VIROLOGY

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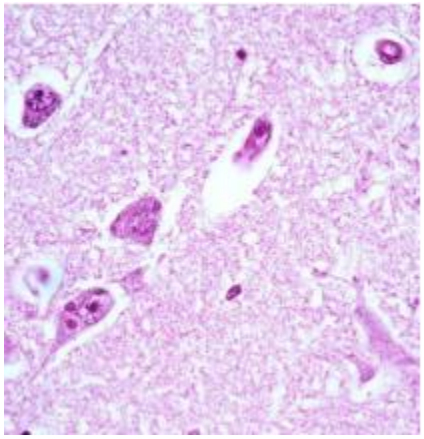
بسم الله الرحمن الرحيم

Firstly, we shall finish Cultivation and Assay of Viruses from the last lecture:



In the course of viral multiplication within cells, virus-specific structures called inclusion bodies may be produced. They become far larger than the individual virus particle and often have an affinity for acid dyes (e.g., eosin). They may be situated in the nucleus (herpesvirus), in the cytoplasm (poxvirus), or in both (measles virus). In many viral infections, the inclusion bodies are the site of development of the virions (the viral factories).

Histopathologic changes can be used for the detection of viral infections, for example: in Autopsy (تشريح الجثة) in rabies (داء الكلب) , the presence of eosinophilic cytoplasmic inclusions that are called nuclear bodies is very specific, and considered pathognomonic, it is a characteristic of infection by rabies virus.

Variations in the appearance of inclusion material depend largely on the tissue fixative used. The presence of inclusion bodies may be of considerable diagnostic aid. The intracytoplasmic inclusion in nerve cells—the Negri body—is pathognomonic for rabies.

Quantitation of Viruses: the number of viruses in a sample.

Extra: why should we know number of viruses in a sample?

This helps us in studying viruses and diagnosing the patient, more viruses means more probability to be infected and more severity of the disease

The unit used for this operation will be the Titer, if I have a solution which contain viruses and a plate which contain cells, the Titer would be the concentration of the viruses in the solution which is able to infect 50% of the cells.

We can use physical of biological methods:

A. Physical methods:
    - PCR, we can depend on quantitative real time PCR
    - Enzyme Immunoassay EIA
    - Radioimmunoassay RIA
    - Agglutination (تلصيق)/hemagglutination (التراص الدموي) {from the word glue}, to define the titer of the virus that present on the sample.
B. Biologic methods:

- End-point biologic assays depend on the measurement of animal death, animal infection, or cytopathic effects in tissue culture at a series of dilutions of the virus being tested.

The titer is expressed as the 50% infectious dose (ID50), which is the reciprocal of the dilution of virus that produces the effect in 50% of the cells or animals inoculated (Plaque assay, IFA). It is used in the plaque assay and immunofluorescence assay.

Purification of Virus Particles

First, we do concertation be precipitation with ammonium sulfate, ethanol, or polyethylene glycol or by ultrafiltration.

Then, viruses could be separated form host material by differential gradient centrifugation, density gradient centrifugation, column chromatography, and electrophoresis.

Viruses Reaction to Physical and Chemical Agents

It is very important topic specially in preparation for vaccines, and in the disinfection and sterilization procedures.

Heat and Cold: There is great variability in the heat stability of different viruses, as a general rule the envelope viruses are more sensitive to changes in temperatures.

Icosahedral viruses tend to be stable, losing little infectivity after several hours at 37°C. Enveloped viruses are much more heat labile, rapidly dropping in titer at 37°C. Viral infectivity is generally destroyed by heating at 50–60°C for 30 minutes, although there are some notable exceptions (e.g., hepatitis B virus, polyomaviruses: polyoma is naked it is intrinsically more stable to changes in temperature).

Viruses can be preserved by storage at subfreezing temperatures, and some may withstand lyophilization and can thus be preserved in the dry state at 4°C or even at room temperature. Viruses are sensitive to repeated freezing and thawing.

For DNA viruses -20 is sufficient, for RNA viruses because of the nature of RNA molecules which is more fragile compared to DNA molecules, the storage is done at -70 to -80.

Important Note: multiple freeze-thaw cycles may affect the stability of RNA viruses, so even if the virus is stored at -70 or -80, 2 to 3 cycles of freeze-thaw will make RNA degradation take place.

