GENERAL CHARACTERISTICS OF VIRUSES

In order to comprehend the nature of viruses and the ways they affect the living world, we first need to learn about their role in nature.

Viruses are one of the smallest infectious agents in nature (20-300 nm in diameter). Viruses contain only one of the nucleic acids, either DNA or RNA in their genome. Nucleic acids are found in membranes. The membrane is called **capsid** - a protein sheath that may enclose one of the nucleic acids or may be empty; **nucleocapsid** - which always encloses one of the nucleic acids. On their surfaces there are **structural units** consisting of basic proteins, the **capsomeres** consisting of groups of different polypeptides and **a sheath** consisting of a lipid membrane which is most often formed during the maturation of the virus by the process of exit (budding) of the virus through the host cell membrane whereby the surface contains glycoproteins encoded by the genome of the virus, which essentially gives it a characteristic genetic signature. The particle defined in this way is called an infectious particle or **virion**. The viral particles are inert in the external environment, they only reproduce in living cells and can be considered as parasites at the gene level.

Virus nucleic acids contain the information necessary for reproduction and they program an infected host cell to synthesize a number of specific viral molecules required to produce viral offspring. Numerous copies of nucleic acids and structural proteins are generated during the replicative process. At the end of replication, the structural proteins, by their assembly, form a capsid, which protects the nucleic acid from the external environment and, upon leaving the host cell, it serves to attach to other suitable cells.

VIRAL EVOLUTION

To date, there are doubts about the origin of the viruses, and two possible hypotheses have been identified so far:

According to the first hypothesis, viruses are assumed to originate from certain components of cells that have become autonomous over time. This assumption came from their characteristic that some genes located on their nucleic acids have the ability to exist and function independently of the host cell (as some organelles in eukaryotic organisms, for example mitochondria). Also, parts of the virus gene are similar to parts of genes of multicellular organisms that encode functional domains of proteins. Today most viruses are thought to have originated in this way.

According to the second hypothesis, some viruses are thought to be derived from acellular (unicellular) organisms. There is no direct evidence that they evolved from e.g. bacteria, but it is possible that some other obligate intracellular organisms (chlamydia) have evolved in this way. One of the main strongholds of this hypothesis is the existence of very large and complex poxviruses that are thought to have actually originated from an acellular (unicellular) ancestor.

THE CLASSIFICATION OF VIRUSES

The classification of viruses is very complex and there is no completely unique system for classifying them. Some of these features are used as a basis in their classification. The amount of information for each of the following properties is not the same for all viruses. For some groups, insights are available for just a few properties, which makes the classification particularly difficult:

- According to nucleic acid type, DNA or RNA, single-stranded or double-stranded and replication mode
- According to morphology and size of the viral particle together with the type of symmetry, number of capsomeres and presence or absence of the sheath
- According to sensitivity to physical and chemical agents
- According to the presence of certain enzymes, RNA or DNA polymerases, neuraminidases, etc.
- According to the immunological properties
- According to the transmission routes (natural)
- According to the tendency to retain in certain tissue or cell types (viral tropism)
- According to the formation of inclusion bodies (pathology)
- According to the symptomatology

The most significant contemporary classification in both human and animal virology is primarily based on **symptomatology**. At the same time, this is the oldest type of classification.

A virus-based symptom classification system is ideal for physicians, however, it is not satisfactory for biologists because the same virus can occur in several groups of animals and can therefore cause more than one disease depending on the organ or system of organs in which it is located.

Due to these facts, it is extremely important to study the natural pathways of spreading the virus and to learn about all possible reservoirs and their transmission vectors in nature!

Significant efforts have been made in recent decades as well as discoveries to identify spread pathways, as well as to find natural reservoirs for certain groups of viruses that are significant in terms of clinical virology and epidemiology in human medicine.

Classification according to symptomatology

- Generalized diseases, which include viruses tending to persist in a number of different organ systems, such as measles virus, chickenpox, rubella, enteroviruses and many others.
- Viruses that attack specific organs and most commonly spread through the bloodstream, along the peripheral nerves, or otherwise through the body.

