General Virology

Fourth Year
Department of Environmental Health
University of Karbalaa

Winter Session

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Events Helped in Virus Discovery:

The first record of a virus infection consists of a hieroglyph from Memphis, the capital of ancient Egypt, drawn in 3700 BC, which depicts a temple priest showing typical clinical signs of paralytic poliomyelitis. Pharaoh Ramses V, who died in 1196 BC and whose extraordinarily well-preserved mummified body is believed to have succumbed to smallpox—a comparison between the pustular lesions on the face of the mummy and those of more recent patients is startling.

- Smallpox was **endemic** in China by 1000 BC and practice of **variolation (similar to Vaccination)** was developed. Survivors of smallpox outbreaks were protected from subsequent infection; the Chinese inhaled the dried crusts from smallpox lesions like snuff or, in later modifications, inoculated the pus from a lesion into a scratch on the forearm.

- Invention of microscope by Antony van Leeuwenhoek (1632–1723), a Dutch merchant, constructed the first simple microscopes and with these identified bacteria as the ‘animalcules’ he saw in his specimens.

- However, it was not until Robert Koch and Louis Pasteur in the 1880s jointly proposed the ‘germ theory’ of disease that the significance of these organisms became apparent.

- **Koch’s Postulates** (proof that an infectious agent is responsible for a specific disease:

1. The agent must be present in every case of the disease

2. The agent must be isolated from the host and grown *in vitro*

3. The disease must be reproduced when a pure culture of the agent is inoculated into a healthy susceptible host.

4. The same agent must be recovered once again from the experimentally infected host.

- Pasteur worked extensively on rabies, which he identified later as being caused by a ‘virus’ but despite this he did not discriminate between bacteria and other agents of disease.

First real evidence on virus identity

- In 1892, Dimitri Iwanowski, a Russian botanist, showed that extracts from diseased tobacco plants could transmit disease to other plants after passage through ceramic filters fine enough to retain the smallest known bacteria.

- A few years later (1898), Martinus Beijerinick confirmed and extended Iwanowski’s results on tobacco mosaic virus (TMV) and was the first to develop the modern idea of the virus, which he referred to as *contagium vivum fluidum* (‘contagious fluid ’).

- Freidrich Loeffler and Paul Frosch (1898) showed that a similar agent was responsible for foot-and-mouth disease in cattle, but, despite the realization that these new-found agents caused disease in animals as well as plants, people would not accept the idea that they might have anything to do with human diseases.

- This resistance was finally dispelled in 1909 by Karl Landsteiner and Erwin Popper, who showed that poliomyelitis was caused by a ‘filterable agent’—the first human disease to be recognized as being caused by a virus.

New Virus infecting Bacteria:

- Frederick Twort (1915) and Felix d'Herelle (1917) were the first to recognize viruses that infect bacteria, which d'Herelle called **bacteriophages** ('eaters of bacteria').

- In the 1930s and subsequent decades, pioneering virologists such as Salvador Luria, Max Delbruck, and many others used these viruses as model systems to investigate many aspects of virology, including virus structure, genetics and replication.

- These relatively simple agents have since proven to be very important to our understanding of all types of viruses, including those of humans which are much more difficult to propagate and study.

- During the Spanish–American War of the late nineteenth century and the subsequent building of the Panama Canal, the number of American deaths due to yellow fever was colossal. The disease also appeared to be spreading slowly northward into the continental United States. In 1902, through experimental transmission to mice, Walter Reed demonstrated that yellow fever was caused by a virus spread by mosquitoes. This discovery eventually enabled Max Theiler in 1937 to propagate the virus in chick embryos and to produce an attenuated vaccine—the17D strain—which is still in use today.

-The success of this approach led many other investigators from the 1930s to the 1950s to develop animal systems to identify and propagate pathogenic viruses.

- **Eukaryotic** cells can be grown *in vitro* (tissue culture) and viruses can be propagated in these cultures, but these techniques are expensive and technically quite demanding.

Nevertheless, they are increasingly being discarded for the following reasons:

1. Breeding and maintenance of animals infected with pathogenic viruses is expensive.
2. Whole animals are complex systems in which it is sometimes difficult to discern events.
3. Results obtained are not always reproducible due to host variation.
4. Unnecessary or wasteful use of experimental animals is morally repugnant.
5. They are rapidly being overtaken by 'modern science'—cell culture and molecular biology.

In recent years, an entirely new technology has been employed to study the effects of viruses on host organisms. This involves the creation of **transgenic** animals and plants by inserting all or part of the virus **genome** into the DNA of the experimental organism, resulting in expression of virus **mRNA** and proteins in somatic cells (and sometimes in the cells of the germ line). Thus, the pathogenic effects of virus proteins, individually and in various combinations, can be studied in living hosts. 'SCID-hu' mice have been constructed from immunodeficient lines of animals transplanted with human tissue.

These mice form an intriguing model to study the pathogenesis of human immunodeficiency virus (HIV) as there is no real alternative to study the properties of this important virus *in vivo*. While these techniques often raise the same moral objections as 'old-fashioned' experimental infection of animals by viruses, they are powerful new tools for the study of virus pathogenicity. This method will become widely used after solving technical difficulties associated with the construction of **transgenic** organisms.

CELL CULTURE METHODS

They began early in the twentieth century with whole-organ cultures, then progressed to methods involving individual cells, either **primary cell** cultures (somatic cells from an experimental animal or taken from a human patient which can be maintained for a short period in culture) or **immortalized** cell

lines, which, given appropriate conditions, continue to grow in culture indefinitely.

In 1949, John Enders and his colleagues were able to propagate poliovirus in primary human cell cultures. This achievement regarded as the ‘Golden Age of Virology’ and led to the identification and isolation during the1950s and 1960s of many viruses and their association with human diseases.

Plaque Assay

Renato Dulbecco in 1952 was the first to quantify accurately animal viruses using a **plaque** assay. In this technique, dilutions of the virus are used to infect a cultured cell **monolayer**, which is then covered with soft agar to restrict diffusion of the virus, resulting in localized cell killing and the appearance of plaques after the monolayer is stained. Counting the number of plaques directly determines the number of infectious virus particles applied to the plate.

The same Plaque technique can also be used biologically to clone a virus (i.e., isolate a pure form from a mixture of types). This technique had been in use for some time to quantify the number of infectious virus particles in **bacteriophage** suspensions applied to confluent ‘lawns’ of bacterial cells on agar plates, but its application to viruses of **eukaryotes** enabled rapid advances in the study of virus replication to be made.

Plaque assays largely replaced earlier endpoint dilution techniques, such as the tissue culture infectious dose (TCID50) assay, which are statistical means of measuring virus populations in culture; however, endpoint techniques may still be used in certain circumstances—for example, for viruses that do not replicate in culture or are not cytopathic and do not produce plaques, (e.g., human immunodeficiency virus)

- George Hirst, in 1941, observed **haemagglutination** of red blood cells by influenza virus. This proved to be an important tool in the study of not only influenza but also several other groups of viruses—for example, rubella virus. In addition to measuring the **titre** (i.e., relative amount) of virus present in any preparation, this technique can also be used to determine the antigenic type of the virus. Haemagglutination will not occur in the presence of antibodies that bind to and block the virus haemagglutinin. If an antiserum is titrated against a given number of haemagglutinating units, the haemagglutination inhibition titre and specificity of the antiserum can be determined.

In the 1960s and subsequent years, many improved detection methods for viruses were developed, such as:

- Complement fixation tests
- Radioimmunoassays
- Immunofluorescence (direct detection of virus antigens in infected cells or tissue)
- Enzyme-linked immunosorbent assays (ELISAs)
- Radioimmune precipitation
- Western blot assays
- PCR techniques

These techniques are sensitive, quick, and quantitative.

Monoclonal Antibodies (MA) Technology

In 1975, George Kohler and Cesar Milstein isolated the first MA from clones of cells selected *in vitro* to produce an antibody of a single specificity directed against a particular antigenic target. This enabled virologists to look not only at the whole virus, but at specific regions—epitopes—of individual virus antigens as well function of individual virus proteins. MA techniques have more applications in other types of serological assays (e.g., ELISAs) to increase their reproducibility, sensitivity, and specificity

- MA are produced by immunization of an animal with an antigen that usually contains a complex mixture of epitopes. Immature B-cells are later prepared from the spleen of the animal, and these are fused with a myeloma cell line, resulting in the formation of transformed cells continuously secreting antibodies. A small proportion of these will make a single type of monoclonal antibody against the desired epitope. Recently, *in vitro* molecular techniques have been developed to speed up the selection of monoclonal antibodies, although these have not yet replaced the original approach shown here

Ultrastructural & Physical Testing

- Physical measurements of virus particles began in the 1930s with the earliest determinations of their proportions by filtration through colloidal membranes of various pore sizes-The first electron micrograph of a virus (TMV) was published in 1939. Over subsequent years, techniques were developed that allowed the direct examination of viruses at magnifications of over 100,000 times.The two fundamental types of electron microscope are the transmission electron microscope (TEM) and the scanning electron microscope (SEM) - Studies of the sedimentation properties of viruses in ultracentrifuges in the 1960s in obtaining purified and highly concentrated preparations of many different viruses, free of contamination from host cell components that can be subjected to chemical analysis. The relative density of particles, measured in solutions of sucrose or CsCl virus

Molecular Biology Technology

- The term ‘molecular biology’ has taken on the new and different meaning of ‘genetic engineering’ or ‘genetic manipulation.’

These techniques for manipulating nucleic acids *in vitro (that is, outside living cells* or organisms)

- This powerful new technology has revolutionized virology and, to a large extent, has shifted the focus of attention away from the virus particle onto the virus **genome.**

Initially, any investigation of a virus genome will usually include questions about the following:

- Composition—DNA or RNA, single-stranded or double-stranded, linear or circular
- Size and number of segments
- Terminal structures
- Nucleotide sequence
- Coding capacity—open reading frames
- Regulatory signals—transcription **enhancers**, **promoters**, and **terminators**

History of Viruses (Summary):

- In 3700 BC: The first record of a virus infection with small pox consists of a hieroglyph from Memphis, the capital of ancient Egypt, drawn in 3700 BC.

- 1000 BC: Smallpox was endemic in China. **Variolation** (similar to Vaccination) was by inhaling the dried crusts from smallpox lesions or inoculated the pus from a lesion on a scratch on the forearm.

- In the first quarter of 18^{th} century: Invention of simple microscope by Antony van Leeuwenhoek, a Dutch merchant, used to identify bacteria as the 'animalcules' he saw in his specimens.

- In 1880s: Robert Koch and Louis Pasteur jointly proposed the 'germ theory' of disease.

- In 1890, Koch's Postulates showed proof that an infectious agent is responsible for a specific disease:

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- The discovery of yellow fever virus eventually enabled Max Theiler in 1937 to propagate the virus in chick embryos and to produce an attenuated vaccine—the17D strain—which is still in use today.

- In 1939: The first electron micrograph of a virus (TMV) was published in using two types electron microscope are the transmission electron microscope (TEM) and the scanning electron microscope (SEM).

- George Hirst, in 1941, observed haemagglutination of red blood cells by influenza virus.

- In 1952: Renato Dulbecco was the first to quantify accurately animal viruses using a plaque assay.

- In 1960s, Studies of the sedimentation properties of viruses in ultracentrifuges in the 1960s in obtaining purified and highly concentrated preparations of many different viruses

- In 1975, George Kohler and Cesar Milstein isolated the first monoclonal antibody from clones of cells selected in vitro to produce an antibody of a single specificity directed against a particular antigenic target.

- In the 1980s, molecular biology' has been used in 'genetic engineering' or 'genetic manipulation.' These techniques for manipulating nucleic acids in vitro (that is, outside living cells or organisms) used in virus diagnosis.

Theories on Origin of viruses:

1. Regressive evolution: This theory states that viruses are degenerate life forms that have lost many functions that other organisms possess and have only retained the genetic information essential to their parasitic way of life.

2. **Cellular origins:** In this theory, viruses are thought to be subcellular, functional assemblies of macromolecules that have escaped their origins inside cells.

