STUDY MATERIAL FOR B.Sc. MICROBIOLOGY

VIROLOGY AND PARASITOLOGY

SEMESTER – V

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PREPARED BY

MICROBIOLOGY DEPARTMENT

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VIROLOGY AND PARASITOLOGY

Unit: 1

General Properties, replication and Classification of viruses (Baltimore classification), Cultivation of viruses- in animals, embryonated eggs and tissue culture, Virus purification assays - collection and transport of clinical specimens for viral infections.

Unit: II

Viral diseases with reference to symptoms, pathogenesis, transmission, prophylaxis and control – Arboviruses (Flavi virus), Picorna viruses (Polio virus and Rhinovirus), Hepatitis viruses (HAV, HBV, HCV, HDV, HEV), Rabies virus, Orthomyoviruses (Influenza virus) and Paramyxoviruses (Mumps and Measles virus), Pox viruses (Variola, Vaccinia), Herpes viruses (Herpes simplex, Varicella zoster), Adeno viruses, Rota viruses and HIV viruses. Oncogenic viruses (Human Papilloma virus): Introduction, characteristics of transformed cells, mechanism of viral oncogenesis and clinical manifestations.

Unit: III

Emerging and reemerging viral infections (SARS, Swine flu, Ebola, Dengue, Chikungunya- and Corona) – causes, spread and preventive measures. Detection of viruses in clinical specimens – Serological and Molecular diagnosis of virus infections – Antiviral agents, Interferons and Viral Vaccines, Immunization schedules.

Unit: IV

General introduction to Medical Parasitology, Classification of medically important parasites. Morphology, life cycle, pathogenesis, clinical features, laboratory diagnosis, prevention and treatment of diseases caused by the following organisms: Entameobahistolytica, flagellates (Giardia lamblia, Leishmaniadonovani), Sporozoa- Plasmodiumspps.

Unit: V

Introduction to Helminthes, Platyhelminthes – Taenia – Fasciola – Paragonimus – Schistosomaspps.. Nemathelminthes – Ascaris– Ankylostoma – Enterobius – Trichuris – Trichinella – Wuchereria – Dracanculus. Collection, transport and examination of specimen Laboratory techniques in parasitology Examination of faeces for ova and cyst by direct wet mount and iodine wet mount, Concentration methods (Floatation and Sedimentation techniques), Examination of blood for parasites. Cultivation of parasites.

UNIT - I

GENERAL PROPERTIES OF VIRUSES

Viruses are uniquely different from the many uni-cellular micro-organisms you have studied so far. Protozoa, yeasts, bacteria, mycoplasmas, rikettsiae and chlamydiae are all living organisms with the following features in common:

• They are all cells

• They store their genetic information as DNA

• Within their cell, they contain all the organelles necessary for producing energy and synthesizing proteins, carbohydrates, cell wall structures etc.

• Replicate by means of binary fission

• Viruses do not share these properties. They are not cells. They are very simple structures consisting essentially of a nucleic acid genome, protected by a shell of protein. They are metabolically inert and can only replicate once they are inside a host cell

. • The genome consists of only one type of nucleic acid: either RNA or DNA. Most DNA viruses are double stranded and most RNA viruses have a single stranded (ss) genome. A ssRNA genome may be either positive sense (this means that it can be used as mRNA to make proteins) or negative sense.

• Negative sense RNA is complimentary to mRNA, in other words, it has to be copied into mRNA. The viral genome codes only for the few proteins necessary for replication: some proteins are non-structural. e.g. polymerase and some are structural, i.e. they form part of the virion structure.

• They have no organelles.

• They are very small, sizes range from 20 to 200 nm, with newly discovered viruses as large as 800nm. Most viruses are beyond the resolving power of the light microscope.

Properties of virus

Some general properties of virus are;

1. Size:

- The size of virus ranges from (20-300) nm in diameter.
- Parvovirus is the smallest virus with size 20nm whereas Poxvirus is largest being 400nm.

2. Shape:

- The overall shape of virus varies in different groups of virus.

- Most of animal viruses are spherical shape, Pox virus is rectangular shape, TMV is rod shape, Poliovirus is bullet shape etc
- Some virus are irregular and pleomorphic in shape.

3. Symmetry:

- Morphological protein subunits of capsid are arranged together to from a symmetrical structure of the virus.
- Two basic symmetry are recognized in virus, they are helical symmetry and icosahedral symmetry.
- In some virus, symmetry is more complex, which is other than helical or icosahedral.

4. Structure and Chemical composition:

i. Genome:

- Viral genome or nucleic acid contains either DNA or RNA but not both.
- The genome can be either ds DNA or ss DNA or ds RNA or ss RNA
- The genome can exist as single piece or segmented. Eg, Influenza virus contains 8 segments of ss RNA genome.
- The genome may be linear or circular. Most virus possess linear genome except Papova virus which contains circular ss DNA.
- Genome helps replication of virus in host cell.

ii. Capsid:

- Capsid is the outer shell of a virus.
- It is chemically a viral protein.
- Capsid is composed of capsomere.
- Structure of capsid gives the symmetry of virus.
- Capsid protects the nuceic acid and also helps in attachments on host cell surface during infection.

iii. Envelope:

- Some virus contains phospholipid bilayer known as envelope.
- Virus lacking envelope is called naked virus.
- Envelope is a lipid bilayer which is acquired from host cell membrane

iv. Glycoprotein spike:

- Envelope of some virus contains viral coded spike projected outside the envelope called glycoprotein spike or peplomers.
- Glycoprotein spike are viral coded protein with carbohydrate head.
- Glycoprotein spikes is an important antigenic structure.
- Neuraminidase and Haemagglutinin are glycoprotein spikes which helps in virus attachment to cellular receptor on host cell to establish infection.