According to the viruses that cause diseases by attacking a specific organ or system of organs, the diseases are classified by symptomatology into:

1) Diseases of the gastro-intestinal tract - causal agents are rotavirus, enteric adenoviruses, etc.
2) Liver disease virus inductors - hepatitis viruses A, B, C or E, enteroviruses, rubella virus, etc.
3) Diseases of the skin and mucous membranes - causal agents are herpes viruses and others
4) Eye diseases - causal agents are adenoviruses, enteroviruses and others
5) Diseases of the salivary glands - causal agents are cytomegaloviruses and others
6) Respiratory tract diseases - causal agents are influenza pneumonia viruses, **coronaviruses**, rhinoviruses and others
7) Nervous system diseases - causal agents are polioviruses, rabies viruses, encephalitis viruses and others

Classification according to biological, chemical and physical characteristics

According to biological characteristics, viruses are basically divided into two large groups of DNA and RNA viruses.

The following virus groups belong to **DNA viruses**:

Papovaviruses are of small size about 45-55 nm. They are stable at higher temperatures and contain double-stranded DNA. The best known of these are papillomaviruses (causal agents of warts). Some of them cause serious diseases of the nervous system. **In animals, there are viruses from this group, namely papillomaviruses, polyoma and vacuolating viruses. The characteristic of these viruses is their extraordinary slow growth. These viruses are the causal agents of latent and chronic infections in natural hosts (animals) and some have been proved to cause even tumors.**

Adenoviruses are of medium size about 70-90 nm. They have double-stranded DNA and have no sheath. More than 40 types have been reported in humans that can cause respiratory tract diseases. **Some of the viruses belonging to this group can cause tumors in animals. Many different virus serotypes of this group cause diseases in animals.**

Herpes viruses are medium-sized viruses with double-stranded DNA. Their size is about 100 nm. A characteristic of this group is that latent infections can be maintained throughout the host's life, primarily in nerve and lymphoid tissue cells. **Some of these viruses are tumor-causal agents in both humans and animals. Many members of this group are infectious agents in many other animal groups such as rodents, ungulates and some primates.**

Poxviruses are large viruses of 230-400 nm. They possess double-stranded DNA, several enzymes, as well as DNA-dependent RNA polymerase. They are characterized by the fact that the entire reproduction cycle takes place in the cytoplasm of the host cell. Almost all viruses in this group cause major lesions in the skin of the infected. The best known representative of this group is the virus causing **smallpox** (in humans), however, a large proportion of them also attack groups of animals such as **ungulates and other primates**. However, human infections are possible by the types that cause diseases in other groups of animals.

Hepadna viruses are of small size about 42 nm. Their characteristic is that they contain incomplete double-stranded circular DNA. In addition, virion also contains the enzyme DNA polymerase, which upon penetration into the host cell serves to complete the DNA of the virus upon its activation. The viruses in this group cause acute and chronic liver inflammation (hepatitis). **The hepatitis caused by this virus in most cases results in the development of liver cancer. Three types of viruses in this group that cause infections in mammals and one that cause infections in birds have been known to date.**

The following virus groups belong to the **RNA viruses**:

Picornaviruses are of small size about 20-30 nm. They contain single-stranded RNA. RNA is a positive chain and can function as an information RNA. The best known representatives of this group that infect humans are rhinoviruses, of which over 100 serotypes have been known so far, causing mainly upper respiratory tract diseases. Enteroviruses are another group to which polio, coxsackie and echoviruses belong. **Picornaviruses cause infections in animals, most commonly in ungulates (hoof-and-mouth disease), while in rodents they mainly cause encephalomyocarditis.**

Caliciviruses are 35-39 nm in size. They are non-enveloped viruses (without sheath) containing single-stranded positive RNA. The viruses belonging to this group mainly cause gastroenteritis, among which the best known is the Norwalk virus, which causes acute epidemic gastroenteritis in humans. **Other representatives of this group cause disease in the cat group (Felidae), in the seals, sea lions and other aquatic mammals (Pinnipedia) as well as in other primate representatives.**

Reoviruses are 60-80 nm in size. They contain double-stranded segmented RNA. The best known among this group is the rotavirus, a causal agent of gastroenteritis in humans. **However, very similar serotypes to this virus cause gastroenteritis in many other groups of animals. A special group within this group consists of orbiviruses belonging to the group of viruses that are transmitted by the arthropods and are the cause of some diseases in the ungulates ("blue tongue" disease).**