3. **Independent entities:** This theory suggests that viruses evolved on a parallel course to cellular organisms from the self-replicating molecules believed to have existed in the primitive, prebiotic RNA world. This is similar to what spontaneous generation stated that viruses created from primitive cellular molecules have replication properties such as plasmids.

Viruses are:

- Submicroscopic, obligate intracellular parasites.

- Particles produced from the assembly of preformed components (replication of nucleic acid and protein synthesis.

- Particles (virions) themselves do not grow or undergo division. Virion: is the complete single virus particle (Nucleic acid and protein coat).

- Lacking the genetic information that encodes apparatus necessary for the generation of metabolic energy or for protein synthesis (ribosomes). They are therefore absolutely dependent on the host cell for this function

- One view said that inside the host cell viruses are alive, whereas outside it they are merely complex assemblages of metabolically inert chemicals.

- Viruses are smallest entities in most cases. While this is true, the largest known virus (Mimivirus, for ‘mimicking microbe’) is 400 nm in diameter.

Exceptional Entities:

There a number of exceptional pathogenic entities are clearly more similar to viruses than other microorganisms. The known entities are:

- Viroids: Viroids are very small (200–400 nucleotides), circular RNA molecules with a rod-like secondary structure. They have no capsid or envelope and are associated with certain plant diseases. Their replication is viruses—they are obligate intracellular parasites.

- Virusoids: Virusoids are satellite, viroid-like molecules, almost larger than viroids (e.g., approximately 1000 nucleotides); they depend on the presence of virus replication for multiplication (e.g. ‘satellite’); they are packaged into virus capsids.

- Prions: are infectious agents believed to consist of a single type of protein molecule with no nucleic acid component. Confusion arises that the prion protein and the gene that encodes it are also found in normal ‘uninfected’ cells. These agents are associated with ‘slow’ virus diseases such as Creutzfeldt–Jakob disease in humans, scrapie in sheep, and bovine spongiform encephalopathy (BSE) in cattle

Basic characteristics of Viruses

1-Although viruses are very heterogeneous, there is a unity of structure, basically protein and nucleic acid (RNA or DNA).

2- They are reproduced by replication..

3- Size: viruses are "filterable" agents.

4- Obligate (genetic) parasites--dependent on host cell genetic material.

Viruses persist in two stages:

1) dormant phase--extracellular; this phase is neither "alive nor dead" rather should be described as functionally active or inactive.

2) Vegetative phase--intracellular.

One should note that the nucleic acid of the virus, in some cases, is infectious. All viruses consist of RNA or DNA and a protein coat. Some viruses are enclosed within envelopes that contain both proteins and lipid.

An interesting question: Is a virus an organism? Is it more of an organism than a chromosome? Could viruses have evolved from chromosomes or some other organelle? What about transposons?

Recent diagnosis method

Comparison between Viruses and other microorganisms:

TABLE 13.1 Viruses and Bacteria Compared

	Bacteria		Viruses
	Typical Bacteria	Rickettsias/ Chlamydias	
Intracellular parasite	No	Yes	Yes
Plasma membrane	Yes	Yes	No
Binary fission	Yes	Yes	No
Pass through bacteriological filters	No	No/Yes	Yes
Possess both DNA and RNA	Yes	Yes	No
ATP-generating metabolism	Yes	Yes/No	No
Ribosomes	Yes	Yes	No
Sensitive to antibiotics	Yes	Yes	No
Sensitive to interferon	No	No	Yes

Chemistry of Viruses:

Virus Particle:

- Submicroscopic entity measured in nanometer (nm) = 1/1000000000, ranged from 20-250 nm
- consists of a nucleic acid covered with a protective protein coat or lipoprotein.
- The protein coat called capsid consists from smaller subunit called capsomer.
- Able to organize its own replication within host cell
- It is dependent on the host's protein- synthesizing system
- They have receptor binding-protein for attaching to cells Nucleic Acids:
- Ribonucleic (RNA) and Deoxyribonucleic (DNA) acids are important genetic materials in both virus and host.
- RNA may take enzyme role during amino acids synthesis to form polypeptides in protein production
- Most plant viruses are RNA and in human and animals are DNA viruses but RNA viruses are dominant.
- Both are unbranched macromolecules polymers that differ primarily in the structure of their monomers (repeating unit).
- Each monomer of N.A. is a nucleotide consists of 3 major parts:

1. Phosphates (PO4-3)
2. Pentose Sugar (Deoxyribose or Ribose)
3. Nitrogenous Bases:

Adenine (A), Guanine (G), Cytosine (C), Thymine (T) in DNA and Uracil (U) in RNA.

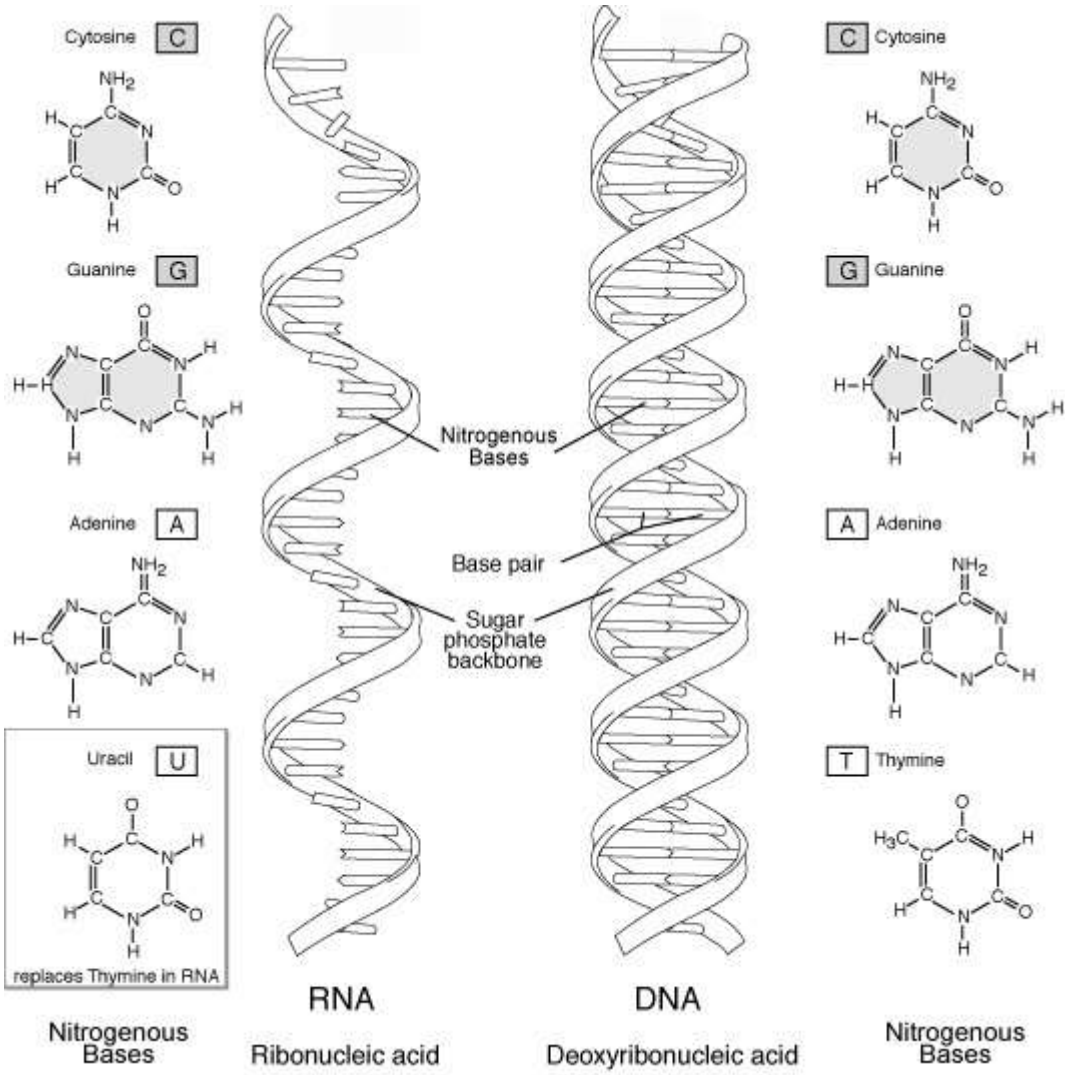
- Bases A + G= purines (double ringed molecule) and

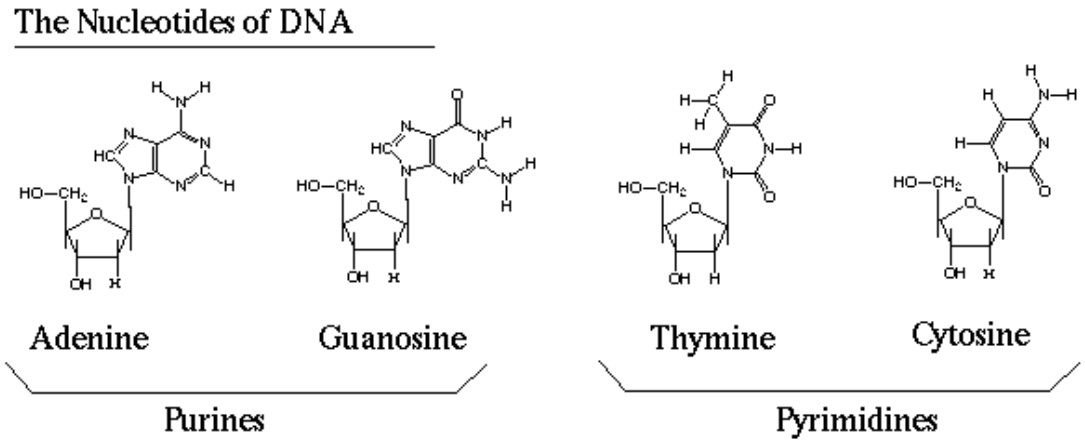
C + T or U= pyrmidines (single ringed molecule).

- Nucleotide lacks phosphate root (PO_4^{-3}) called Nucleoside.

Relationship between RNA role and amino acids synthesis:

- Triplet Code or 3 letter code first suggested by
  the Physicist Gamow in 1954.
- It is called now Codon which specify each amino acid: Example: AGA
- Synthesis of 20 amino acids are determined by 64 bases (4X4x4) of purine or pyramidine bases which makes distinct triplets (codon) with assistance of transfer RNA.





Proteins:

- Are macromolecules consists of amino acids connected by polypeptides to form the protein coat

- The protein coat of virus particle which called capsid consists of capsomers which may be covered by a lipid layer.

- Sometimes, there are protein spikes originate from the capsid.

- Protein represent the major part of the virus particle about 95% of the size.

- Amino acids are the repeating units in the protein synthesis using the genetic code or codon (triplet letters) as described earlier.