v. Enzymes:

- Some virus possess their own enzymes.
- Retrovirus possess reverse transcriptase

5. Viral replication:

Virus only replicates inside host cell

6. Metabolism:

- Viruses are metabolically inert outside host cell.
- They are also called as obligate intracellular parasite

7. Resistance:

i. Temperature:

- Most viruses are heat labile.
- Viruses are inactivated by heating at 60°C for 30 minutes or 100°C for few seconds.

ii. Cold:

- Viruses are stable and resistant to cooling.
- Virus can be stored for long duration at -40°C to -70°C by lyophilization or freeze drying.

iii. Radiation:

- Both non-ionizing and ionizing radiation can kill virus.
- UV rays causes pyrimidine dimer formation while ionizing radiation eg, X-rays causes lethal break of viral genome.

iv. Organic solvent:

- Chloroform, ether and bile salt can destroy all viruses by lipid solubiliation.

v. Disinfectant:

- Most viruses are destroyed by oxidizing agents such as chlorine, H2O2, iodine etc.
- Many viruses are resistant to phenol and chlorination. The phenol and chlorine do not always inactivates enterovirus, particularly if they are present in faecal materials.

vi. Antibiotics:

- Viruses are resistant to antibiotics.

Virus: STRUCTURAL CHARACTERISTICS

Size:

Variable. Most viruses are much smaller than bacteria. The size ranges in between 100A to 250 mu. Some viruses are larger than bacteria, for example the psittacos is a virus measuring 0.75 mu in diameter.

- Virus are very small infectious agents with size ranging from 20-300nm in diameter.
- Viruses are non-cellular entities so they are also called as particles.
- Virus lacks their own independent metabolism and cannot replicate outside the host cell. So they are also called as obligate intracellular parasites.
- Virus that infects bacteria are called bacteriophage or simply phage. Animal virus infects animals and similarly plant virus infects plants.

Structure of virus

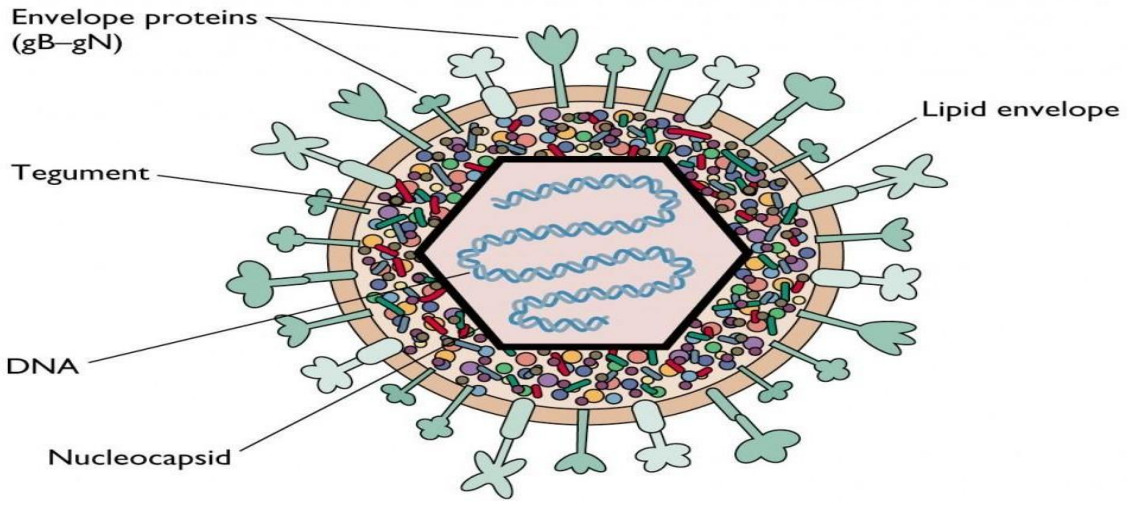


Figure: a diagrammatic sketch of an icosahedral virus

- A basic structure of virus is nucleic acid core (either DNA or RNA but not both) surrounded by protein coat.
- Central core of nucleic acid of a virus is called genome and the protein coat surrounding is called as capsid.
- In some virus, an envelope made up of glycoprotein and phospholipid bilayer is present outside the capsid.

The basic structural components of a virus are;

1. Genome:

- Virus contains either DNA or RNA as genetic material but not both. Virus which contains DNA as genetic material are called DNA virus and those containing RNA are called RNA virus.
- Unlike other living cell where ds DNA is always a genetic material, a viral genome may consists of linear or circular ds DNA, single stranded DNA, ss linear RNA or ds linear RNA.
- Examples; Reo virus is a RNA virus which contains ds RNA genome. Parvovirus contains ss DNA, Papovavirus contains ds circular DNA as genetic materials.

2. Capsid:

- Capsid is the outer layer. Sometime it is refered as coat or shell.
- Capsid serves as impenetrable shell around the nucleic acid core.
- Capsid also helps to introduce viral genome into host cell during infection.
- The protein coat or capsid is made up of number of morphological similar sub units called capsomere. Each capsomere is further composed of protomere.
- Capsomere are arranged precisely and tightly together in a repetitive pattern to form complete capsid.
- The number of capsomere in a capsid varies from virus to virus.
- The complete complex of nucleic acid and protein coat of a virus particle is called as virus nucleo-capsid.
- Structure of capsid give the symmetry to the virus. Virus particle may be either cubicl or helical or binal or complex symmetry.