Arboviruses are smaller than 350 nm in size. Viruses with different physical and chemical characteristics belong to this ecological group. One of the important common characteristics of this group of viruses is that the vectors of transmission are the arthropods, most commonly representatives of the Arachnida class, primarily ticks and species from the Insecta class, above all mosquitoes. An interesting fact is that the replication of the virus in the organism of the carriers (arthropods) does not harm the hosts. When transmitted, these viruses **infect reptiles, mammals and birds**. For primates, and therefore for humans, the most significant diseases caused by viruses from this group are yellow fever, dengue fever, various encephalitis, and more. Representatives of arboviruses belong to many other families such as togaviruses, flaviviruses, bunyaviruses, rhabdoviruses, arenaviruses and reoviruses.

Togaviruses are representative of a group of arboviruses of 50-70 nm in size. They contain positive single-stranded RNA. The best known virus in this group is the rubella virus.

Flaviviruses are 45-50 nm in size. They contain single-stranded positive RNA. A characteristic of this group is that mature virus particles accumulate in endoplasmic reticulum cisternae. The best known representative is the yellow fever virus.

Coronaviruses are 80-160 nm in size. They contain single-stranded, non-segmented positive RNA. On the surface, they have outgrowths in the form of petals or sun corona (crowns), after which they are named. Several types are known to **cause the most common acute upper respiratory tract infections in humans. In animals** they cause long and **persistent respiratory tract infections, of which the best studied are those in birds.** Toroviruses are a special genus within the coronavirus family and they cause **gastroenteritis** in both humans and animals.

Arenaviruses are 50-300 nm in size. They contain single-stranded negative RNA. Representatives of this group use ribosomes of the host cell in their replication, so that the electron micrographs indicate that the appearance of the virus in the cytoplasm is "sandy" during replication. The largest number of this group viruses cause diseases in the Central American region (also known as the Takaribe Complex), as well as in parts of some other tropical and subtropical areas. **All members of this group, besides causing diseases in humans, also cause chronic diseases in other groups of animals, and especially in rodents.** The most common transmission vectors are ticks.

Bunyaviruses are 90-100 nm in size. They contain single-stranded segmented negative RNA. As with most representatives of the arboviruses group, they are usually transmitted by arthropods. One of the representatives of this group, a hantavirus, is not transmitted by the arthropods, but by **rodents** and it is the cause of severe hemorrhagic fever, nephropathy and severe pulmonary syndromes.

Retroviruses are 90-120 nm in size. They contain two copies of single-stranded RNA and the reverse transcriptase enzyme. The virus genome is incorporated into the DNA of the host cell and replicated together with the host cell. The hosts remain chronically (lifelong) infected. **Viruses causing leukemia and sarcoma in humans and animals** belong to this group. Some of these viruses cause acquired immunodeficiency syndrome (**AIDS**) such as **HIV** in humans or **SIV** in other primates.

Orthomyxoviruses are 80-120 nm in size. They contain segmented single-stranded negative RNA. All members of this group are influenza viruses and cause infections in **humans and animals**. A special characteristic of this group of viruses are surface "shoots" that exhibit hemagglutinin or neuraminidase activity. **Segmented genetic material allows this group to easily recombine when two viruses with different genomes infect a single cell. This explains the high degree of natural variation among influenza viruses.**

Paramyxoviruses are 150-300 nm in size. They contain single-stranded non-segmented negative RNA. Like orthomyxoviruses, they possess hemagglutinins. The best known are mumps, measles, parainfluenza viruses and others. Unlike orthomyxoviruses, they are genetically stable.

Rhabdoviruses are 75-180 nm in size. They contain single-stranded, non-segmented negative RNA. The best known of these viruses causes **rabies.**

Viroids are small infectious agents that cause plant diseases. So far, the viroids have not been determined to cause human or animal diseases.

Other viruses are viruses for which there is insufficient information to enable accurate classification. Diseases caused by some of the viruses that are not precisely classified are the so-called unconventional viral diseases, such as **mad cow disease**, or the same disease in humans, called **Creutzfeldt-Jakob disease**. Also, diseases caused by unclassified viruses have been attributed to some animal diseases, such as ungulates disease (**sheep scrapie**), as well as some types of **non-specific gastroenteritis** in animals.