- The gene expression process includes:

1. Transcription: dsDNA orders to make a complimentary copy of RNA

GTG CAT CTG ACT CCT GAG GAG AAG

GUG CAU CUG ACU CCU GAG GAG AAG

Valine Histidin Lucine Trypto Phenyl Gluta Gluta Lys

2. Translation: amino acids formation and protein synthesis

Virus Morphology and Symmetry:

helical symmetry A form of symmetry in which many RNA virus capsids are constructed.
Each capsomere on the helix consists of a single polypeptide molecule and establishes bonds with two capsomeres on each of the adjacent turns, giving stability to the capsid.
The overall length of the helix is determined by the length of the RNA molecule. In all animal viruses with helical symmetry the nucleocapsid is folded and packed within a lipoprotein envelope, e.g. *Bunyaviridae,Orthomyxoviridae*, *Paramyxoviridae* and *Rhabdoviridae*.

helical viruses Viruses whose morphology displays helical symmetry

icosahedral symmetry One of the two types of symmetry found in viral capsids, the other being helical symmetry. Crystallographic considerations prescribe that the identical units forming the capsid of an isometric particle must be arranged with cubic symmetry. Of the possible forms that this may take, icosahedral symmetry provides the facility to make a range of viral capsids with different numbers of structural units. An icosahedron has 20 triangular faces and 12 vertices.
The simplest has 60 identical structural units in regular relation to each other, three to a triangular face. To make a large virus in this simple form from 60 units would require a large protein, which raises difficulties with genome coding capacity, and an alternative is to use a larger number of small units (i.e. more than 60). This inevitably means that the units cannot all have identical relationships to each other.
Those not surrounding a vertex form groups of six called ‘hexons’, and those at each vertex are in groups of five called ‘pentons’. Only certain multiples of 60 units are possible, and the numbers which make up different viral capsid structures are defined by the triangulation number,
T. There are always 60T units, where $T = h2 + hk + k2$ (h and k are integers having no common factors).
Examples are $T = 3$ (caliciviruses), $T = 4$ (alphaviruses),
$T = 13$ (rotaviruses and orbiviruses) and $T = 16$ (herpesviruses).
The structural units form into morphological units on the virus surface.
In general, the number of morphological units (capsomeres) which can be visualized on the surface of an icosahedral virion is $10T + 2$ (e.g. 162 for herpesviruses).

icosahedron A solid with 20 triangular faces and 12 vertices. In a regular icosahedrons the faces are equilateral triangles and there are axes of two-fold, three-fold and five-fold rotational symmetry.

isometric particle Particles with identical linear dimensions, distinct from the rodshaped and bullet-shaped virus particles and viruses enclosed by irregular capsules. They appear spherical; however, their capsids are constructed with icosahedral symmetry.

quasi-equivalence: A theory invoked to account for the surface morphology of spherical viruses. It requires that subunits forming the icosahedral capsid should be capable of assembling into both hexamers and pentamers. The insertion of 12 pentamers produces curvature in the sheet of hexamers where they are inserted, resulting in a closed icosahedral shell that is not strictly equivalent, but forms a more stable structure. Thus icosahedral viruses have a capsid composed of 12 pentamers and a variable number of hexamers, e.g. herpesviruses have 150 hexamers and 12 pentamers, making up the capsid; adenoviruses have 240 hexamers and 12 pentamers.

quasi-species A term that describes the nature of most RNA viruses, which are populations of genetic variants within which one (the quasi-species) predominates.

Virus Genome:

The hereditary information of the virus is encoded in the sequence of nucleotides in the RNA or DNA. This information has to be passed on to new viruses through replication of the viral nucleic acid. This genetic information directs the synthesis of viral proteins. The nucleic acid of DNA viruses does not direct protein synthesis itself. RNA copies of the appropriate segments (genes) of DNA are used as template to direct the synthesis of the protein. Some RNA viruses contain a positive-strand (or positive sense) RNA genome that acts directly as mRNA. By contrast, negative-strand RNA viruses posses an enzyme that copies the viral negative-sense RNA genome into a positive-strand copy, which is then used as mRNA to direct protein synthesis.

Genome size:

Genomes sizes of viruses vary greatly because of the difficulty of packaging the genes into the small space within the virion particle.

- Human cells have 30,000 genes, in comparison the bacterium E. coli has 4000 genes.
- The largest viruses (e.g. poxviruses) may contain only 200 or fewer genes.
- The smallest virus may have the equivalent of only 3 or 4 genes.
- In general, RNA viruses have smaller genomes and code for fewer proteins than DNA viruses.
- RNA genomes are more fragile than DNA genomes and this characteristic limits the size of RNA genomes.

The size of viral genome is measured in the number of bases in their nucleic acids.
In case of large genome, these numbers are expressed are thousands of bases (Kilobases or Kb).
For single-stranded (SS) genomes the notation kb is used, for example single stranded measles virus genome is 16,000 bp (16 Kb) in length.
For double-stranded genomes, the bases number are expressed as Kilobase pair (Kbp), for example the double-stranded adenovirus genome is about 36,000 bp (36 Kbp).

The structure and complexity of virus genome

The compositions and structures of virus **genomes** are more varied than any of those seen in the entire bacterial, plant, or animal kingdoms.

- The nucleic acid comprising the genome may be
  - Single stranded or
  - Double stranded and
- The shape may have a linear, circular, or segmented configuration.

Single-stranded virus genomes may be either

- Positive (+) sense (i.e., of the same polarity, or nucleotide sequence, as the **mRNA**),
- Negative (-) sense, or **ambisense** (a mixture of the two).

  Many of the DNA viruses of **eukaryotes** closely resemble their host cells in terms of the biology of their genomes.

Some DNA virus genomes are complexed with cellular histones to form a **chromatin**-like structure inside the virus particle. Once inside the nucleus of the host cell, these genomes behave like miniature satellite chromosomes, following the dictates of cellular enzymes and the cell cycle.

Virus genetic and mutants

Although nucleotide sequencing now dominates the analysis of virus genomes, functional genetic analysis of viruses is based largely on the isolation and analysis of mutants, usually achieved using plaque purification ('biological cloning'). In the case of viruses for which no such systems exist (because they either are not cytopathic or do not replicate in culture), little genetic analysis was possible before the development of molecular genetics

VIRUS MUTANTS

'Mutant,' 'strain,' 'type,' 'variant,' and even 'isolate' are all terms used rather loosely by virologists to differentiate particular viruses from each other and from the original 'parental,' 'wild-type,' or 'street' isolates of that virus. More accurately, these terms are generally applied as follows:

Strain: Different lines or isolates of the same virus (e.g., from different geographical locations or patients)

Type: Different serotypes of the same virus (e.g., various antibody neutralization phenotypes)

Variant: A virus whose phenotype differs from the original wild-type strain but the genetic basis for the difference is not known

Viruses Classification:

Virus classification is the process of naming viruses and placing them into a taxonomic system. There are 5 classification systems of viruses.

1. Classification based on disease:

The first, and most common, experience of viruses is as agents of disease and it is possible to group viruses according to the nature of the disease with which they are associated. This method of grouping viruses is useful, but it suffers from serious deficiencies:

First, this approach is focusing as it does on diseases that we recognize because they affect humans or our domestic livestock.

Second, this approach ignores the fact that most viruses either do not cause disease or cause a disease that we do not recognize because of a lack of

understanding of the host; for example we understand little of the diseases caused by viruses of fish or amphibians. Similarly, it is possible for a single virus to cause more than one type of disease; a good example of this is varicella zoster virus which causes chickenpox in a first infection but when reactivated later in life causes shingles.

2. **Classification based on host:**
This has the attraction that it emphasizes the parasitic nature of the virus–host interaction. However, there are several difficulties with this approach. This form of classification implies a fixed, unchanging, link between the virus and host in question. Some viruses are very restricted in their host range, infecting only one species, such as hepatitis B virus infecting humans, and so a designation based on the host is appropriate. There other viruses may infect a small range of hosts, such as poliovirus which can infect human and monkeys, and the designation here must reflect this rather than name a single species.

3. Classification based on the virus particle morphology:
Using electron microscope assisted in classification of viruses based on the size and shape of virus particles. Then based on the presence of envelope and lipids, three morphological categories are defined, isometric, filamentous, and complex. Isometric viruses appear approximately spherical but are actually icosahedrons. Filamentous viruses have a simple, helical, morphology. The complex viruses are those which do not neatly fit within the other two categories.
Complex shapes for virus particles may be made up of a combination of isometric and filamentous components, such as is seen with bacteriophage T2.
While a classification scheme based on morphology is simple and describes an unchanging feature of the virus, it suffers from several drawbacks. Primary amongst these is that knowing the shape of a virus particle does not allow us to predict anything about the biology, pathology, or molecular biology of similarly shaped viruses. Thus, two viruses with very similar morphologies may differ in all of their other fundamental characteristics.

4. Classification based on nucleic acid
The nucleic acid content of a virus has been used as a basis for classifying viruses. The key aspect of this classification scheme is that it considers the nature of the virus genome in terms of the mechanisms used to replicate the nucleic acid and transcribe mRNA encoding proteins.
The nature of a particular nucleic acid sample is assessed by determining its base composition, sensitivity to DNase or RNase, buoyant density, etc. Single-stranded nucleic acids are distinguished from double-stranded by the absence of a sharp increase in absorbance of ultraviolet light upon

heating and the nonequivalence of the molar proportions of adenine (A) and thymine (T) (or uracil (U)) or guanine (G) and cytosine (C). From these types of analysis, it appears that viruses utilize four possible kinds of viral nucleic acid: single-stranded DNA, single-stranded RNA, doublestranded DNA, and double-stranded RNA. Each kind of genome is found in many virus families, which between them contain members that infect a diverse array of animals, plants, and bacteria.

Classifying viruses – the Baltimore Scheme

As considered above, viruses exhibit great diversity in terms of morphology, genome structure, mode of infection, host range, tissue tropism, disease (pathology), etc. While, as we have seen, each of these properties can be used to place viruses into groups, classifying viruses solely on the basis of one or even two of these parameters does not lead to a system where studying one virus in a particular group can be used to draw inferences about other members of the same group. Also, classification on these grounds does not give a good basis for unifying discussions of virus replication processes. To circumvent these problems Nobel laureate David Baltimore proposed a classification scheme which encompasses all viruses, based on the nature of their genomes, and their modes of replication and gene expression. This system provides an opportunity to make inferences and predictions about the fundamental nature of all viruses within each defined group.

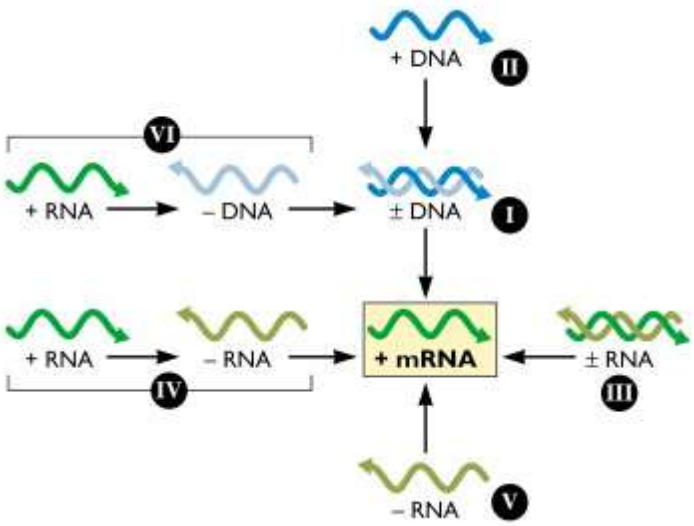
The original Baltimore classification scheme was based on the fundamental importance of messenger RNA (mRNA) in the replication cycle of viruses. Viruses do not contain the molecules necessary to translate mRNA and rely on the host cell to provide these. They must therefore synthesize mRNAs which are recognized by the host cell ribosomes. In the Baltimore scheme, viruses are grouped according to the mechanism of mRNA synthesis which they employ (Fig. 4.1). By convention, all mRNA is designated as positive (or “plus”) sense RNA. Strands of viral DNA and RNA which are complementary to the mRNA are designated as negative (or “minus”) sense and those that have the same sequence are termed positive sense. Using this terminology, coupled with some additional information about the replication process, a modified classification scheme based on the original proposed by Baltimore defines seven groups of viruses, with each commonly being referred to by the nature of the virus genomes it includes:

Class 1 contains all viruses that have double-stranded (ds) DNA genomes. In this class, the designation of positive and negative sense is not meaningful since mRNAs may come from either strand. Transcription can occur using a process similar to that found in the host cells.