3. Envelope:

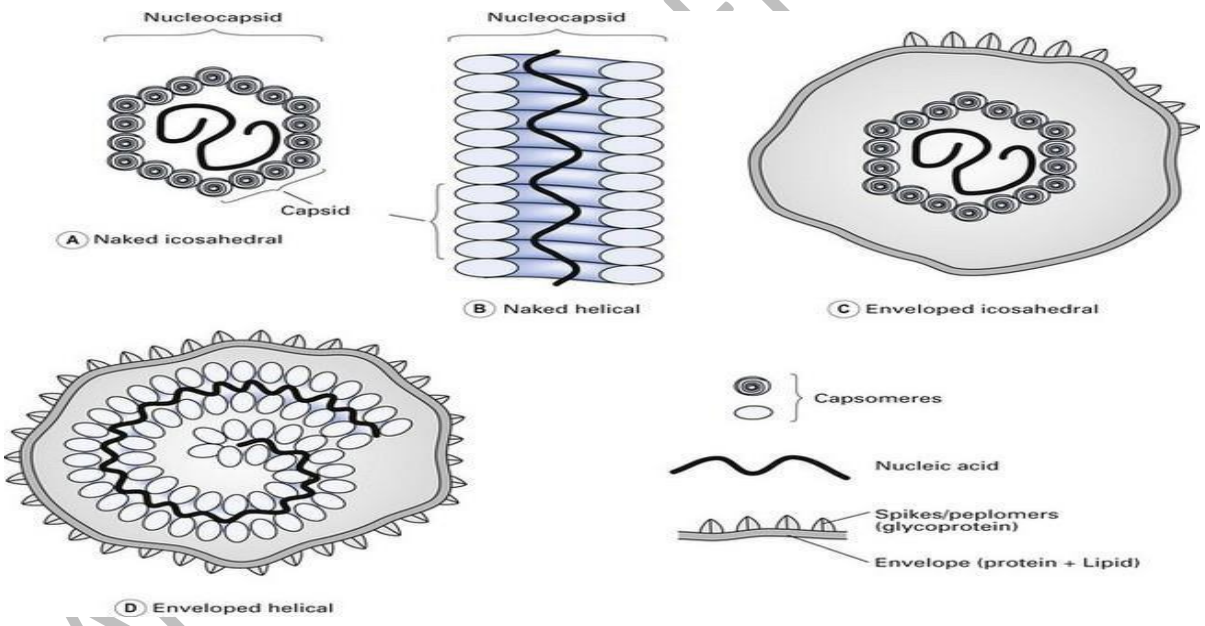
- Some virus contains envelope that surrounds nucleocapsid. The virus without envelope is called naked virus.

- The envelope is a bilayer of lipoprotein and glycoprotein.
- The envelope is acquired by the progeny virus from host cell during virus release by budding process.
- In some virus the glycoprotein projects out in the form of spike called peplomere. Some of the peplomers or glycoprotein spike such as Haemaglutinin and Neuraminidase which are involved in binding of virus to host cell.

4. Enzymes:

- Some virus contains enzymes which play central role during infection process. Eg. Some bacteriophage contains an enzyme lysozyme, which makes small hole in bacterial cell that allows viral nucleic acid to get in.
- Some virus contains their own nucleic acid polymerase which transcribe the viral genome into mRNA during replication process. Eg. Retro virus are RNA virus that replicates inside host cell as DNA intermediate. These virus possess an RNA dependent DNA polymerase called reverse transcriptase.

Symmetry of virus



- Symmetry refers to the way in which capsomere units are arranged in viral capsid.
- Two kinds of symmetry are recognized in the viruses which corresponds to two primary shape ie. Rod and spherical shape of virus.
- Rod shaped virus have helical symmetry and spherical shaped virus have icosahedral symmetry.

i. Helical (spiral) symmetry:

- The capsomere and nucleic acid are wined together to form helical or spiral tube like structure.
- Most of the helical viruses are enveloped and all are RNA viruses.
- The typical virus with helical symmetry is tobacco mosaic virus (TMV), which is a RNA virus with 2130 identical capsomeres arranged in a helix.

ii. Icosahedral (cubical) symmetry:

- An icosahedral is a polygon with 12 vertices (corner), 20 facet (sides) and 30 edges.
- Each facet is an equilateral triange.
- Icosahedral capsid is the most stable and found in human pathogenic virus eg. Adenovirus, Picornavirus, Papovavirus, herpes virus etc.
- Icosahedral capsid are of two types;
- Pentagon; Pentagonal capsomere at the vertices
- Hexagon; Hexagonal capsomere at the vertices

iii. Complex symmetry:

- Some virus are more complex, being composed of several separate capsomere with separate shape and symmetry.
- They do not have either icosahedral or helical symmetry due to complexity of their capsid structure. Eg. Pox virus, Bacteriophage.

Binal symmetry:

- It is a type of complex symmetry
- Some viruses such as T-phage (T2,T4 etc) have compex symmetry including head and tail
- The most complicated virus in terms of structure are some bacteriophage which possess icosahedral head and helical tail. Such structure is called binal symmetry.