Viral replication

Viruses reproduce only in living host cells. Host cells during virus replication provide all the necessary components for the synthesis of molecules (proteins) and nucleic acids of the virus itself. The virus nucleic acid directs the cell's operation in a very organized manner in favor of the virus. After penetration of the viral particle into the host cell, it is not possible to detect the particle or that anything is going on. This stage in virus replication is called the eclipse phase. It lasts depending on the type of virus and the host. At this stage, intense physiological changes and synthesis of viral components occur. The whole mechanism of the host cell is redirected to meet the needs of the virus. In some cases, the metabolism of the cell is not significantly altered, although everything needed for the virus is being synthesized.

Processes in virus replication take place in several stages.

One of the steps is the **penetration** of the virus into the host cell and the **release** of nucleic acid and enzymes. In order for the virus to enter the cell, the existence of receptors on the cell is necessary, which are required for the virus to recognize the target organ or tissue. The receptors can be protein in nature, glycoprotein, lipid or combined. The presence or absence of receptors plays a significant role in the viral tropism. After the process of finding an adequate receptor for the penetration of the virus into the cell, the viral particle enters it. The most common way of penetrating a viral particle into a cell is receptor-mediated endocytosis, and in some cases the mode of viral particle penetrating is unknown. The release of viral nucleic acid occurs shortly after the virus has penetrated the cell. Decomposition of the viral sheath cancels infectivity.

After this stage, a stage of **viral component synthesis** occurs. From the viral nucleic acid, all the elements necessary for the formation of a new generation of virus are synthesized. On the type of nucleic acid depends the order of synthesis of the viral elements, as well as the replication of the nucleic acid itself, which is synthesized in some viruses at the beginning and in some other viruses at the end of this stage. There are differences in gene expression during virus replication in RNA and DNA viruses.

Virus release and morphogenesis is the stage in which newly formed viral genomes are formed by the collection of capsids, or in the case of viruses with the sheath, they are formed by the budding process. After accumulation of newly formed viral particles with their genome occurs the lysis (decomposition) of the host cells, thereby releasing the virus into the external environment.

In some cases, during the maturation of the viral particles, the host cell does not decompose, but the viral particles, as well as other components of the virus, accumulate in the host cell and lead to cell death. However, in some cases, the inclusion bodies are created in the cell and the cell does not die, resulting in long-term persistent infection.

INFLUENCE OF PHYSICAL AND CHEMICAL AGENTS ON VIRUSES

Acidity (pH) of the environment in some cases can significantly affect the inactivation of viral particles. Most viruses are stable at pH values of 5.0 to 9.0. Some viruses are resistant in slightly acidic environments, while alkaline environments with pH values above 10 and more destroy them all.

Chemicals (detergents), depending on whether they are non-ionic or ionic, generally dissolve the membranes of the viral particles, while some anionic detergents denature proteins contained in the virus capsid.

Ether mainly affects the viruses containing a sheath (enveloped viruses). The following virus groups are inactivated in the presence of ether: herpesviruses, orthomyxoviruses, paramyxoviruses, rhabdoviruses, **coronaviruses**, retroviruses, arenaviruses, togaviruses, flaviviruses and bunyaviruses.

Formaldehyde is an agent that mainly reacts with a nucleic acid of a viral particle. The only difference in the effect of formaldehyde on viral particles is that it is much easier to inactivate single-stranded genomes as opposed to viruses with double-stranded genomes.

The effect of **temperature** on viruses varies. Most viruses are inactivated at 60 ^{o}C when exposed for 30 minutes. However, most viruses are resistant to low temperatures and generally do not lose their infectivity.

It is this feature that allows them to be stored in laboratories.

Radiation at certain wavelengths, such as ultraviolet light and high frequency X-rays, can in some cases inactivate viral particles. The dosage, as well as the time of exposure to this radiation, is different for different groups of viruses. Even irradiated particles may be able to penetrate and exhibit their characteristics in the host cell.

Antibiotics do not affect viral particles.

Organic iodine compounds are not effective for inactivating viruses on solid or organic substrates.

Chlorine from inorganic compounds as well as in the gaseous state is effective in large quantities. It takes longer in order for chlorine to work and inactivate viral particles.

Alcohol (ethanol) or other alcohols are not particularly effective in combating viruses (inactivation), and some alcohols are not effective at all (picornaviruses).