Class 2 contains viruses that have single-stranded (ss) DNA genomes. The DNA can be of positive or negative sense, depending on the virus being

studied. For viruses in class 2 the DNA must be converted to a doublestranded form before the synthesis of mRNA can proceed.
Class 3 contains viruses that have dsRNA genomes. All known viruses of this type have segmented genomes and mRNA is only synthesized from one template strand of each segment. The process of transcription from a dsRNA genome can be envisioned as occurring using a mechanism similar to that for transcription from a dsDNA genome. However, the enzymes necessary to carry out such a process do not exist in normal, uninfected, cells. Consequently, these enzymes must be encoded by the virus genome and must be carried into the cell by the virus to initiate the infectious process.
Class 4 contains viruses with ssRNA genomes of the same (positive) sense as mRNA and which can be translated. Synthesis of a complementary strand, generating a dsRNA intermediate, precedes synthesis of mRNA. As with the class 3 viruses the RNA synthesis must be carried out using virus-encoded enzymes, although these are not carried in the virus particle.
Class 5 contains viruses that have ssRNA genomes which are complementary in base sequence to the mRNA (negative-strand RNA viruses). Synthesis of mRNA requires novel virus-encoded enzymes, and generation of new virus genomes requires the synthesis of a dsRNA intermediate, the positive sense strand of which is used as a template for replication. Viral RNA-synthesizing enzymes are carried in the virion. Some class 5 viruses use the newly synthesized “antigenome” RNA strand as a template for the production of an mRNA and are referred to as “ambisense” viruses.
Class 6 contains viruses that have ssRNA genomes and which generate a dsDNA intermediate as a prelude to replication, using an enzyme carried in the virion.
Class 7 More recently, it has been suggested that some viruses, termed reversiviruses, should be transferred from class 1 into a new class 7. This is based on their replication from dsDNA via a positive sense ssRNA intermediate back to dsDNA. This represents the inverse of the class 6 replication strategy, with which class 7 has many similarities.
The Baltimore scheme has both strengths and weaknesses as a tool for understanding virus properties. A particular strength is that assignment to a class is based on fundamental, unchanging, characteristics of a virus. Once assigned to a class, certain predictions about the molecular processes of nucleic acid synthesis can be made, such as the requirement for novel virus-encoded enzymes. A weakness is that, whilst it brings together viruses with similarities of replication mechanism, the scheme takes no account of their biological properties. For example, bacteriophage T2 and variola virus (the cause of smallpox) are classified together in class 1 although they are totally dissimilar in both structure and biology. Similarly, the identification of a positive sense RNA genome is not sufficient to classify the virus

unambiguously since viruses of classes 4 and 6 have similar genome nucleic acids.



5. Classification based on traditional taxonomy

The International Committee on Taxonomy of Viruses (ICTV), first founded in the late 1960s, has established a taxonomic classification scheme for viruses. This uses the familiar systematic taxonomy scheme of *Order, Family, Subfamily*, and *Genus* (no Kingdoms, Phyla, or Class of viruses.

In assigning a virus to a taxonomic group the ICTV considers a range of characteristics. These include host range (eukaryote or prokaryote, animal, plant, etc.), morphological features of the virion (enveloped, shape of capsid or nucleocapsid, etc.), and nature of the genome nucleic acid (DNA or RNA, single stranded or double stranded, positive or negative sense, etc.). Within these parameters additional features are considered.

These include such things as the length of the tail of a phage or the presence or absence of specific genes in the genomes of similar viruses, and these aspects allow allocation of subdivisions in the taxonomic designation.

For each of the individual genera defined by the ICTV a single virus has been designated as the type member. The type member is used essentially as the reference for the genus.

Viral classification starts at the level of order and continues to the species as follows, with the taxon suffixes given in *italics*:

Order (*-virales*)

Family (*-viridae*)

Subfamily (*-virinae*)

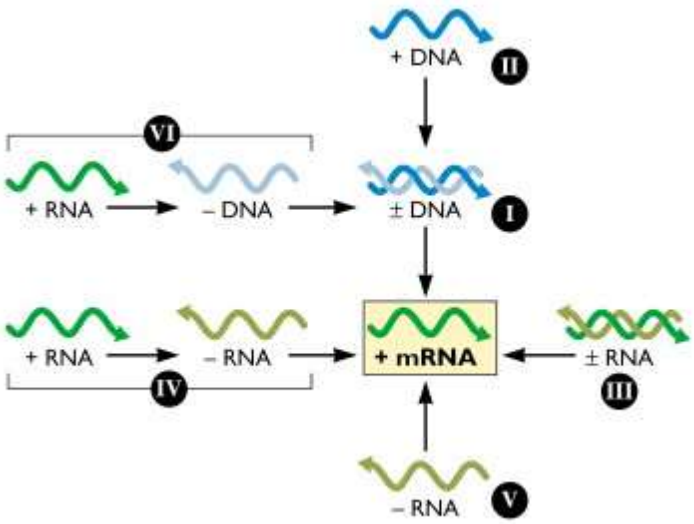
Genus (*-virus*)

Species

Species names generally take the form of *[Disease] virus*

The ICTV defined "A species is a monophyletic group of viruses whose properties can be distinguished from those of other species by multiple criteria.

2. The Baltimore system



Although many viruses are classified into individual families based on a variety of physical and biological criteria, they may also be placed in groups according to the type of genome in the virion. Over 30 years ago virologist David Baltimore devised an alternative classification scheme that takes into account the nature of the viral nucleic acid.

One of the most significant advances in virology of the past 30 years has been the understanding of how viral genomes are expressed. Cellular genes are encoded in dsDNA, from which mRNAs are produced to direct the synthesis of protein. Francis Crick conceptualized this flow of information as the central dogma of molecular biology:

DNA —> RNA —> protein

All viruses must direct the synthesis of mRNA to produce proteins. No viral genome encodes a complete system for translating proteins; therefore all viral

protein synthesis is completely dependent upon the translational machinery of the cell. Baltimore created his virus classification scheme based on the central role of the translational machinery and the importance of viral mRNAs in programming viral protein synthesis. In this scheme, he placed mRNA in the center, and described the pathways to mRNA from DNA or RNA genomes. This arrangement highlights the obligatory relationship between the viral genome and its mRNA.

By convention, mRNA is defined as a positive (+) strand because it is the template for protein synthesis. A strand of DNA of the equivalent sequence is also called the (+) strand. RNA and DNA strands that are complementary to the (+) strand are, of course, called negative (-) strands.

According to Baltimore classification, viruses can be placed in one of the seven following groups

- I: **dsDNA viruses** (e.g. Adenoviruses, Herpesviruses, Poxviruses)
- II: **ssDNA viruses** (+ strand or "sense") DNA (e.g. Parvoviruses)
- III: **dsRNA viruses** (e.g. Reoviruses)
- IV: **(+)ssRNA viruses** (+ strand or sense) RNA (e.g. Picornaviruses, Togaviruses)
- V: **(−)ssRNA viruses** (− strand or antisense) RNA (e.g. Orthomyxoviruses, Rhabdoviruses)
- VI: **ssRNA-RT viruses** (+ strand or sense) RNA with DNA intermediate in life-cycle (e.g. Retroviruses)
- VII: **dsDNA-RT viruses** (e.g. Hepadnaviruses)

Virus Disease Development:

There are certain terms are commonly used with virus disease development.

The disease is any abnormal function in the metabolism of living cell due to infectious or noninfectious agent and virus is the agent here.

The capability of infectious virus particle to initiate a disease in a susceptible host is called pathogencity.

The stages of initiation infection until symptoms formation is called disease development.

Incubation period: time required after virus penetration to symptoms development in the infected host.

The virus diseases general is associated with symptoms which are expressions of virus pathological activity in living cells.

Viral Infection: the establishment of a virion in a susceptible host and causing a disease.

Latent infection: a delayed infection in the susceptible host due to environmental and/or host-related factors such as age and genetic.

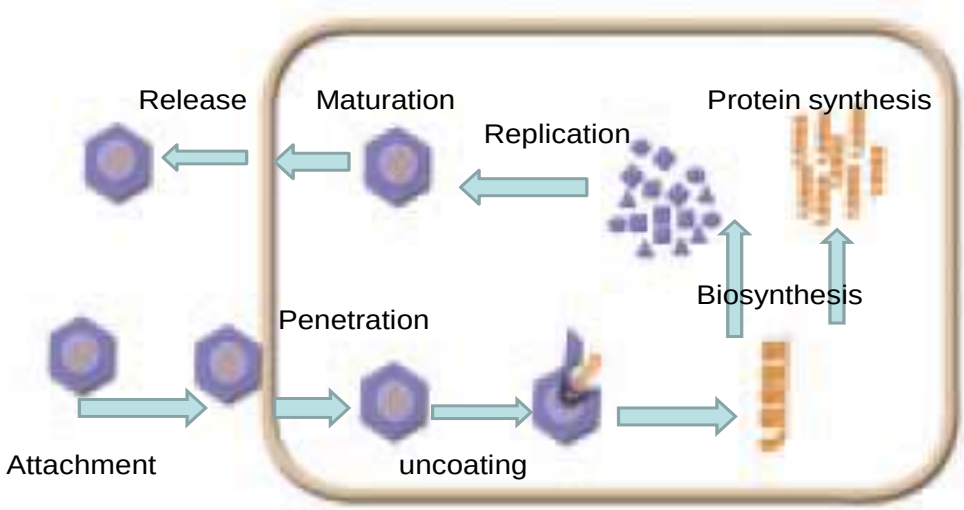
Symptoms are external and/or internal expressions of pathological activity in a living host.

Virus Disease Cycle:

Disease cycle is the process of disease development in the infected living host.

This involves three broad stages carried out by all types of viruses:

1. The initiation of infection in 2 steps:
    - Attachment
    - Penetration
2. Biosynthesis process;
    - Replication of nucleic acid
    - Expression of the genome (protein synthesis)
3. Release of mature virions from the infected cell to outside.



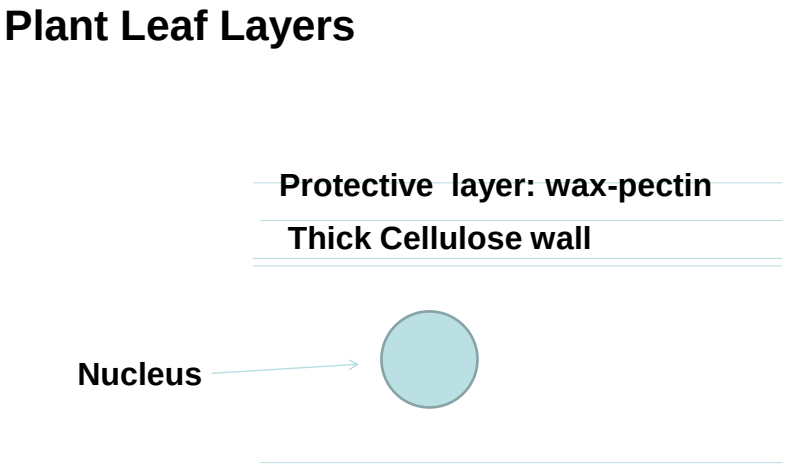
- Virus attachment consists of specific binding of a virus-attachment protein (or 'antireceptor') to a cellular receptor molecule (except plant viruses).

- Target receptor molecules on cell surfaces may be proteins (usually glycoproteins) or the carbohydrate residues present on glycoproteins or glycolipids. The former are usually specific receptors in that a virus may use a particular protein as a receptor. Carbohydrate groups are usually less specific (not common) receptors because the same configuration of side-chains may occur on many different glycosylated membrane bound molecules.

- Plant viruses face special problems initiating an infection. The outer surfaces of plants have **protective layers** of waxes and pectin, and each cell is surrounded by a **thick wall** of cellulose overlying the cytoplasmic membrane.

- To date, no plant virus is known to use a specific cellular receptor of the type that animal and bacterial viruses use to attach to cells; instead, plant viruses rely on a mechanical damage integrity of a cell wall to introduce a virus particle directly into a cell. This is achieved either by

the vector transmitting the virus or simply by mechanical damage to cells. These entries can be called **infectable sites**.



- Penetration: normally occurs a very short time after attachment of the virus to its receptor in the cell membrane. Unlike attachment, cell penetration is generally an energy-dependent process; that is, the cell must be metabolically

active for this to occur.

Three main mechanisms are involved:

1.**Translocation** of the entire virus particle across the cytoplasmic membrane of the cell

2. **Endocytosis** of the virus into intracellular vacuoles . This is the most common mechanism of a virus entry into cells. It does not require any specific virus proteins (compared to attachment).

- It relies on the normal formation and internalization of coated pits at the cell

membrane. Receptor-mediated endocytosis is an efficient process for taking up and concentrating extracellular macromolecule.

3. **Fusion:** of the virus envelope (only applicable to enveloped viruses) with the cell membrane, either directly at the cell surface or following endocytosis in a cytoplasmic vesicle, which requires the presence of a specific fusion protein

in the virus envelope. These proteins promote the joining of the cellular and virus membranes which results in the nucleocapsid deposited directly in the cytoplasm.

- The process of endocytosis is almost universal in animal and human cells.