Cultivation Methods

Techniques of virus cultivation

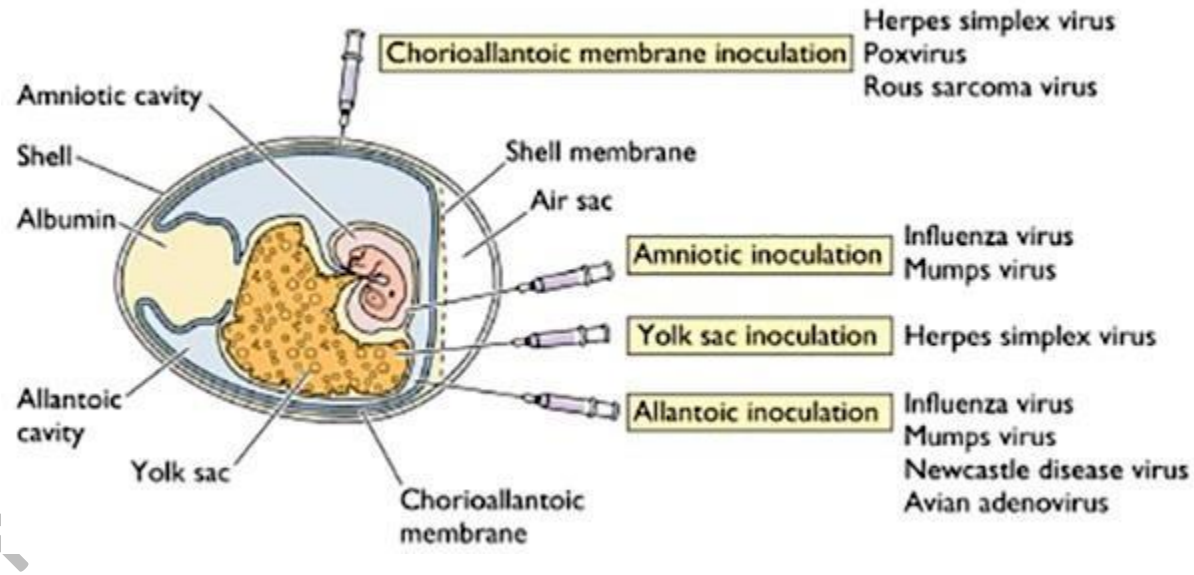
1. Animal inoculation
2. Embryonated egg culture
3. Cell culture

1. Animal inoculation:

- Animal inoculation is one of the primary method for isolation of certain viruses and for study of pathogenesis of certain viral diseases.
- Lab mice (white mice) particularly suckling one are animal of choice for virus cultivation. Suckling mice of age less than 48 hrs are used for culture of Toga virus and Coxsackie virus.
- Other animals such as hamsters, Guinae pig, Chimpanzee etc are sometimes used as alternative for virus culture.
- After inoculation of virus sample, the animals are observed for symptoms of disease till death. And finally virus is isolated from tissue of animal.

2. Embryonated egg culture:

- For virus cultivation, an egg embryo of 7-12 days is used.
- At first egg is kept in incubator for embryo development up to 7-12 days and then virus sample is inoculated into the egg.
- Opening in egg should be shield with paraffin and it is incubated for sufficient time.
- Virus can be cultured in different parts of embryonated egg, such as choriallontoic membrane, amniotic sac, allantoic cavty or yolk sac depending upon types of virus.



i. Chorioallontoic membrane (CAM):

- Pox virus are cultured in choriallontoic membrane.
- The growing virus produce grey-white lesions called Pocks.

- Each Pock is developed by a single virus.
- The number of pocks indicates the number of virus present in inoculated sample.

ii. Allantoic cavity:

- Viruses such as Influenza virus, Mumps virus, Yellow fever virus and Rabies virus are cultivated in allantoic cavity.Allantoic cavity culture of virus is mainly done for vaccine preparation, to obtain large amount of virus load.

iii. Amniotic cavity:

- Influenza virus are cultured in amniotic cavity for isolation of virus from clinical sample.

iv. Yolk sac:

- Herpes virus is cultured by inoculating in yolk sac.
- This is also used for cultivation of some bacteria such as Chlamydia and Rickettsia.

3. Cell culture (tissue culture) technique:

- This technique is most commonly used technique for cultivation of virus.
- There are three types of cell culture technique.

i. Organ culture:

- Small bits of organ from human or animal is maintained in tissue culture media.
- This technique is used in specific purposes only. For eg, to culture Corona virus tracheal ring culture is done.

ii. Explant culture:

- In this small fragment of tissue is extracted from human or animal and used for virus culture.
- This technique is very rarely used.

iii. Cell line culture:

- This is the most commonly used technique.
- Cell line culture is routinely used in lab for virus culture, isolation and identification.
- In cell line culture, at first growth media is prepared by maintaining balanced salt concentration, all essential aminoacids, glucose, buffering agents, some antibiotic, serum etc.
- Some tissue fragment is obtained, it is trypsionised to dissociate cells.

- The dissociated cell are washed and suspended in culture media in a tube or petriplates and incubated for sufficient time.
- On incubation, cell divides and spread out on the glass surface to form a confluent mono-layer of cells, which is now used for virus culture.
- On the basis of origin, chromosomal characteristics and number of generation through which cell culture can be maintained, cell line culture are of three types.

I. Primary cell line

II. Semi-continuous (diploid) cell line

III. Continuous cell line

I. Primary cell line:

- These are normal cells, obtained from fresh organ of animals or human and cultured.
- Once the cell attached to the surface of culture vessel, they divides by mitosis until confluent mono-layer of cells covers the surface.
- These cells are capable of limited growth for limited generation. They cannot be maintained in serial sub culture.
- These primary cell line culture is used for isolation of virus and for preparation of vaccines.
- Examples: Monkey kidney cell line, Human amnion cell line, etc

II. Semi-continuous cell line (Diploid cell):

- These cell are fibroblastic cell.
- They are diploid cell containing same number of chromosome as the parent cell.
- Fibroblastic cells are obtained from embryo tissue.
- These diploid cell can be sub cultured for limited generation.
- There is a rapid cell division and after 50 serial sub culture, they undergoes senescence.
- The diploid cell are susceptible for wide range of Human virus culture and also used for vaccine production.
- Examples: Rhesus embryo cell, human embryonic lung strain, etc

III. Continuous cell line:

- These are cells of single type capable of infinite growth in vitro.
- These are usually cancer cell derived from cancerous tissue. These cells grow faster and they are haploid cells.