Many viruses can be stabilized by salts in concentrations of 1 mol/L (i.e., the viruses are not inactivated even by heating at 50°C for 1 hour) and the mechanism by which the salts stabilize viral preparations is not known.

Viruses are preferentially stabilized by certain salts. $MgCl_2$, 1 mol/L, stabilizes picornaviruses and reoviruses; $MgSO_4$, 1 mol/L, stabilizes orthomyxoviruses and paramyxoviruses; and Na_2SO_4, 1 mol/L, stabilizes herpesviruses.

The stability of viruses is important in the preparation of vaccines. The ordinary non-stabilized oral polio vaccine must be stored at freezing temperatures to preserve its potency. However, with the addition of salts for stabilization of the virus, potency can be maintained for weeks at ambient temperatures even in the high temperatures of the tropics, and this is very helpful in low-income settings.

Viruses are usually stable between pH values of 5.0 and 9.0. Some viruses (e.g., enteroviruses) are resistant to acidic conditions. All viruses are destroyed by alkaline conditions. In hemagglutination reactions, variations of less than 1 pH unit may influence the result. As a general rule, naked viruses are more stable at lower PH.

Ultraviolet, x-ray, and high-energy particles inactivate viruses. The dose varies for different viruses (we will talk about that when we talk about the specific virus infections). Infectivity is the most radiosensitive property because replication requires expression of the entire genetic contents. Irradiated particles that are unable to replicate may still be able to express some specific functions in host cells.

Ether susceptibility can be used to distinguish viruses that possess an envelope from those that do not.

Non-ionic detergents (e.g., Triton X-100) solubilize lipid constituents of viral membranes. The viral proteins in the envelope are released (undenatured).

Anionic detergents (e.g., sodium dodecyl sulfate) also solubilize viral envelopes; in addition, they disrupt capsids into separated polypeptides.

Viruses are penetrable to a varying degree by vital dyes such as toluidine blue, neutral red, and proflavine. These dyes bind to the viral nucleic acid, and the virus then becomes susceptible to inactivation by visible light.

Neutral red is commonly used to stain plaque assays so that plaques are more readily seen. The assay plates must be protected from bright light after the neutral red has been added; otherwise, there is the risk that progeny virus will be inactivated and plaque development will cease. So, the bright light can make the virus culture or the plaque assay as if there is no infection because of the altered susceptibility to bright light if we add neutral red.

▪ Antibacterial antibiotics have no effect on viruses.

▪ Some antiviral drugs are available.

▪ Quaternary ammonium compounds are not effective against viruses.

▪ Organic iodine compounds are also ineffective.

▪ Larger concentrations of chlorine are required to destroy viruses than to kill bacteria, especially in the presence of extraneous proteins. Usually if we want to get rid of viruses from biological samples usually it is accompanied by blood containing a lot or proteins so larger concentration of chlorine are needed.

▪ For example, the chlorine treatment of stools adequate to inactivate typhoid bacilli is inadequate to destroy poliomyelitis virus present in feces.

▪ Alcohols, such as isopropanol and ethanol, are relatively ineffective against certain viruses, especially picornaviruses(naked).

Common Methods of Inactivating Viruses for Various Purpose

▪ Viruses may be inactivated for various reasons, such as to sterilize laboratory supplies and equipment, disinfect surfaces or skin, make drinking water safe, and produce inactivated virus vaccines.

▪ Sterilization may be accomplished by steam under pressure, dry heat, ethylene oxide, and γirradiation.

▪ Surface disinfectants include sodium hypochlorite, glutaraldehyde, formaldehyde, and peracetic acid.

▪ Skin disinfectants include chlorhexidine, 70% ethanol (less effective), and iodophores.