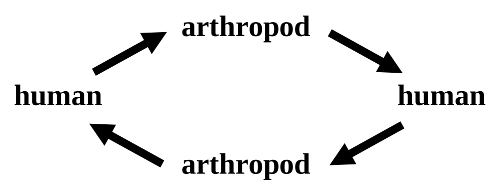
VIRUS ECOLOGY AND EPIDEMIOLOGY

Virus ecology refers to the part of epidemiology that deals with the way viruses are maintained and transmitted in nature. Unlike epidemiology, which deals with the clinical manifestation of infection, the ecology of the virus is concerned with the inter-epidemic phase of the virus movement and its maintenance in nature. For a long time it was thought that viruses were present only in living organisms and were unstable in the environment. Contemporary research has shown that viruses can survive for a long time and retain infectious properties in the environment. Viruses reach the environment from the infected organism in various ways. From the respiratory tract they reach the external environment via cough in the form of droplets. Most viruses causing respiratory infections are very sensitive and rapidly decompose. Dry air and organic material favor the long-term maintenance of these viruses in the external environment. Viruses that are expelled from the body in excrement or other forms of excreta can retain their infectious properties for a long time, depending on whether they are on the ground or in water. Many viruses can survive very long in water and can be transmitted with water. Virus survival in water depends on many factors (temperature, pH, virus group, etc.), as well as on seasonal variations. Some virus groups have been determined to be able to survive for up to 200 days at moderate temperatures.

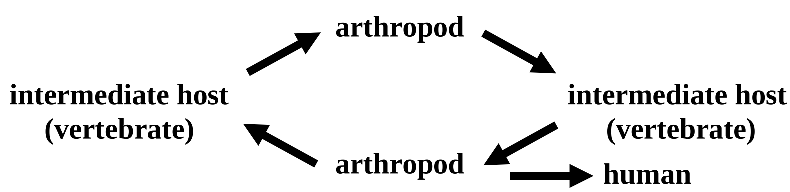
Viruses from soil or water enter the body of various animals, thus becoming reservoirs of infections and hosts in which the viruses are maintained. The presence of viruses in some animals for which viruses are non-pathogenic is very important in studying the ecology of the virus. After discovering that certain types of viruses survive, that is, they are hosted by different species of both wild and domestic animals, it became known how the outbreaks of certain diseases occurred. The virus most studied is influenza A virus, all recombination of which have been found to occur in bird organisms. This virus is also very important when it comes to infecting humans. It was found to be transmitted over long distances by birds in their migration. In addition to the virus mentioned, one of the best studied groups is the group of arboviruses transmitted by haematophagous groups of the arthropods. The virus that remains in the arthropods is transmitted to other animals and is thus maintained in nature. In some cases, it has been observed in the arthropods that the virus is transmitted in a transovarian manner (via the egg), so the presence of vertebrates is not necessary to maintain the virus in nature.

Ways of virus transmitting:

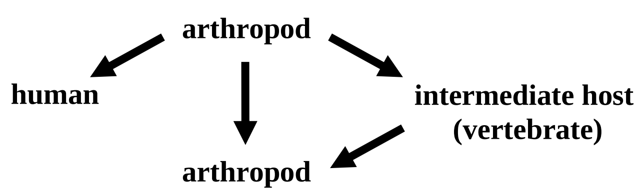
1. Huaman – arthropod cycle



2. Intermediate host cycle (vertebrate) - arthropod - human



3. Arthropod cycle - arthropod – intermediate host (vertebrate) - human



In the study of diseases caused by viruses, **epidemiology** deals with data obtained from epidemiological studies during epidemic or pandemic outbreaks. In order for the data to be valid, continuous monitoring of the occurrence of certain diseases and regular reporting on them are required. The morbidity of a particular viral disease depends on collective immunity. The immunity of a population or a particular part of the population (age structure) depends on many factors, including whether that population or part of the population has been in contact with this or a similar disease before. The rate of spread of the disease depends on the virulence of the agent, population density, age structure, season, transmission vector, etc.

Of great importance in epidemiology is the way of spreading the virus, for which there are basically two possibilities.

- viruses maintained in only one type of host and
- viruses that are maintained in multiple hosts.