- They are termed as continuous cell line as they can be serially sub culture for infinite generation without going senescence.
- Examples; HeLa cell is obtained from cervical cancer, HEP-2 (Humman Epithelioma of larynx cell line), Vero (Vervet monkey) kidney cell lines, BHK-21 (Baby Hamster Kidney cell line).
- Continuous cell line is maintained by serial sub culture or by deep freezing at -70C, so that these cell be reused when necessary.
- Continuous cell line is used for virus culture but it is not used for vaccine preparation because vaccine prepared by continuous cell culture are not considered safe to Human use.

Classification of Animal Viruses

Baltimore (2008) classified the animal viruses in the following seven groups according to the relationships between virion, nucleic acid and mRNA transcription Table (17.1).

The RNA within the virion is known as plus (+) or sense strand because it acts as mRNA, whereas the newly synthesized RNA which is complementary in base-sequence to the original infectious strand is called minus (-) or antisense strand. It acts as template to produce additional (+) strand which may act as mRNA.

Table 17.1 : The animals viruses.

Family	*Virions*	*Genome (kb)*	*Example*
1. dsDNA viruses			
Adenoviruses	Naked, icosahedral	35-40	Human and Animal adenoviruses
Herpes virus	Enveloped, icosahedral	120-200	Herpes simplex, chicken-pox, Epstein-Bar virus
Papovavirus	Naked, icosahedral	5-8	SV40, polyoma, papilomaviruses
Poxvirus	Enveloped, complex	120-300	Small pox, vaccinia
2. ssDNA viruses			
Parvovirus	Naked, icosahedral	4-5	Adeno-associated virus, Human parvovirus
3. (+) ssRNA viruses			
Coronavirus	Enveloped, helical	16-21	Human common cold like diseases, mouse hepatitis virus
Picorna virus	Naked, icosahedral	7	Polio, common cold virus, foot and mouth disease virus
Togavirus	Enveloped, icosahedral	17	Encephalitis virus
4. (-) ssRNA virus			
Paramyxoviruses	Enveloped, helical	15	Measles, mumps
Rhabdovirus	Enveloped, helical (Bullet shaped)	12-15	Rabies virus
Orthomyxovirus	Enveloped, helical	14	Influenza
5. dsRNA viruses			
Reovirus	Naked, icosahedral	18-30	Reovirus, human and animal diarrhoea virus
6. (+)ssRNA-RT viruses			
Retrovirus	Enveloped, icosahedral (diploid)	7-10	Rous sarcoma, Avian mouse, Mammary tumour virus
7. dsDNA-RT viruses			
Hepadnavirus	Enveloped, icosahedral	13	Hepatitis-B virus, humans, rodents and birds

Baltimore classification (first defined in 1971) is a classification system that places viruses into one of seven groups depending on a combination of their nucleic acid (DNA or RNA), strandedness (single-stranded or double-stranded), sense, and method of replication. Named after David Baltimore, a Nobel Prize-winning biologist, these groups are designated by Roman numerals. Other classifications are determined by the disease caused by the virus or its morphology, neither of which are satisfactory due to different viruses either causing the same disease or looking very similar. In addition, viral structures are often difficult to determine under the microscope. Classifying viruses according to their genome means that those in a given category will all behave in a similar fashion, offering some indication of how to proceed with further research. 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. Coronaviruses, 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 DNA with RNA intermediate in life-cycle (e.g. Hepadnaviruses)

DNA viruses

Viruses with a DNA genome, except for the DNA reverse transcribing viruses, are members of three of the four recognized viral realms: Duplodnaviria, Monodnaviria, and Varidnaviria. But the incertae sedis order Ligamenvirales, and many other incertae sedis families and genera, are also used to classify DNA viruses. The domains Duplodnaviria and Varidnaviria consist of double-stranded DNA viruses; other double-stranded DNA viruses are incertae sedis. The domain Monodnaviria consists of single-stranded DNA viruses that generally encode a HUH endonuclease; other single-stranded DNA viruses are incertae sedis.[15]

- Group I: viruses possess double-stranded DNA. Viruses that cause chickenpox and herpes are found here.
- Group II: viruses possess single-stranded DNA.

Examples of DNA viruses

Virus family	Examples (common names)	Virion naked/ enveloped	Capsid symmetry	Nucleic acid type	Group
1. Adenoviridae	Canine hepatitis virus, Some types of the common cold	Naked	Icosahedral	Ds	I
2. Papovaviridae	JC virus, HPV	Naked	Icosahedral	ds circular	I
3. Parvoviridae	Human parvovirus B19, canine parvovirus	Naked	Icosahedral	Ss	II
4. Herpesviridae	Herpes simplex virus, varicella-zoster virus, cytomegalovirus, Epstein–Barr virus	Enveloped	Icosahedral	ds	I
5. Poxviridae	Smallpox virus, cowpox, myxoma virus, monkeypox, vaccinia virus	Complex coats	Complex	ds	I
6. Anelloviridae	Torque teno virus	Naked	Icosahedral	ss circular	II
7. Pleolipoviridae	HHPV1, HRPV1	Enveloped		ss/ds linear/circular	I/II

RNA viruses

All viruses that have an RNA genome, and that encode an RNA-dependent RNA polymerase (RdRp), are members of the kingdom Orthornavirae, within the realm Riboviria.[16]

- **Group III:** viruses possess double-stranded RNA genomes, e.g. rotavirus.
- **Group IV:** viruses possess positive-sense single-stranded RNA genomes. Many well known viruses are found in this group, including the picornaviruses (which is a family of viruses that includes well-known viruses like Hepatitis A virus, enteroviruses, rhinoviruses, poliovirus, and foot-and-mouth virus), SARS virus, hepatitis C virus, yellow fever virus, and rubella virus.