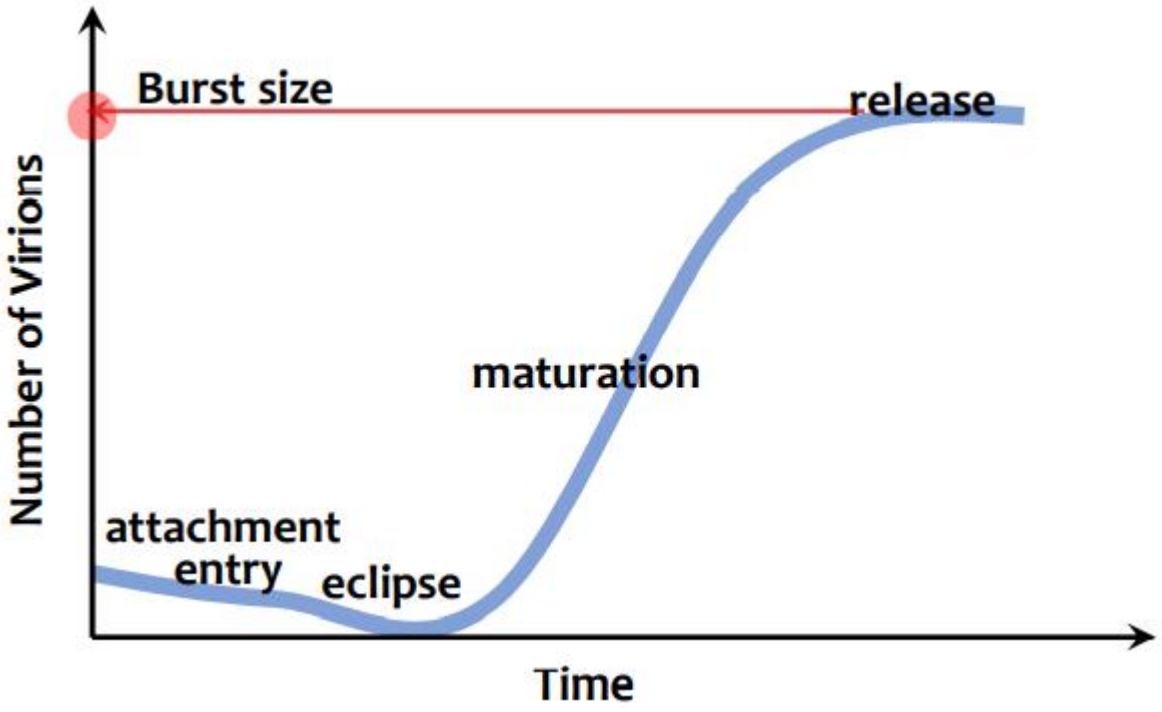
▪ Vaccine production may involve the use of formaldehyde, β-propiolactone, psoralen + ultraviolet irradiation, or detergents (subunit vaccines) to inactivate the vaccine virus.

Replication of Viruses

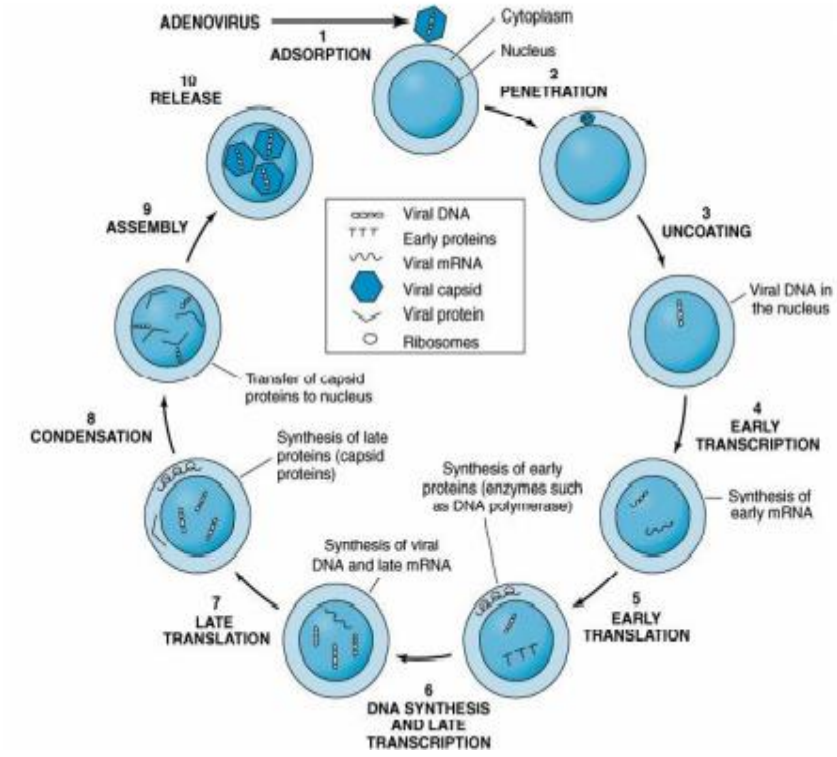
▪ The unique feature of viral multiplication is that soon after interaction with a host cell (once the virion enter the target cell) the infecting virion is disrupted and its measurable infectivity is lost (the total number of infectious virions decrease). Usually, infection starts with a single of very few number of virions, after that there will be disappearance of all infectious virions at this stage).