- The formation of coated pits results in the engulfment of a membrane-bounded vesicle by the cytoplasm of the cell. The lifetime of these initial coated vesicles is very short, within seconds, most fuse with endosomes, releasing their contents into these larger vesicles.

The release of virus particles from endosomes and their passage into the cytoplasm is intimately connected with (and often impossible to separate from) the process of uncoating.

- **Uncoating** is a general term for the events that occur after penetration, in which the virus capsid is completely or partially removed and the virus genome exposed, usually in the form of a nucleoprotein complex. The removal of a virus envelope that occurs during membrane fusion is part of the uncoating process.

The initial events in uncoating may occur inside endosomes, being triggered by the change in pH as the endosome is acidified or directly in the cytoplasm.

After uncoating or protein coat removal, two biosynthesis process may occur:

1. Replication

2. Gene Expression:

The most critical interaction between a virus and its host cell is the requirement by the virus for the cellular apparatus of nucleic acid and protein synthesis. The course of virus replication is

determined by tight control of gene expression.

- Therefore, gene expression is a multistep process by which the protein product of a gene is synthesized.

The replication process of any virus depends on the nature of its genetic material.

For viruses with RNA genomes in particular, genome replication and the expression of genetic information or gene expression are completely linked;

- The control of gene expression determines the overall course of a virus infection:

- Latent or masked

- Acute or sharp,

- Chronic or persistent,

Virus Replication

- The objective of a virus after entering a host is to replicate its genetic information.

- Replication occurs in eukaryotic cells in nuclear and/or cytoplasmic compartments using genetic information and biochemical capacity between the virus genome and that of the host cell.

- Viruses with an RNA lifestyle (i.e., an RNA genome plus messenger RNAs) have no apparent need to enter the nucleus, although during the course of replication some do.

- DNA viruses, mostly replicate in the nucleus, where host-cell DNA is replicated and where the biochemical apparatus necessary for this process is located.

During Gene Expression, viruses make use of the biochemical apparatus of their host cells to express their genetic information as proteins and, consequently, utilize the appropriate biochemical language recognized by the cell.

Therefore, viruses of prokaryotes produce polycistronic mRNAs (a mRNA that encodes more than one protein which means several different polypeptide chains), while viruses with eukaryotic hosts produce mainly monocistronic mRNAs (a mRNA that encodes only one kind of protein which means one polypeptide chain).

Assembly process involves the collection of all the components necessary for the formation of the mature virion at a particular site in the cell. During assembly, the basic structure of the virus particle is formed. The site of

assembly depends on the site of replication within the cell and on the mechanism by which the virus is eventually released from the cell and varies for different viruses. For example,

- In picornaviruses, poxviruses, and reoviruses, assembly occurs in the cytoplasm;

- In adenoviruses, polyomaviruses, and parvoviruses, it occurs in the nucleus.

Maturation is the stage of the life-cycle at which the virus becomes infectious.

Maturation usually involves structural changes in the virus particle that may result from specific cleavages of capsid proteins to form the mature products or conformational changes in proteins during assembly. Such events frequently lead to substantial structural changes in the capsid that may be detectable by such criteria as differences in the antigenicity of incomplete and mature virus particles, which in some cases (e.g., picornaviruses) alters radically. Alternatively, internal structural alterations—for example, the condensation of nucleoproteins with the virus genome—often result in such changes. Virus-encoded proteases are frequently involved in maturation.

Virus Release:

Viruses escape the cell by one of two mechanisms:

- For **lytic viruses** (such as most non-enveloped viruses), release is a simple process—the infected cell breaks open and releases the virus.

- For **Enveloped viruses:** acquire their lipid membrane as the virus buds out of the cell through the cell membrane or into an intracellular vesicle prior to subsequent release. **Virion envelope proteins** are picked up during this process as the virus particle is extruded. This process is known as **budding.**

- Plant viruses face particular difficulties imposed by the structure of plant cell walls when it comes to leaving cells and infecting others. In response, they have evolved particular strategies to overcome this problem using mechanical and vector mechanisms

- There is no known plant virus employs a specific cellular receptor of the types that animal and bacterial viruses use to attach to host cells.

- Transmission of plant viruses by vectors is the main and most common biological transmission method in the fields to infect and cause disease in healthy plants.

Main transmission methods of human viruses:

1. Blood transfusion like hepatitis virus C
2. Direct shaking or other contact like herpes viruses
3. Sexual relationship like HIV
4. Using contaminated medical or dental tools and equipments like HIV, herpes
5. By air: Coughing and sneezing like flu viruses
6. Contaminated foods like stomach viral flue
7. Insect sucking blood like mosquitoes transmitting west nile virus.
8. Using same utensils of sick people like virus flu or viral stomach flu
9. Using clothes and shoes of infected persons like feet warts.

ZOONOSES

Any disease or infection that is naturally transmissible from vertebrate animals to humans and vice-versa is classified as a zoonosis. Many **emergent virus diseases are zoonoses (i.e., transmitted from animals to** humans).This emphasises the importance of the 'species barrier' in preventing transmission of infectious diseases, and several recent examples illustrate the potentially disastrous consequences that can occur when this is breached. Typical example is severe acute respiratory syndrome (SARS) is a type of viral pneumonia, with symptoms including fever, a dry cough, shortness of breath, and headaches. Death may result from progressive respiratory failure due to lung damage. The first SARS outbreak originated in the Guangdong province of China in 2003, where 300 people became ill and at least five died. The cause was found to be a novel corona virus, SARS-CoV. The SARS virus is

believed to be spread by droplets produced by coughing and sneezing, and faecal contamination.

Where did the SARS virus come from? Coronaviruses with 99% sequence similarity to the surface spike protein of human SARS isolates have been isolated in Guangdong, China, from apparently healthy masked palm civets (a cat-like mammal closely related to the mongoose). The palm civet is regarded as a delicacy in Guangdong and it is believed that humans became infected as they raised and slaughtered the animals rather than by consumption of infected meat.

Other examples of zoonoses is rabies virus transmitted by dogs as the primary host. Rabies can be passed on infected saliva by biting human and causing the disease unless vaccination was used in advance.

Yellow fever virus transmitted by female mosquito as vectors from monkeys to human.

Swine flue virus which was isolated first from pigs as a primary host causing this virus disease.

Mechanisms of Viruses Transmission

The process of transfer between hosts is referred to as transmission and this is an important step in the life cycle of viruses.

Viruses are intracellular parasites and have to find a new host before the original host mounts an effective immune response or dies. However virus infectivity is generally unstable and transmission has to be achieved usually within a few hours.

All transmission strategies adopted by viruses have in common the ability to penetrate the outer layer of the skin which is impermeable to viruses, and to bring virus into contact with the naked cells. Person-to-person infections are said to take place by ***horizontal*** **transmission**,

while those viruses transmitted from mother to fetus or baby are put in a separate category and described as ***vertical* transmission**. Rubella virus (a togavirus) is the classic example of a vertically transmitted virus. Other examples are hepatitis B and HIV-1.

Some of horizontal transmission routes are:

- Respiratory route: occurs commonly, e.g. rhinoviruses, influenza viruses
- Conjunctival route: occurrence rates are not known, e.g. respiratory viruses
- Fecal route: occurs commonly, e.g. poliovirus
- Sexual route: used by specific viruses, e.g. HIV-1, HBV, papillomaviruses
- Via urine: used by specific viruses, e.g. Lassa fever virus, cytomegalovirus
- *Mechanical route: common with tropical arthropods that feed on humans, e.g. arboviruses, and with high risk behaviors, e.g. HIV-1, HBV*

General Guidelines for using a clinical Laboratory:

1- Always wear your lab coat during laboratory work.
2- Discard used or contaminated materials in the proper garbage bags.
3- Keep your working area on your bench clean and disinfected with disinfectants like 95% Ethanol or 10% Javex.
4- Keep all the sterilized water or buffer in the right place according to the instructions.

5- Label every specimen or reagent or buffer with name and dates before starting the diagnosis or after preparation for chemicals.
6- Avoid eating or smoking in the laboratory.
7- Don’t put food or drink in the fridges used for keeping chemicals or reagents or cultures.
8- Keep all sterilized glassware and clean micropipettes arranged on their racks or shelfs.
9- Keep all the tips and microtubes sterilized and handy to use.
10- Wear gloves before you touch any tool or machine or during specimens collection and handling.
11- Learn how to read the manuals for operating the laboratory equipments.
12- Follow and record each protocol steps you completed in a special note book for future work or revision and avoid relying on memory.
13- Make buffer or stain stocks with high concentration from chemical reagents or stains to have them ready in the diagnosis by making diluted sub-stocks using the right concentration.

Respiratory System Viruses

Respiratory Viruses:

The viruses infect the mucous membranes of upper and lower respiratory tracts. Most of these viruses are not causing serious threat unless other factors are involved like immunity disease syndrome, malnutrition and complex infection (mixed infection).

The reason for no real threat of most of these viruses affecting only conjungtiva is they are localized in the mucous membrane and absorbed directly to the epithelial cells where they start initial cycle of replication.

These viruses belong to main groups divided between DNA and RNA:

Adenviruses is only DNA viruses infecting respiratory tract, while most of these are RNA viruses such as:

1. Rhinoviruses: like common cold
2. Orthomyxoviruses like Influenza
3. Paramyxoviruses like measles, mumps, parainfluenza

4. Coronaviruses: like SARS

Adenoviruses (members of the family ***Adenoviridae***) are:

- Their name derives from their initial isolation from human adenoids in 1953.
- They are medium-sized (90–100 nm),
- They are nonenveloped viruses with icosahedral nucleocapsid containing a double stranded DNA genome.
- Adenoviruses are spread primarily via respiratory droplets and sometimes they can also be spread by fecal routes.
- Symptoms with most infections with adenovirus result in infections of the upper respiratory tract. Adenovirus infections often show up as conjunctivitis, tonsilitis, an ear infection, especially in children.

Rhinoviruses (from the Greek (gen.) "nose") are the most common viral infective agents in humans and are the predominant cause of the common cold. Rhinovirus infection proliferates in temperatures between 33–35 °C, the temperatures found in the nose.

There are two methods of transmission: via aerosols of respiratory droplets and from contaminated surfaces, including direct person-to-person contact. Crowded areas like buses, classes, rooms are helpful environment for transmitting common cold.

Symptoms include sore throat, runny nose, nasal congestion, sneezing and cough.

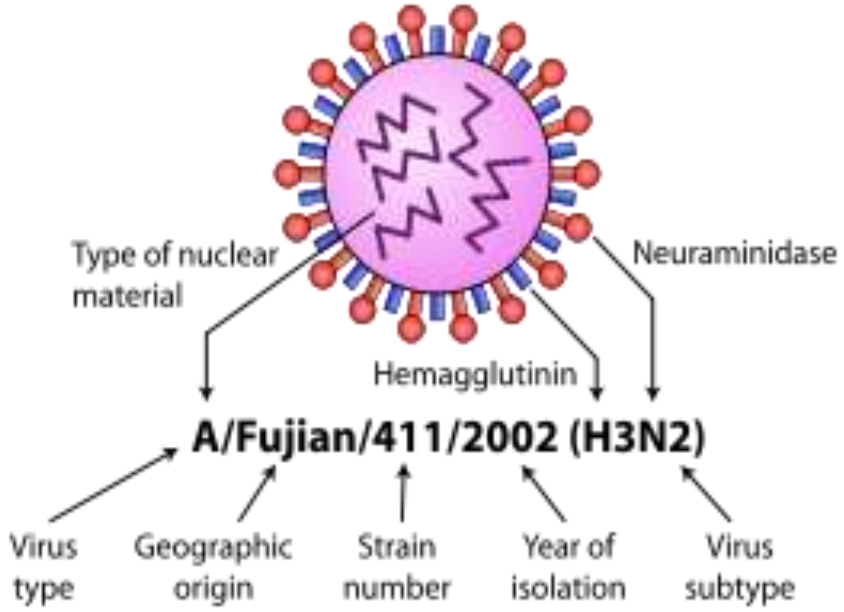
The **Orthomyxoviruses** (*orthos*, Greek for "straight"; *myxa*, Greek for "mucus") are a family of RNA viruses that includes six genera: Influenzavirus A, Influenzavirus B, Influenzavirus C, Isavirus, Thogotovirus and a recently discovered, still undescribed genus.[2] The first three genera contain viruses that cause influenza in vertebrates, including birds (called avian influenza), humans, and other mammals. Isaviruses infect salmon; thogotoviruses infect vertebrates and invertebrates, such as mosquitoes and sea lice.