- **Group V:** viruses possess negative-sense single-stranded RNA genomes. Ebola and Marburg viruses are well known members of this group, along with influenza virus, measles, mumps and rabies.

Examples of RNA viruses					
Virus Family	**Examples (common names)**	**Capsid naked/enveloped**	**Capsid Symmetry**	**Nucleic acid type**	**Group**
1. Reoviridae	Reovirus, rotavirus	Naked	Icosahedral	ds	III
2. Picornaviridae	Enterovirus, rhinovirus, hepatovirus, cardiovirus, aphthovirus, poliovirus, parechovirus, erbovirus, kobuvirus, teschovirus, coxsackie	Naked	Icosahedral	ss	IV
3. Caliciviridae	Norwalk virus	Naked	Icosahedral	ss	IV
4. Togaviridae	Eastern equine encephalitis, Chikungunya	Enveloped	Icosahedral	ss	IV
5. Arenaviridae	Lymphocytic choriomeningitis virus, Lassa fever	Enveloped	Complex	ss(−)	V
6. Flaviviridae	Dengue virus, hepatitis C virus, yellow fever virus, Zika virus	Enveloped	Icosahedral	ss	IV
7. Orthomyxoviridae	Influenzavirus A, influenzavirus B, influenzavirus C, isavirus, thogotovirus	Enveloped	Helical	ss(−)	V
8. Paramyxoviridae	Measles virus, mumps virus, respiratory syncytial virus, Rinderpest virus, canine distemper virus	Enveloped	Helical	ss(−)	V
9. Bunyaviridae	California encephalitis virus, Sin nombre virus	Enveloped	Helical	ss(−)	V
10. Rhabdoviridae	Rabies virus, Vesicular stomatitis	Enveloped	Helical	ss(−)	V

11. Filoviridae	Ebola virus, Marburg virus	Enveloped	Helical	ss(−)	V
12. Coronaviridae	Human coronavirus 229E, Human coronavirus NL63, Human coronavirus OC43, Human coronavirus HKU1, Middle East respiratory syndrome-related coronavirus, Severe acute respiratory syndrome coronavirus, and Severe acute respiratory syndrome coronavirus 2	Enveloped	Helical	ss	IV
13. Astroviridae	Astrovirus	Naked	Icosahedral	ss	IV
14. Bornaviridae	Borna disease virus	Enveloped	Helical	ss(−)	V
15. Arteriviridae	Arterivirus, equine arteritis virus	Enveloped	Icosahedral	ss	IV
16. Hepeviridae	Hepatitis E virus	Naked	Icosahedral	ss	IV

The balitmore scheme for viral classification. Viruses within the Baltimore scheme are grouped based on the composition of their genomes, and their method for genome replication.

Bacteriophage are viruses that specifically infect bacterial cells. Bacteriophage, even within similar Baltimore taxa are extremely varied, with wide diversity in both their genomic and coat structures.

On the basis of presence of single or double strands of genetic material, the bacteriophages are categorized as under:

1. The ssDNA Bacteriophages:

(i) Icosahedral phages = ϕx 174, St-1, ϕR, BR2, 6SR U3 and G series, e.g., G4, G6, G13, G16. All are like ϕx 174.

(ii) Helical (filamentous)

(a) The Ft group: They are F specific phages and absorb to the tip of F type sex pilus e g E. coli phages (fd, fl, M13).

(b) If group: They are absorbed to I-type sex pilus specified by R factors, e.g.. If1,IF2, etc.

(c) The third group is specific to strains carrying RF1 sex factor.

2. The dsDNA Phages:

Following are the examples of dsDNA phages:

i. T-odd phage of E. coli, e.g., T1, T3, T5, T7.

ii. T-even phage of E. coli, e.g., T2, T4, T6.

iii. The other E. coli phages, e.g., P1, P2, Mu, ϕ80.

iv. The phages of Bacillus subtilis, e.g., PBS 1, PBSX, SPO1, SPO2.

v. The phage of Shigella a, e.g., P2.

vi. The phage of Salmonella; e.g., PI, P22.

vii. The phage of Haemophilus, e.g., HP1.

viii. The phage of Pseudomonas, e.g., PM2.

3. The ssRNA phages.

Examples of the ssRNA bacteriophages are as below:

(i) Group I:

E. coli. phages such as f2, MS2, M12, R17, fr, etc.

(ii) Group II:

The Qβ phages.

4. The dsRNA phages.

Example:

The ϕ6 bacteriophage.

Morphological Groups of Bacteriophages:

On the basis of EM studies, Bradley (1967) has described the following six morphological types of bacteriophages.

Type A:

This type of virus has hexagonal head, a rigid tail with contractile sheath and tail fibers dsRNA, T-even (T2, T4, T6) phages.

Type B:

This type of phage contains a hexagonal head but lacks contractile sheath. Its tail is flexible and may or may have tail fiber, for example dsDNA phages, e.g., T1, T5 phages.