▪ This phase of the growth cycle is called the eclipse period; its duration varies depending on both the particular virus and the host cell, and it is followed by an interval of rapid accumulation of infectious progeny virus particles.

▪ The eclipse period is actually one of intense synthetic activity as the cell is redirected toward fulfilling the needs of the viral "pirate". (Protein synthesis, transcripts and replication of the viral genome, this will end by a large number of viral particles)



So the number of virions is very low at the beginning, those who attack the cell are usually one or very low number of virions, and following the attachment and entry the total number of infective virions in the cell reaches zero, and this is called eclipsed phase, followed by maturation during which the assembly of infective virions takes place in an exponential manner, followed by release of infective virions form the infected cell, and the burst size is the total number of virions that are released from a single target cell.



So, the stages of viral replication include absorption, penetration and coating of the virus (capsid). Early transcription which result in synthesis of early mRNA that give rise to early proteins that are involved in viral replication like the production of polymerases, and this stage is followed by DNA, RNA synthesis and late transcription which gives rise to following late translation to late proteins which are structural proteins of the virus followed by condensation, assembly and release of the mature variants from the infected cells the release can be by lysis of the infected cells, if the virus is a naked virus or the release can be by budding process in the enveloped viruses.

▪ Productive infections occur in permissive cells and result in the production of infectious virus. (It allows and support the replication of the virus)

▪ Abortive infections fail to produce infectious progeny, either because the cell may be non-permissive and unable to support the expression of all viral genes or because the infecting virus may be defective, lacking some functional viral gene. (It means the failure of the cell to support the replication of the virus)

▪ A latent infection (It can occur in some cases as we will see) may ensue, with the persistence of viral genomes, the expression of no or a few viral genes, and the survival of the infected cell. The pattern of replication may vary for a given virus, depending on the type of host cell infected.

▪ The yield of infectious virus per cell ranges widely, from modest numbers to more than 100,000 particles. The duration of the virus replication cycle also varies widely, from 6 to 8 hours (picornaviruses) to more than 40 hours (some herpesviruses).

TABLE 29-2 Pathways of Nucleic Acid Transcription for Various Virus Classes

Type of Viral Nucleic Acid	Intermediates	Type of mRNA	Example	Comments
± ds DNA	None	+ mRNA	Most DNA viruses (eg, herpesvirus, adenovirus)	
+ ss DNA	± ds DNA	+ mRNA	Parvoviruses	
± ds RNA	None	+ mRNA	Reoviruses	Virion contains RNA polymerase that transcribes each segment to mRNA
+ ss RNA	± ds RNA	+ mRNA	Picornaviruses, togaviruses, flaviviruses	Viral nucleic acid is infectious and serves as mRNA. For togaviruses, smaller + mRNA is also formed for certain proteins
− ss RNA	None	+ mRNA	Rhabdoviruses, paramyxoviruses, orthomyxoviruses	Viral nucleic acid is not infectious; virion contains RNA polymerase, which forms + mRNAs smaller than the genome. For orthomyxoviruses, + mRNAs are transcribed from each segment
+ ss RNA	− DNA, ± DNA	+ mRNA	Retroviruses	Virion contains reverse transcriptase; viral RNA is not infectious, but complementary DNA from transformed cell is

−, negative strand; +, positive strand; ±, a helix containing a positive and a negative strand; ds, double stranded; ss, single stranded.

The pathways of nucleic acid transcription of various virus classed will be discussed in details when we present the specific virus families, but you have to get a general idea about the needed intermediates, the type of messenger RNA and the examples for DNA & RNA viruses.

