets in Zhejiang Province, eastern China Scientific RepoRts 5:17508 (2015)

Dalziel, B. D. et al. Urbanization and humidity shape the intensity of influenza epidemics in U.S. cities Science 362, 75-79 (2018)

Wong & Webby, Traditional and New Influenza Vaccines, Clin Microbiol Rev. 26(3): 476–492 (2013).

Zimmer, C. A planet of viruses, 2nd edition, The University of Chicago Press (2015)