Type C:

Type C characterized by a hexagonal head and a tail shorter than head. Tail lacks contractile sheath and may or may not have tail fiber, for example dsDNA phages, e.g., T3, T7.

Type D:

Type D contains a head which is made up of capsomers but lacks tail, for example ssDNA phages (e.g., ϕX174).

Type E:

This type consists of a head made up of small capsomers but contains no tail, for example ssRNA phages (e.g., F2, MS2).

Type F:

Type F is a filamentous phage, for example ss

DNA phages (e.g., fd, f1).

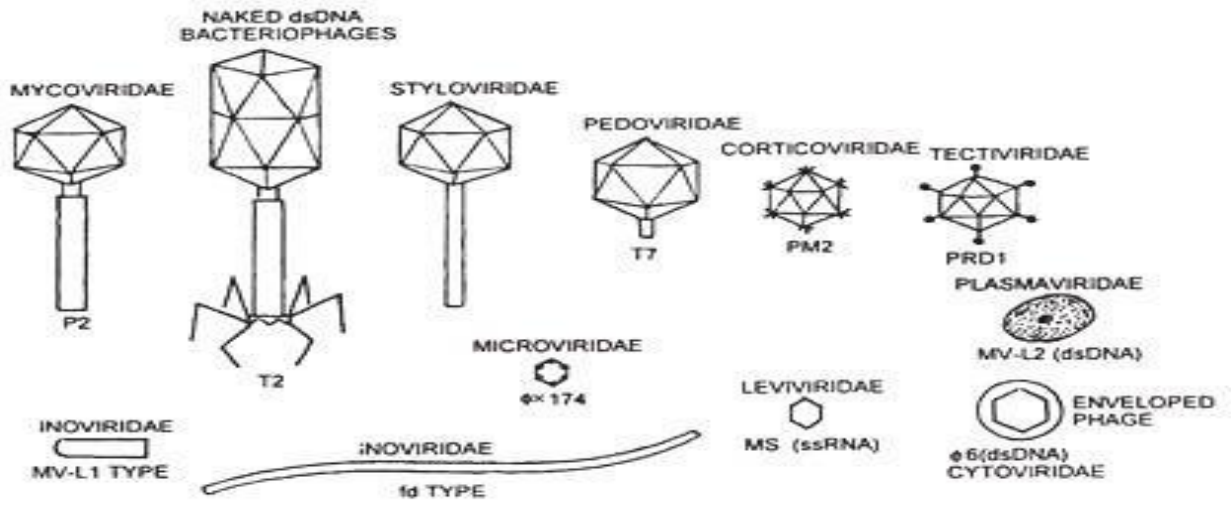


FIG. 13.1. Families of bactenophages (diagrammatic).

Viral replication

Viruses multiply only in living cells. The host cell must provide the energy and synthetic machinery and the low-molecular-weight precursors for the synthesis of viral proteins and nucleic acids.[2]

Virus replication occurs in seven stages:

- Attachment
- Entry (penetration)
- Uncoating
- Replication

- Assembly
- Maturation
- Release (liberation stage).

Attachment

It is the first step of viral replication. Some viruses attach to the cell membrane of the host cell and inject its DNA or RNA into the host to initiate infection. Attachment to a host cell is often achieved by a virus attachment protein that extends from the protein shell (capsid), of a virus. This protein is responsible for binding to a surface receptor on the plasma membrane (or membrane carbohydrates) of a host cell. Viruses can exploit normal cell receptor functions to allow attachment to occur by mimicking molecules that bind to host cell receptors. For example, the rhinovirus uses their virus attachment protein to bind to the receptor ICAM-1on host cells that is normally used to facilitate adhesion between other host cells.

Entry

Entry, or penetration, is the second step in viral replication. This step is characterized by the virus passing through the plasma membrane of the host cell. The most common way a virus gains entry to the host cell is by receptor-mediated endocytosis, which comes at no energy cost to the virus, only the host cell. Receptor-mediated endocytosis occurs when a molecule (in this case a virus) binds to receptor on the membrane of the cell. A series of chemical signals from this binding causes the cell to wrap the attached virus in the plasma membrane around it forming a virus-containing vesicle inside the cell.

Viruses enter host cells using a variety of mechanisms, including the endocytic and non-endocytic routes. They can also fuse at the plasma membrane and can spread within the host via fusion or cell-cell fusion.[5] Viruses attach to proteins on the host cell surface known as cellular receptors or attachment factors to aid entry. Evidence shows that viruses utilize ion channels on the host cells during viral entry. Fusion: External viral proteins promote the fusion of the virion with the plasma membrane. This forms a pore in the host membrane, and after entry, the virion becomes uncoated, and its genomic material is then transferred into the cytoplasm. Cell-to-cell fusion: Some viruses prompt specific protein expression on the surfaces of infected cells to attract uninfected cells. This interaction causes the uninfected cell to fuse with the infected cell at lower pH levels to form a multinuclear cell known as a syncytium. Endocytic routes: the process by which an intracellular vesicle is formed by membrane invagination, which results in the engulfment of extracellular and membrane-bound components, in this context, a virus. Non-endocytic routes: the process by which viral particles are released into the cell by fusion of the extracellular viral envelope and the membrane of the host cell.

Uncoating

Uncoating is the third step in viral replication. Uncoating is defined by the removal of the virion's protein "coat" and the release of its genetic material. This step occurs in the same area that viral transcription occurs. Different viruses have various mechanisms for uncoating. Some RNA viruses such as Rhinoviruses use the low pH in a host cell's endosomes to activate their uncoating mechanism. This involves the rhinovirus releasing a protein that creates holes in the endosome, and allows the virus to release its genome through the holes. Many DNA viruses travel to the host cells nucleus and release their genetic material through nuclear pores.