TABLE 29-3 Comparison of Replication Strategies of Several Important RNA Virus Families

	Grouping Based on Genomic RNA[a]					
	Positive-Strand Viruses			Negative-Strand Viruses		Double-Stranded Viruses
Characteristic	Picornaviridae	Togaviridae	Retroviridae	Orthomyxoviridae	Paramyxoviridae and Rhabdoviridae	Reoviridae
Structure of genomic RNA	ss	ss	ss	ss	ss	ds
Sense of genomic RNA	Positive	Positive	Positive	Negative	Negative	
Segmented genome	0	0	0[b]	+	0	+
Genomic RNA infectious	+	+	0	0	0	0
Genomic RNA acts as messenger	+	+	+	0	0	0
Virion-associated polymerase	0	0	+[c]	+	+	+
Subgenomic messages	0	+	+	+	+	+
Polyprotein precursors	+	+	+	0	0	0

The doctor passed over this table in a split of second (مرور الكرام).

TABLE 29-4 Summary of Replication Cycles of Major Virus Families

Virus Family	Presence of Virion Envelope	Intracellular Location			Multiplication Cycle (Hours)[b]
		Replication of Genome	Formation of Nucleocapsid[a]	Virion Maturation	
DNA viruses					
Parvoviridae	0	N	N	N	
Polyomaviridae	0	N	N	N	48
Adenoviridae	0	N	N	N	25
Hepadnaviridae	+	N	C	M–E	
Herpesviridae	+	N	N	M	15–72
Poxviridae	0	C	C	C	20
RNA viruses					
Picornaviridae	0	C	C	C	6–8
Reoviridae	0	C	C	C	15
Togaviridae	+	C	C	M–P	10–24
Flaviviridae	+	C	C	M–E	
Retroviridae	+	N	C	M–P	
Bunyaviridae	+	C	C	M–G	24
Orthomyxoviridae	+	N	N	M–P	15–30
Paramyxoviridae	+	C	C	M–P	10–48
Rhabdoviridae	+	C	C	M–P	6–10

[a]The synthesis of viral proteins always occurs in the cytoplasm.

[b]The values shown for duration of the multiplication cycle are approximate; ranges indicate that various members within a given family replicate with different kinetics. Different host cell types also influence the kinetics of viral replication.

C, cytoplasm; M, membranes; M–E, endoplasmic reticulum membranes; M–G, Golgi membranes; M–P, plasma membranes; N, nucleus.

This table present a summary for the replication cycles.

For example, in Herbies viruses the envelop is taken form the nuclear membrane rather than the cell membrane.

It shows where the formation of the nuclear capsid and replication of the genome takes place (cytoplasm or the nucleus), and the multiplication cycle range in hours, for Herbies viruses for example it can be as long as 72 hours, for the coronaviruses 6-8 hours, raptor viruses 6-10 hours.

The doctor: This Table Is Very Important to know.

Genetics of Animal Viruses

▪ Genetic analysis is a powerful approach toward understanding the structure and function of the viral genome, its gene products, and their roles in infection and disease, the epidemiology, evolution of viruses, pathogenesis of diseases, the severity of certain virions, etc.

▪ Viruses that have stable antigens on their surfaces (poliovirus, measles virus) can be controlled by vaccination. Other viruses that exist as many antigenic types (rhinoviruses) or change frequently (influenza virus A) are difficult to control by vaccination.

▪ Genetic analysis will help identify virus-specific processes that may be appropriate targets for the development of antiviral therapy.