Viruses that infect humans can basically be divided into two groups:

- viruses that are only maintained in humans and
- viruses that are maintained in other organisms, and humans or other vertebrates represent the last host in which the viral life cycle ends.

For viruses characterized by rapidly short-term (acute) infections, rapid transmission is very important. These groups of viruses are most commonly secreted by aerosols (coughing) and there is a large amount of viral particles in the aerosol. In contrast, viruses that cause long-lasting infections are secreted constantly and can be secreted for years. They are transmitted in various ways (horizontally), by direct contact, sexual contact, orally, by blood, by the bite of an animal, or by arthropods. Vertical transmission pathway involves transplacental, perinatal and postnatal transmission, or breastfeeding.

Viral diseases can be epidemic or endemic in nature. The nature of viral diseases depends on age, gender, socioeconomic living conditions, geographical area, climatic factors, presence of transmission vectors, etc.

THE CONTEMPORARY RESEARCH OF CORONAVIRUSES

To the coronavirus group (Coronavirus) belong significant mammal and bird pathogens, including human pathogens. They are basically divided into three groups (alpha-, beta- and gamacorona viruses). The first two groups were found in mammals, while the third group of gamma coronaviruses was present in birds. What is observed in this group of bird corona viruses, as well as in other known infections with some other types of viruses (avian influenza, etc.), is that the infected species are mainly from the ecological group of aquatic bird species (Anseriformes, Ciconiiformes and Pelecaniformes). The aquatic environment has proven to be a very favourable for maintaining and surviving of the viruses (see the ecology and epidemiology of the virus). On the other hand, it can be said that birds in the case of SARS-CoV2 (Covid-19) are not a vector in the transmission of the virus due to significantly different physiological characteristics as compared to the mammals. As in the case of many other viral pandemics where the reservoirs or one of the vectors were birds, here as well domestic farmed birds (poultry) and species cohabitating with poultry are directly associated with the process of transmission and spread of avian coronavirus types.

According to papers published in the past few months, the new coronavirus was found to belong to the betacorona virus family (SARS-CoV2) (as MERS CoV and SARS-CoV). Also, at a site where the SARS virus has genes that do not encode specific proteins, the new virus has a sequence (12 nucleotides) in its S protein that recognizes human protein furin, which is found in high concentration in lung epithelial cells. This discovery found that, this virus, which only resided in certain groups of animals for a long period of time, has for the first time "learned" by spontaneous mutation to recognize host cells of another species (human).

The entry point for this virus is ACE2 receptors. There is a number of research and assumptions from which group of animals this virus could pass to humans. There has been much speculation that this virus has passed from bats or snakes, which has not been reliably confirmed. The study found that some species such as pigs, monkeys, civets, cats and some bats have more or less similar affinity levels based on the structure of the ACE2 receptor, and it is suggested that some of these species could be intermediary hosts. There is also speculation that Chinese pangolin (*Manis pentadactyla*) was intermediary host in the virus transmission from bats, which has also not been confirmed.

Preliminary studies have found that coronaviruses can be found in some snakes that inhabit Serbia, but do not pose threat to human health. The research began in 2016 and is still ongoing. (the unpublished authors'data).

In addition to the presence of these viruses in snakes, their presence in bats inhabiting Serbia has also been determined (data obtained from experts with the Museum of Natural History). **The presence of the virus in the representatives of these animal groups does not pose a threat to wild or domestic animals, as well as to humans.**

Coronaviruses also include many strains that cause disease in both domestic and wild species.

Some of these diseases are the following:

- transmissible gastroenteritis virus – swine disease
- infectious viral bronchitis in poultry
- bovine coronavirus
- mouse hepatitis virus
- feline coronavirus
- enteritis in turkeys caused by coronavirus
- gastroenteritis in hares caused by coronavirus and many others

The study of coronaviruses is relatively complicated since their cultivation requires substrates with living cells or tissues. Some strains reproduce only on cultures of human embryonic trachea, some strains only on cultures of human embryonic kidney, and some only on the nervous tissue of mice.

The reason for the poor research of this virus group is the fact that these viruses were not of particular clinical importance from the point of view of human medicine until the emergence of the first pathogenic and potentially lethal strains in the early 21st century.

Authors of the text:
Rastko Ajtić
Miloš Radaković