The three genera of Influenzavirus, which are identified by antigenic differences in their nucleoprotein and matrix protein, infect vertebrates as follows:

- Influenzavirus A infects humans, other mammals, and birds, and causes all flu pandemics
- Influenzavirus B infects humans and seals
- Influenzavirus C infects humans and pigs.

The type A viruses are the most virulent human pathogens among the three influenza types and cause the most severe disease. The serotypes that have been confirmed in humans, ordered by the number of known human pandemic deaths, are:

- H1N1 caused "Spanish Flu" in 1918, "Swine flu" in 2009
- H2N2 caused "Asian Flu".
- H3N2 caused "Hong Kong Flu".
- H5N1 is a pandemic threat.
- H7N7 has unusual zoonotic potential
- H1N2 is endemic in humans and pigs.
- H9N2, H7N2, H7N3, H10N7.



Paramyxoviruses (from Greek para-, beyond, -myxo-, mucus or slime, plus virus, from Latin poison, slime) are viruses of the *Paramyxoviridae*. - They are negative-sense single-stranded RNA viruses responsible for a number of human and animal diseases.

- A number of important human diseases are caused by paramyxoviruses. These include mumps, measles, which caused 745,000 deaths in 2001 and respiratory syncytial virus (RSV), which is the major cause of bronchiolitis and pneumonia in infants and children.

The parainfluenza viruses are the second most common causes of respiratory tract disease in infants and children. They can cause pneumonia, bronchitis and croup in children and the elderly.

Measles, Mumps and Rubella (MMR)

Measles:

- The causal agent is ssRNA virus (Paramyxovirus family, genus Morbillivirus)
- There is no animal reservoir

- The Infective Parts: Nasal secretion , Respiratory tract &Throat
- Communicability- Highly infectious during prodromal period (early symptoms) and at the time of eruption (outbreak).

Secondary attack rate- > 80%

Host Requirements and factors:

- Age 6 months to 3 years even up to 10 years
- Incidence equal in both sexes (Male and Female)
- Immunity – life long immunity
- Malnourished children are susceptible

Environmental Factors:

Winter season, over crowding places

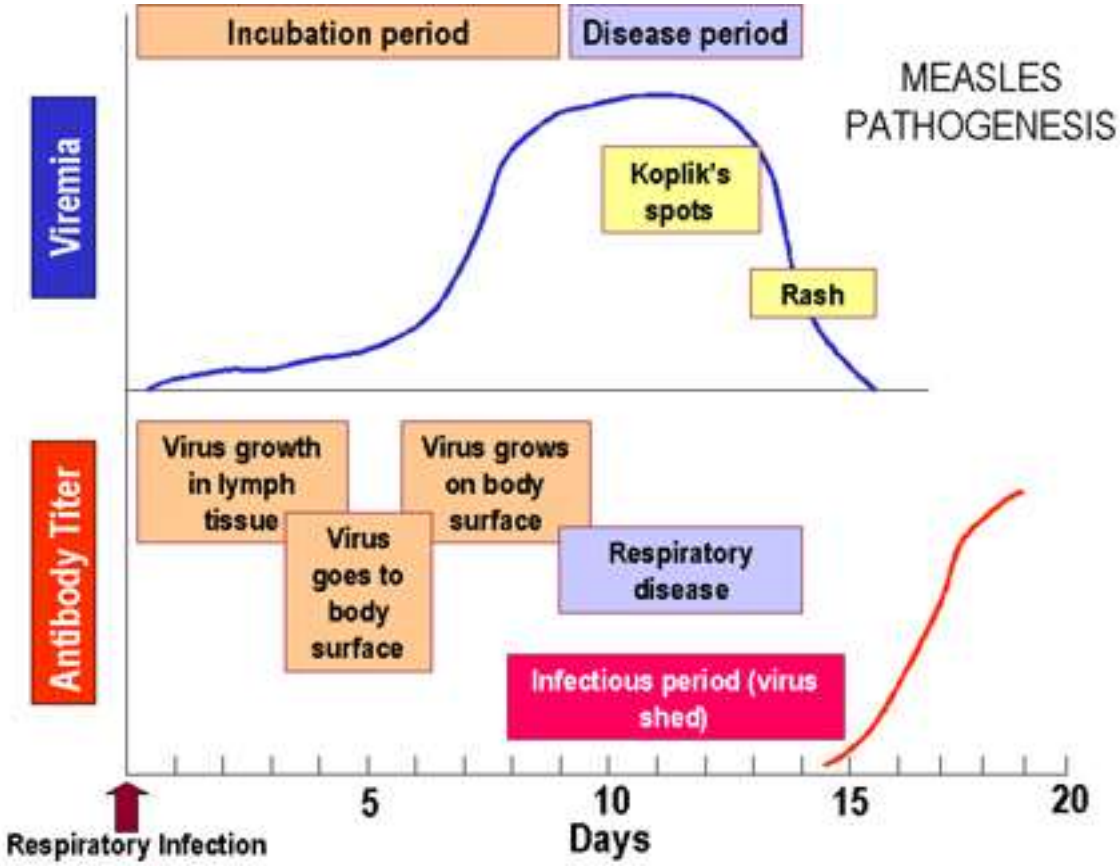
Transmission: Droplet infection

Infection period: 4 days before and 4 days after rash

Incubation period: 7 days

Measles Symptoms Development:

The symptoms development caused by measles can be described in the following diagram:



Measles Diagnosis:

A. Clinical Diagnosis:

Rash is the main symptoms of measles viral disease.

The clinical symptoms severity can be divided in the 3 stages:

- Prodromal stage (Early symptoms)
- Eruptive stage (outbreak)
- Post-measles stage

The other major symptoms includes

- Represented by 3 Cs (Cough, Coryza & Conjunctivitis)
- Koplik spots
- Four days fever (40^0c)
- Generalized, maculopapular, erythematous rash.
- In case of complicated cases, the following clinical symptoms are developed

Diarrhea,

Pneumonia

Otitis media (Ear infection)

Convulsions (Spasm),

SSPE (sub acute sclerosing panencephalitis)

WHO World Health Organization described the strategy for control and prevention of Measles:

1) Catch up

2) Keep up

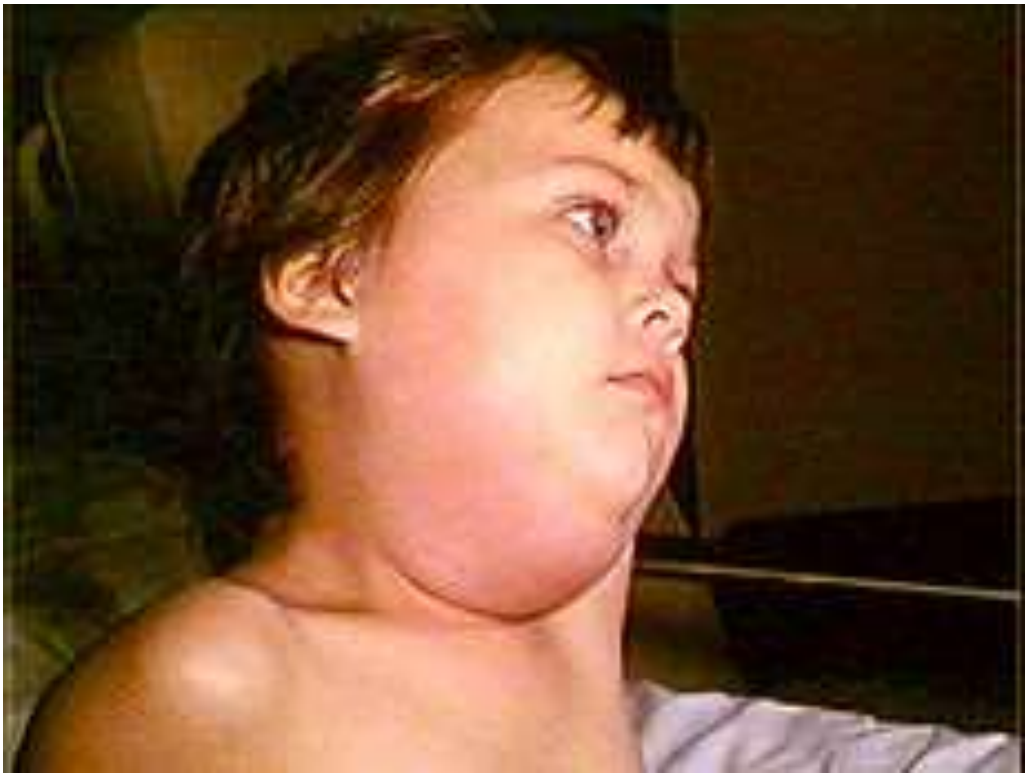
3) Follow up

B. Laboratory Diagnosis

Laboratory confirmation is not required since the rash symptoms are so typical on the skin. In case of unclear symptoms, serological diagnosis can be made by detecting specific IgM by enzyme-linked immunosorbent assay (ELISA).

MUMP:

The name comes from the British word "to mump", that is grimace or grin The appearance of the patient as a result of parotid gland swelling seems to be in grin (miserable face).



Paramyxovirus parotidis is -ssRNA virus.

Source of infection: Spread by saliva and respiratory secretions

Period of communicability : 4-6 days of onset of symptoms

Secondary attack rate : 86%

Age & gender: 5-15 years and girls more common

Immunity: life long

Environmental factor: winter and spring season favors

Mode of transmission: saliva and respiratory droplets or secreations or aerosols and hand contact

Incubation Period: 2 to 3 weeks

Virus Disease Development:

Virus enter through respiratory tract

Virus particles multiplied in the salivary gland

Virus particles spread to spleen, lymphoid tissue

Viremia is the acute stage when the virus spread to other parts of the body like testes or ovaries, pancreas, thyroids it takes the last 15 days of the incubation period.

Symptoms (infectious stage): 4-6 days

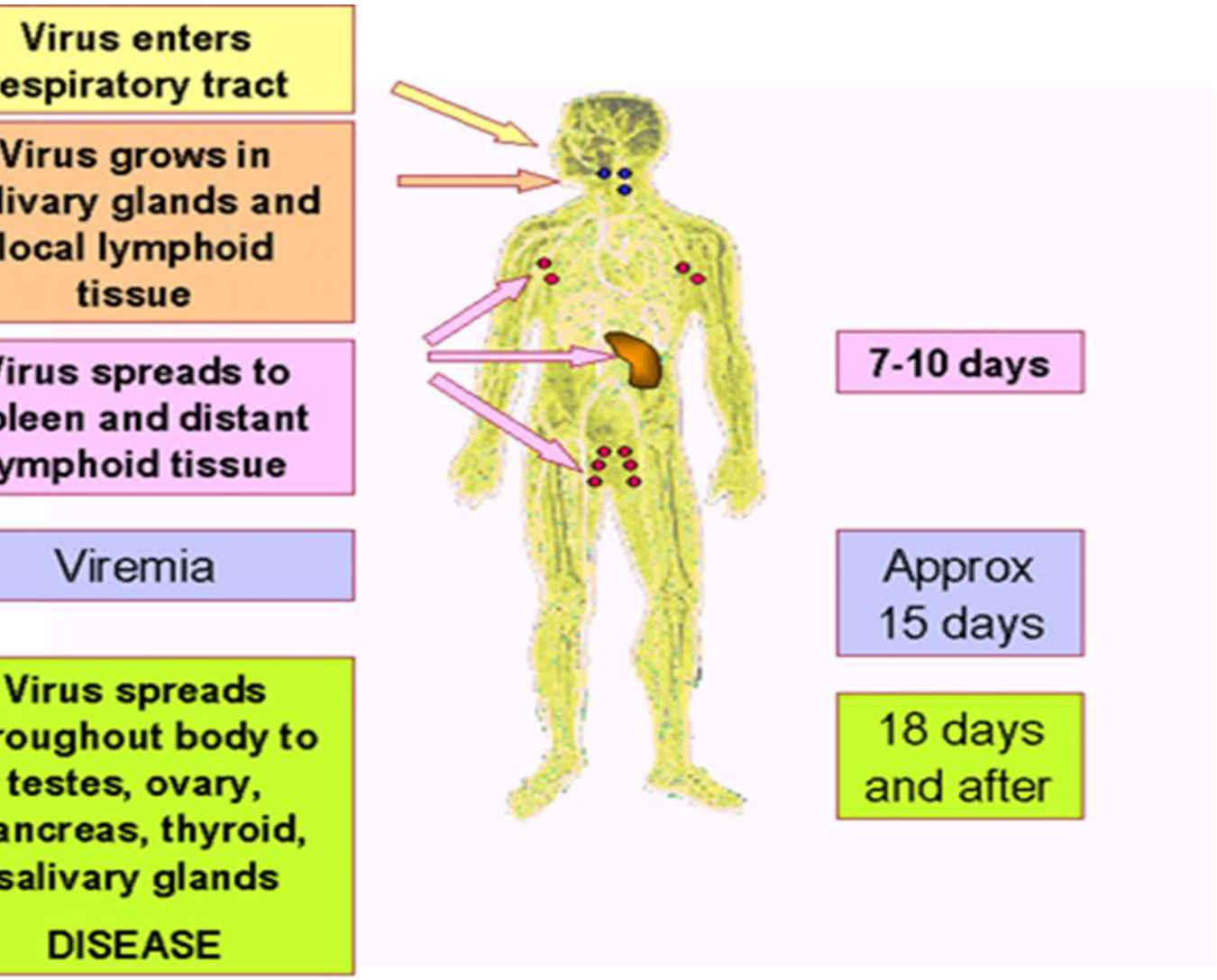
Parotid swelling (salivary glands swelling)