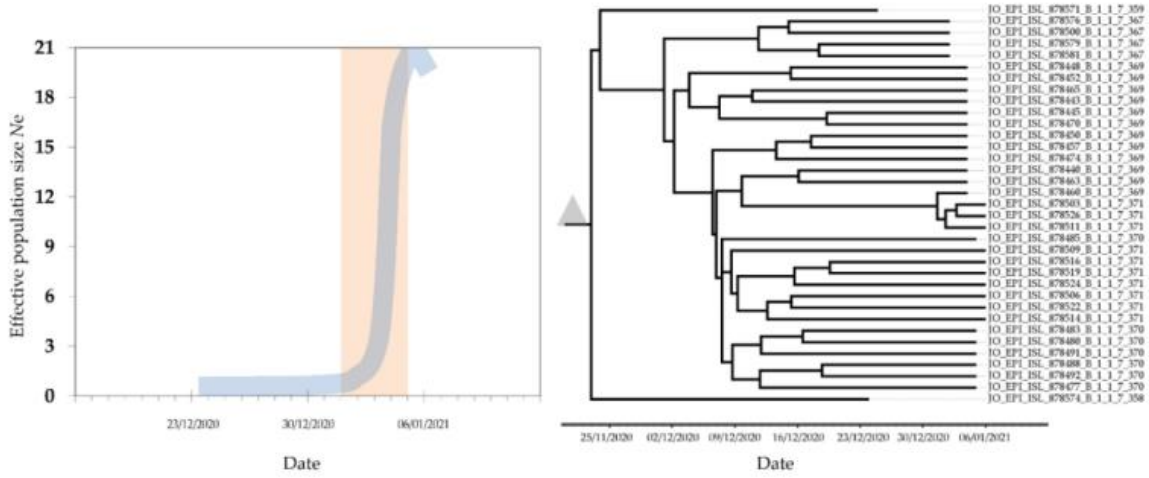


Figure 3. Maximum clade credibility (MCC) tree of the lineage B.1.1.7 (UK variant of concern) in Jordan, with the mean estimate for the tMRCA shown as the grey triangle (**right**). The median effective population size (*Ne*) shown in blue displayed a lag phase in December 2020 followed by an exponential increase in infections starting on 1 January 2021 highlighted in orange rectangle (**left**).

This is a real-life example of the use of genetic analysis in tracking the introduction and the spread of Sars coronavirus in Jordan. On the right is the UK variant of concern of the Alpha variant.

▪ Genotype: the genetic constitution of an organism.

▪ Phenotype: the observable properties of an organism, which are produced by the genotype in cooperation with the environment. (It is the set of proteins and traits defined by the genotype)

▪ A mutation is a heritable change in the genotype.

▪ The genome is the sum of the genes of an organism.

▪ Wildtype virus denotes the original virus from which mutants are derived and with which the mutants are compared; the term may not accurately characterize the virus as it is isolated in nature. Fresh virus isolates from the natural host are referred to as field isolates or primary isolates.

Mapping of Viral Genomes

Biochemical and physical mapping can be done much more rapidly than genetic mapping using classic genetic techniques. However, the accurate results are obtained from genetic analysis by genotyping and genetic analysis of the viruses.

For isolates that can be cloned, sequence analysis and comparison with known viruses is often used, and these comparisons are to draw entrances about the evolutionary relatedness between different types of viruses or viruses strains of variants or linages is done throw phylogenetic analysis.

Restriction endonucleases can be used for identification of specific strains of DNA viruses.

Types of Virus Mutants

▪ Classic genetic studies with animal viruses require a sensitive and accurate quantitative assay method, to compare the features (the virulence, the time) to produce the effect for mutants compared to wild type) such as a plaque assay for viral infectivity, and good mutants (resulting from single mutations) that are easily scored and reasonably stable.

▪ Some markers commonly used include plaque morphology, antibody escape or resistance to neutralizing antisera, loss of a virus protein, drug resistance, host range, and inability to grow at low or high temperatures.

▪ Conditional-lethal mutants are mutants that are lethal (in that no infectious virus is produced) (It is lethal for the virus itself under a set of conditions that is why it is called conditional) under one set of conditions—termed nonpermissive conditions (because no infectious variants are produced)—but that yield normal infectious progeny under other conditions— termed permissive conditions

Defective Viruses

A defective virus is one that lacks one or more functional genes required for viral replication (it might be completely defective if there is for example a certain deletion mutant, or it could be able to replicate in the presence of a helper virus). Defective viruses require helper activity from another virus for some step-in replication or maturation.

One type of defective virus lacks a portion of its genome (i.e., deletion mutant).

Spontaneous deletion mutants may interfere with the replication of homologous virus and are called defective interfering virus particles.

DIPs have lost essential segments of genome but contain normal capsid proteins; they require infectious homologous virus as helper for replication, and they interfere with the multiplication of that homologous virus**.**

Another category of defective virus requires an unrelated replication-competent virus as helper.

Examples include the adeno-associated satellite viruses and *hepatitis D virus (delta agent*), which replicate only in the presence of coinfecting human adenovirus or hepatitis B virus, respectively.

The essential helper function supplied by the helper virus varies, depending on the system.

Another type of defective viruses are Pseudovirions, they contain host cell DNA rather than the viral genome, enclosed in within the viral capsid and its other proteins.

During viral replication, the capsid sometimes encloses random pieces of host nucleic acid rather than viral nucleic acid.

Such particles look like ordinary virus particles when observed by electron microscopy, because they have the capsid and the other structural components of the virus, but they are not able to replicate.

Interactions Among Viruses, which can result in production novel variants

Recombination results in the production of progeny virus (recombinant) that carries traits not found together in either parent. The classic mechanism is that the nucleic acid strands break, and part of the genome of one parent is joined to part of the genome of the second parent, or there is jumping between different strands during the replication of the viral genome which is seen in a verity of RNA viruses, there is also the jumping between the two copies of RNA genome during the reverse transcription process and DNA production and HIV replication cycle.

Complementation is the interaction of viral gene products in cells infected with two viruses, one or both of which may be defective. It results in the replication of one or both under conditions in which replication would not ordinarily occur. The basis for complementation is that one virus provides a gene product in which the second is defective, allowing the second virus to grow.

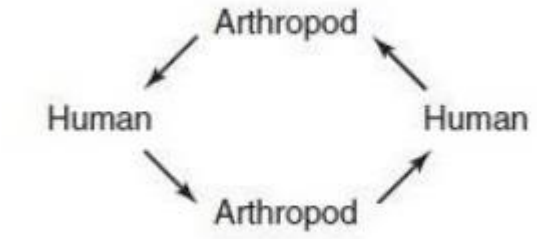
Infection of either cell cultures or whole animals with two viruses often leads to an inhibition of multiplication of one of the viruses, an effect called Interference.

Natural History (Ecology) and Modes of Transmission of Viruses

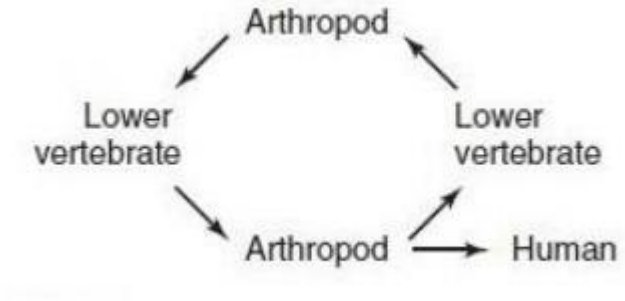
Viruses may be transmitted in the following ways:

- Direct transmission.
- Indirect transmission.
- Transmission from animal to animal, with human as accidental host.
- Arthropod vector.

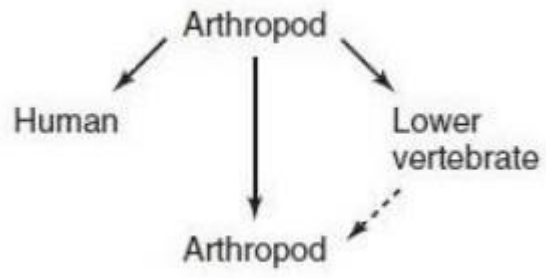
Transmission patters among arboviruses



It could be transmitted directly from the human to the arthropod and vice versa, example include yellow fever dengue fever.



It could be from arthropod to lower vertebrate to arthropod, with human as an accidental host, like Saint Louis encephalitis.



From arthropod to lower vertebrate or to human and from the lower vertebrate to arthropod, so it is here involving the arthropod mainly, include lacrosse encephalitis and Colorado tick fever. Here the viruses have their main host as arthropod, and lower vertebrate and human as accidental.

Emerging Viral Diseases

An important concept to consider and we will touch upon that when we discuss several virous infections like Ebola and Zika fever and of course discussing Sars coronavirus ii

We have contributing factors that might help increase the number of emerging viral infections in human, and these are:

- Environmental changes
- Human behavior
- Socioeconomic and demographic phenomena
- Travel and commerce
- Food production
- Health care
- Microbial adaptation
- Public health measures

Examples of Emerging Viral Infections

Ebola virus

Nipah virus

Hantavirus pulmonary disease

Hyman immunodeficiency virus

West Nile virus: its transmission from the middle-east into the US in late 1990s

Rift Valley fever

Emerging Coronaviruses

Bioterrorism Agents

▪ Microorganisms (or toxins) that could be used to produce death and disease in humans, animals, or plants for terrorist purposes.

▪ Potential bioterrorism agents are classified into risk categories based on the ease of dissemination or transmission from person to person, mortality rates, ability to cause public panic, and requirement for public health preparedness.

▪ Viral agents in the highest risk category are smallpox and the viral haemorrhagic fever.

This will be discussed in details when we touch upon the viral hemargic fevers and when we discuss smallpox.