Ovaritis (ovary infection)

Pancreatitis

Ear ache (ear infection)

Orchitis (testes infection)



In complicated cases additional clinical symptoms include:

Orchitis (inflammation of testis)

Epididymitis (sperm sac or storage)

Oophoiritis (Inflammation of ovary)

Spontaneous abortion

Sensori neural hearing loss, (uni- or bilateral).

Mild form of meningitis

Encephalitis (inflammation of brain)

Diagnosis:

Clinical symptoms includes mainly painful swollen one or both parotid glands.

Laboratory testing for confirmation includes using:

Serological tests by ELISA for specific IgM

Molecular technique using RT-PCR to detect viral RNA in salaiva, blood and urine.

Mump virus prevention:

Vaccine is available in mixture with measles and rubella as MMR. Mump vaccine is prepared from Jeryl Lynn attenuated strain

Rubella (German measles)

The name rubella is derived from a Latin term meaning "little red."

Rubella is sometime called German Measles or 3-day Measles.

The synonym "3-day measles" derives from the typical course of rubella exanthema that starts initially on the face and neck and spreads centrifugally to the trunk and extremities within 24 hours.

It then begins to fade on the face on the second day and disappears throughout the body by the end of the third day.

It is a generally mild disease caused by the rubella virus.

The causal Agent is: +ssRNA virus (Togo virus family), Genus Rubivirus.

Source of infection: Respiratory secretion

Host (Children): 3-10 years

Immunity: life long

Environmental factors : winter and spring season

Transmission: droplet, vertical transmission (from mother to the child)

Incubation Period: 2-3 weeks

Major Symptoms:

Temperature

Fever is usually not higher than 38.5°C (101.5°F).

Lymph nodes

Enlarged posterior auricular and suboccipital lymph nodes are usually found on physical examination.

Mouth

The Forchheimer sign may still be present on the soft palate.

Diagnosis:

Clinical diagnosis is difficult to diagnose on purely clinical grounds due to the disappearance of rash symptoms after 2-3 days.

Laboratory diagnosis

Molecular techniques using RT-PCR is used by getting RNA from throat swab.

IgG antibody can be used in ELISA technique

Tests for rubella infection, past or present can be considered in 3 cases:

Test for Rubella in women of child-bearing age

Test for acute infection in pregnancy

Tests on infants for congenital infection

Rubella prevention:

Vaccine is available in mixture with measles and mumps vaccines.

Measles Vaccines composition:

Live attenuated measles virus (Edmonston-zagreb strain) Propagated on human diploid cell (MRC-5)

0.5 ml of vaccine

Not less than 1000 CC ID_{50} of measles virus

2.5% of gelatin

5% of sorbitol as stabilizers

0.5 ml of sterile water

Dose: 0.5 ml

Route of administration: Sub-cutaneously

3 to 5 weeks antibody level : 200mLU/ml

Mump Vaccine:

10 strains of the mumps virus are in use throughout the world for the preparation of live attenuated vaccine.

Jeryl Lynn strain which was named after the child from whom the virus was isolated.

Leningrad-3 strain

Urabe strain

Hoshino, Torii and NKM - 46 strains

L-Zagreb

MMR Vaccine

Live attenuated strains of Edmonston-Zagreb Measles virus propagated on human diploid cell culture,

L-Zagreb Mumps virus propagated on chick embryo fibroblast cells

Wistar RA 27/3 Rubella virus propagated on human diploid cell culture

Age	Vaccines	Note
9 months	Measles	Deep subcutaneous injection into the upper arm.
12-15 months	MMR -1	Deep subcutaneous injection into the upper arm.

5 years	MMR -2	Deep subcutaneous injection into the upper arm.

Hepatitis Viruses

- Hepatocytes (liver cells) are the primary target of true hepatitis viruses.
- Earlier studies showed that there were 2 forms of transmissible hepatitis: infectious or cararrhal hepatitis (Type A) and Serum Hepatitis (Type B) or HBV

The major differences between the HAV and HBV

cararrhal hepatitis (Type A) or HAV	Serum Hepatitis (Type B) or HBV
- Acquired by the oral route (enteric). - Incubation period 2-6 weeks - It is RNA virus of picornaviridae - Diagnosis easy by serology - Can not be propagated in usual cell culture - HAV is not a serious virus and has a vaccine approved in 1991	- HBV spread by needle injection, blood transfusion and sexual contact - Incubation period 2-5 months - It is a circular dsDNA virus of Hepadnaviridae - Diagnosis by serology. Molecular methods and biopsy test - Discovery of RNA associated agent, Delta virus (HDV) with HBV called satellite which depends on HBV to synthesize its protein coat - Vaccine has been developed since 1981

- HDV may be transmitted as satellite virus along with HBV or as super-infection to cause complex liver disease.

- Mortality rate by HDV are 10 fold higher than for hepatitis HBV. Development of cirrhosis is 3 times than HBV and 80% of patterns develop this in 3-5 years. Incubation period of HDV is 3-7 weeks.
- Different shapes of particles found in blood of acute and chronic cases of HBV. One type is 42 nm in diameter with double shelled. Others are spherical or tubular 20-22 nm in diameter. They consist only of excess surface antigen (the glycoprotein forming the outer layer of the double-shelled Dane particle (HBcAg). The core of the virus particle is an icsohedral nucleocapsid.

3. HBV is a circular dsDNA virus some 3.2 Kb in size. The genome is extremely compact has a complex organization with 4 overlapping open reading frames (ORFs) RNA associated agent, Delta virus (HDV) with HBV called satellite which depends on HBV to synthesize its protein coat.

4. HDV may be transmitted along with HBV or as super-infection to cause complex liver disease.

- Mortality rate by HDV are 10 fold higher than for hepatitis HBV. Development of cirrhosis is 3 times than HBV and 80% of patterns develop this in 3-5 years. Incubation period of HDV is 3-7 weeks.

5. In highly epidemic areas HBV can be acquired by infants during or after birth. The virus concentration is: high in blood and serum, moderate in sexual organs and saliva and low or undetectable in urine, feces, tears, sweat and milk breast.

- Because the titer is high in body fluids (between 1 and 100 million per ml) but less than 0.00001 can transmit the infection.

6. There are 8 genotypes of hepatitis (A-H) in the world: Type A in Europe and Africa, B and C in Asia, D in the Mediterranean, E in Africa, F in south America, G in France and USA and H in Central America and India.

- Replication occurs when: virus attaches to hepatocytes using the virion S protein and enters by endocytosis. The virus nucleocapsid moves to the nucleus where the relaxed circular (RC) DNA genome containing the nick is converted into closed circular DNA for transcription.

The minus strand is transcribed to give mRNAs plus a 3.4 kb RNA transcript called pregenome.

The pregenome and shorter mRNA move to the cytoplasm and translated into virus protein. DNA formed from RNA intermediate through reverse transcription. The RNA is digested away and a plus DNA strand produced. This occurs in the virion. The mature virion contains genomic DNA.

7. The virus nucleocapsid moves to the nucleus where the relaxed circular (RC) DNA genome containing the nick is converted into closed circular DNA for transcription.

Hepatitis B control:

- Acute infection with HBV does not normally need treatment.
- The threat is HBeAg-positive carrier state demands action. This is required with deficient in Interferon (IFN) which treated with large doses of IFN-α for 6 months.

8. In acute infection with HBV, the threat is HBeAg-positive carrier demands action due to deficient in IFN which treated with large doses of IFN-α for 6 months.

- DNA polymerase inhibitors are often used in combination of interferon α with lamivudine and famiciclovir especially for patient with transplanted liver

9. In HBV-infected Patients with transplanted liver, treatment required inhibition of DNA polymerase by using a combination of IFN-α lamivudine and famiciclovir.

- Immunization is a preventing measure against HBV using vaccines prepared from HBsAg. This can be used with human immunoglobulin (HBIG).

10. In the immunization against HBV, vaccine is prepared from HBsAg (i.e. has S protein).

- Laboratory diagnosis of HBV:

1. The first test is for surface antigen. This is normally done by ELISA.

10A- ELISA is the first test used for surface antigen

2. For rapid cases, it can be done by reverse passive haemagglutination in which commercially available erythrocytes coated with anti-HBsAg which takes 20 minutes.

10B- In rapid cases, HBV can be tested by reverse passive haemagglutination in which commercially available erythrocytes coated with anti-HBsAg which takes 20 minutes.

3. Latex slide test based on the same principle can be read in 5 minutes.

10C- Latex slide test is a faster test (5 minutes) than reverse passive haemagglutination test (20 minutes) in diagnosis of HBV.

4. Test of viral DNA and DNA polymerase as a measure of virus replication.

10D- The most reliable method to study HBV replication is using DNA polymerase.

5. Electron microscopy to test quickly both for HBsAg and infective Dane particles.

10E- Best test for quick diagnosis of both HBsAg and infective Dane particles of HBV is electron microscopy.

6. Using real time PCR to confirm HB virus genome

10F- Real time PCR is the most reliable method to confirm HB virus genome.

Hepatitis C Virus Epidemiology

- Hepatitis C is a disease with a significant global impact.
- According to the World Health Organization there are 170 million people infected with hepatitis C virus (HCV).
- There are considerable regional differences. In some countries, e.g., - Egypt, the prevalence is as high as 20%.
- It is estimated that there are 2-5 million HCV-positive persons in Europe. Certain groups are preferentially affected, like injection drug users. In

Europe and the United States chronic hepatitis C is the most common chronic liver disease.

- Transmission means virus transfer from infected person to a healthy person.
- Parenteral exposure to the hepatitis C virus is the most efficient means of transmission. The majority of patients infected with HCV in Europe and the United States acquired the disease through intravenous drug use or blood transfusion, which has become rare since routine testing of the blood supply for HCV began.

11. The most common method in transmitting HCV is injecting intravenous drugs.

11A. HCV transmission by Blood transfusion becomes rare in Europe and USA due to starting routine testing of the blood supply for HCV.

11B. The least method of HCV transmission is immunoglobulin injection.

11C. Transmission of HCV through Pierced ears or body port is more common than religious scarification.

The following possible routes of infection through blood donors (starting with

• Injection drug use
• Blood transfusion
• Sex with an intravenous drug user
• Having been in jail more than three days
• Religious scarification
• Having been struck or cut with a bloody object
• Pierced ears or body parts
• Immunoglobulin injection

Hepatitis C Virus Development:

Acute Hepatitis

- After HCV inoculation, there is a variable incubation period.
- HCV RNA in blood (or liver) can be detected by PCR within several days to eight weeks.
- HCV antibodies can be found about 8 weeks after exposure although it may take several months. The majority of newly infected patients will be asymptomatic and have a clinically non-apparent or mild course.

Disease progression

Chronic HCV progression may differ due to several factors such as the following:

- Age and gender: More rapid progression is seen in males older than 40-55 years, while a less rapid progression is seen in children.
- Ethnic background: A slower progression has been noted in African-Americans.
- HCV-specific cellular immune response: Genetic determinants like HLA expression.
- Alcohol intake: Even moderate amounts of alcohol increase HCV replication, enhance the progression of chronic HCV, and accelerate liver injury.
- Daily use of marijuana: may cause a more rapid progression.

HCV Symptoms Types:

1. Acute Symptoms

Symptoms include malaise, nausea, and right upper quadrant pain. In patients who experience such symptoms, the illness typically lasts for 2-12 weeks. Along with clinical resolution of symptoms, aminotransferases will normalize in about 40% of patients. Loss of HCV RNA, which indicates a hepatitis C cure, occurs in fewer than 20% of patients.

Fulminate hepatic failure due to acute HCV infection may happen in patients with underlying chronic hepatitis B virus infection

2. Chronic Hepatitis

The risk of chronic HCV infection is high**. About 75% of patients with acute hepatitis C do not eliminate HCV RNA and progress to chronic infection**. **Most of these will have persistently elevated liver enzymes in follow-up. Hepatitis C is considered to be chronic after six months**. Once chronic infection is established, there is a very low rate of spontaneous clearance.

Patients with chronic infection are asymptomatic or have only mild nonspecific symptoms since cirrhosis is not present. The most frequent complaint is fatigue. Less common manifestations are nausea, weakness, myalgia, arthralgia, and weight loss.

Viral coinfection: HCV progression is more rapid in HIV-infected patients. Acute hepatitis B in a patient with chronic hepatitis C may be more severe. **Liver damage is usually worse and progression faster in patients with dual HBV/HCV infections.**

Geography and environmental factors: Clear, but not understood.

Use of steroids: increases HCV viral load.

Viral factors: There seems to be no significant role of different genotypes and quasispecies on fibrosis progression or outcome. However, coinfection with several genotypes may have a worse outcome as compared to monoinfection. Liver biopsy is the best predictor of disease progression

Hepatitis Virus C Transmission:

- Transmission means virus transfer from infected person (patient) to a healthy person.
- Parenteral exposure to the hepatitis C virus is the most efficient means of transmission. The majority of patients infected with HCV in Europe and the United States acquired the disease through intravenous drug use or blood transfusion, which has become rare since routine testing of the blood supply for HCV.

Viral Lifecycle (Infection development)

The recent development of small animal models and more efficient in vitro HCV replication systems has offered the opportunity to analyze in detail the different steps of viral replication.

The following figure describes these steps such as:

1. Adsorption after attachment of HCV to the hepatocyte
2. Penetration by Endocytosis
3. Fusion
4. Uncoating
5. Translation and RNA replication
6. Virus assembly
7. Virion maturation
8. Virion release

13A. The particles of HCV started the infection after attachment to the hepatocyte cells.

13B. Infection development after attachment include penetration by Endocytosis.

Adsorption, viral entry and fusion

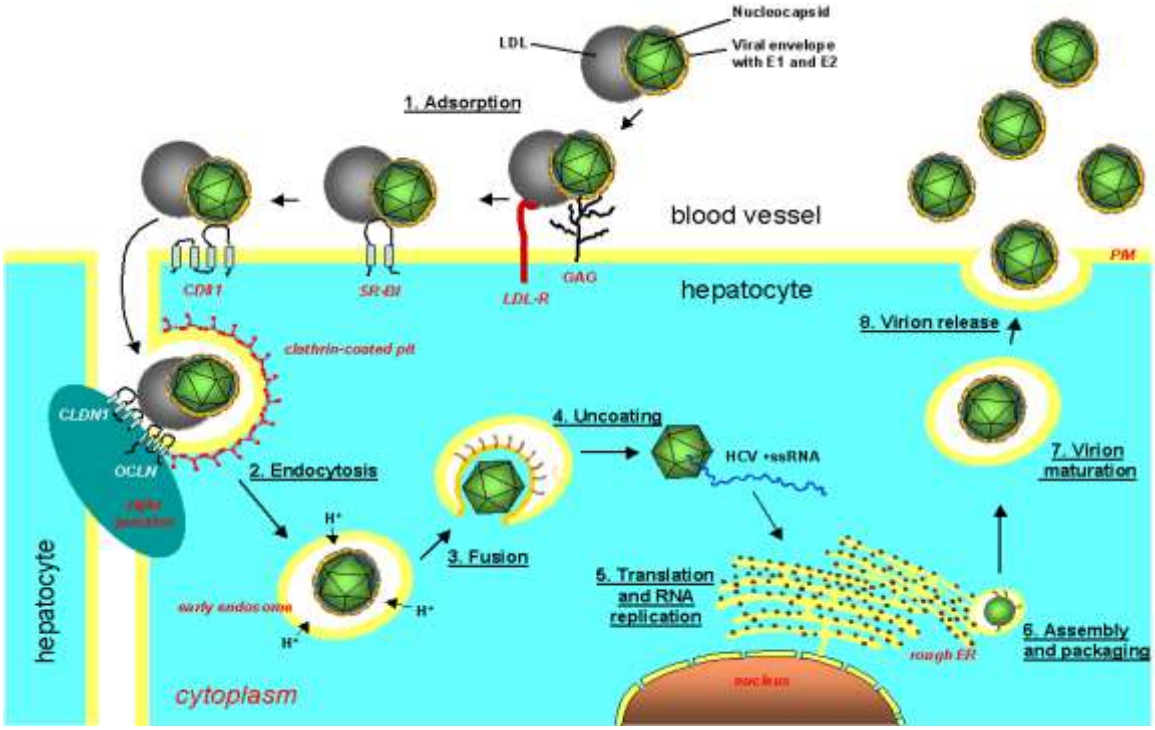
Entry of HCV into a target cell is complex. A cascade of virus-cell interactions is necessary for the infection of hepatocytes and the precise mechanism of viral entry is not completely understood.

The current model of viral adsorption assumes that HCV is associated with low-density lipoproteins (LDL). The binding step includes binding of the LDL component to the LDL-receptor (LDL-R) on the cell surface, and simultaneous interaction of the viral glycoproteins with cellular glycosaminoglycans (GAG)

This initiation step is followed by consecutive interactions of HCV with scavenger receptor B type I (SR-BI) and the tetraspanin CD81

Interaction of HCV with CLDN1 and OCLN seems to induce the internalisation of the virion via clathrin-mediated **endocytosis**.

Subsequent HCV E1-E2 glycoprotein mediation fuses the viral envelope with the endosome membrane



Taxonomy and Genotypes

The hepatitis C virus (HCV) is in the Hepacivirus genus of the Flaviviridae family.

To date, six major HCV genotypes with a large number of subtypes within each genotype are known. The high replication rate of the virus together with the error-prone RNA polymerase of HCV is responsible for the large interpatient genetic diversity of HCV strains.

Moreover, the extent of viral diversification of HCV strains within a single HCV-positive individual increases significantly over time resulting in the development of quasispecies.

Genome Organization

The genome of the hepatitis C virus consists of one 9.6 kb single-stranded RNA molecule with positive polarity.

Similar to other positive-strand RNA viruses, the genomic RNA of hepatitis C virus serves as messenger RNA (mRNA) for the translation of viral proteins.

The linear molecule contains a single open reading frame (ORF) coding for a precursor polyprotein of approximately 3000 amino acid residues flanked by two regulatory nontranslated regions (NTR)

HCV RNA replication

The complex process of HCV RNA replication is poorly understood. **The key enzyme for viral RNA replication is NS5B: an RNA-dependent RNA polymerase (RdRp) of HCV.**

- After the RdRp has bound to its template the NS3 helicase is assumed to unwind putative secondary structures of the template RNA in order to facilitate the synthesis of minus-strand RNA. In turn, the newly synthesized antisense RNA molecule serves as the template for the synthesis of numerous plus-stranded RNA. The resulting sense RNA

may be used subsequently as genomic RNA for HCV progeny as well as for polyprotein translation. Another important viral factor for the formation of the replication complex appears to be NS4B, Which is able to induce an ER-derived membranous web containing most of the non-structural HCV proteins including NS5B.

Assembly and release

After the viral proteins, glycoproteins, and the genomic HCV RNA have been synthesized these components have to be arranged in order to produce infectious virions.

Viral assembly is a multi-step procedure involving most viral components along with many cellular factors. Recent findings suggest that viral assembly takes place within the endoplasmic reticulum and that lipid droplets are involved in particle formation.

N.B. some of the information and pictures are quoted from the internet

Additional Reading:

Collier leslie et al. 2011. Human Virology. Fourth Edition, Oxford. PP 365

PREVENTION AND CONTROL OF VIRAL DISEASES:

(Source: http://www2.gsu.edu/~biotkf/bio475/475lecture15.htm)

A number of preventative measures are routinely used to prevent and control viral diseases. For the success of any comprehensive prevention and control policy, an adequate surveillance system is necessary.

The major measures includes the following:

1. Vaccination: Vaccines are not available for all viruses. For those that there are, public policy decisions must be made as to whether at-risk populations are targeted or whether universal vaccination is pursued. Currently in the U.S., universal vaccination is pursued against polio, measles, mumps, rubella, and hepatitis B. With universal vaccination,

the original goal was to induce *herd immunity*, which theoretically would stop transmission of the virus in the population. In reality, importation leads to resurgence of the disease among non-immunes. Therefore, second vaccinations are generally required to achieve universal coverage.

Worldwide, the WHO initiated the Expanded Program for Immunization in 1977 to protect children against several vaccine preventable diseases: polio and measles are included.

2. Quarantine: Quarantine is used to restrict the importation of exotic diseases and vaccine preventable diseases under control in the U.S.

3. Hygiene and Sanitation: Proper waste water treatment is important in keeping viruses out of the water supply since virus contamination in sewage is between 10^3 and 10^4 particles per liter. Typically, activated sludge followed by anaerobic digestion removes most virus particles (some countries require pasteurization). Where recycled waste water is used for domestic purposes, additional steps of coagulation, adsorption, and chlorination or ozonation are required.

In underdeveloped countries, the level of virus in wastewater is higher. In countries without "reticulated" sewerage systems, contamination of drinking water with waste water is a big problem, particularly after flooding when waterborne epidemics of enteric disease can occur. Personal hygiene is of importance in lowering transmission of both enteric and respiratory viruses, particularly in family and workplace settings. Proper hygiene is also important among food handlers.

4. Vector control: Mosquito control is often more effective than vaccination given the sporadic nature of most arboviral diseases. In the tropics, vaccination is practiced against yellow fever in some countries and development of a Dengue fever vaccine would be highly beneficial.

5. Lifestyle changes: For diseases transmitted sexually and by intravenous drug use, lifestyle change should change transmission patterns if it can be implemented.

6. Eradication:

Not all viral disease are eradicable. The necessary features for eradication are:

- An effective vaccine that optimally doesn't require a cold chain and is easily administered
- No animal reservoir
- Lack of recurrent infection
- One or a few stable serotypes
- No infectivity before symptoms and no inapparent infections, making early containment possible

Additionally, the disease must be of dread enough nature and exact a high enough cost to make the cost of eradication worthwhile.

Smallpox fit all of these criteria and was eradicated in 1977. The next two diseases considered were polio and measles, which do not fit all of these criteria. However, in 1988 the WHO endorsed a worldwide eradication of polio program to be completed by 2000. Polio was eliminated from the Western Hemisphere in 1991; by 1997 the world case rate of paralytic polio was a few thousand cases per year (a 99% decrease). Currently, polio remains endemic only in Central Africa and Southeast Asia. The final elimination effort relies on: worldwide implementation of routine vaccination schedules, maintenance of a worldwide surveillance system with regional offices; National Immunization Days; and mop up vaccination efforts in areas with instability, refugees, or high rates of migration. It is hoped that the infrastructure developed in the polio eradication effort will then provide the basis for measles elimination.