Replication

The fourth step in the viral cycle is replication, which is defined by the rapid production of the viral genome. How a virus undergoes replication relies on the type of genetic material the virus possesses. Based on their genetic material, viruses will hijack the corresponding cellular machinery for said genetic material. Viruses that contain double-stranded DNA (dsDNA) share the same kind of genetic material as all organisms, and can therefore use the replication enzymes in the host cell nucleus to replicate the viral genome. Many RNA viruses typically replicate in the cytosol, and can directly access the host cell's ribosomes to manufacture viral proteins once the RNA is in a replicative form.

Viruses may undergo two types of life cycles: the lytic cycle and the lysogenic cycle. In the lytic cycle, the virus introduces its genome into a host cell and initiates replication by hijacking the host's cellular machinery to make new copies of the virus. In the lysogenic life cycle, the viral genome is incorporated into the host genome. The host genome will undergo its normal life cycle, replicating and dividing replicating the viral genome along with its own. The viral genome can be triggered to begin viral production via chemical and environmental stimulants. Once a lysogenic virus enters the lytic life cycle, it will continue in the viral production pathways and proceed with transcription / mRNA production. (ex: Cold sores, herpes simplex virus (HSV)-1, lysogenic bacteriophages, etc.)

Assembly

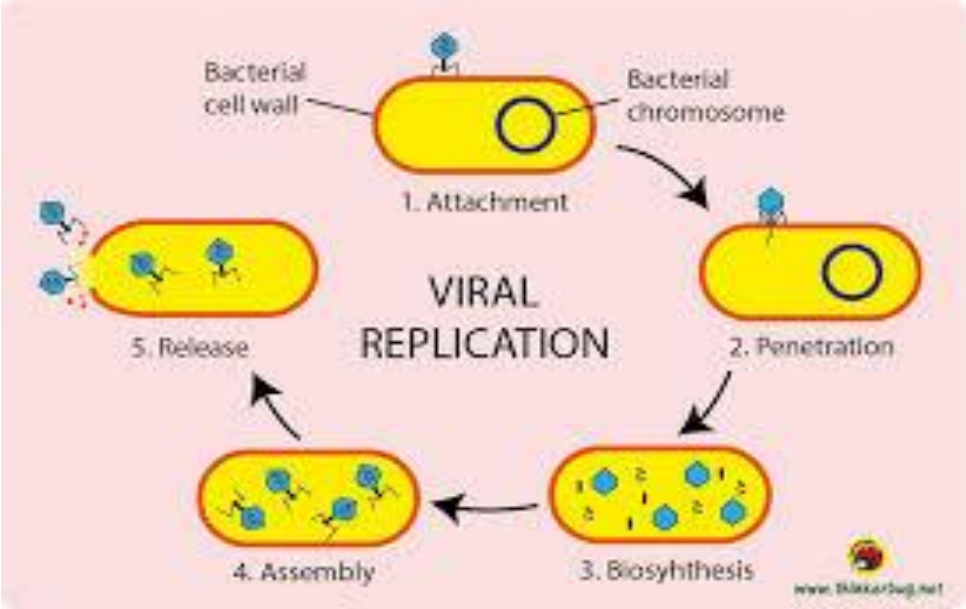
Assembly is when the newly manufactured viral proteins and genomes are gathered and put together to form immature viruses. Like the other steps, how a particular virus is assembled is dependent on what type of virus it is. Assembly can occur in the plasma membrane, cytosol, nucleus, golgi apparatus, and other locations within the host cell. Some viruses only insert their genome into a capsid once the capsid is completed, while in other viruses the capsid will wrap around the genome as it is being copied.

Maturation

This is the final step before a competent virus is formed. This typically involves capsid modifications that are provided by enzymes (host or virus-encoded).

Release (liberation stage)

The final step in viral replication is release, which is when the newly assembled and mature viruses leave the host cell. How a virus releases from the host cell is dependent on the type of virus it is. One common type of release is budding. This occurs when viruses that form their envelope from the host's plasma membrane bend the membrane around the capsid. As the virus bends the plasma membrane it begins to wrap around the whole capsid until the virus is no longer attached to the host cell. Another common way viruses leave the host cell is through cell lysis, where the viruses lyse the cell causing it to burst which releases mature viruses that were in the host cell.



Purification of virus and components:

Ultracentrifugation: The viruses are usually purified with the help of ultracentrifugation. The machine is capable of rotating the samples at 20,000-100,000 rpm under the density gradient of CsCl2 or sucrose. Density at which viruses neither sink nor float when suspended in a density gradient is called as buoyant density. The rate at which viral particles sediment under a defined gravitational force is called as sedimentation coefficient. The basic unit is the Svedberg (S) which is 10-13 sec. The S value of a virus is used to estimate its molecular weight. Types of sedimentation medium:

A. Sucrose cushions or gradient - A fixed concentration or a linear gradient of sucrose is used. Increasing the density and viscosity of the medium decreases the rate at which virus sediments through them. In general a "cushion" of sucrose is prepared at the bottom of the centrifuge tube and the sample containing virus is overlaid over the cushion. Since most viruses have greater

densities than sucrose, separation is based on S values. of 18 method can be used to separate molecules with relatively close S values. Sometime glycerol is also used in place of sucrose.

B. CsCl2 gradient centrifugation - A linear gradient of CsCl2 in buffer is prepared in the ultracentrifuge tube. As the concentration of the CsCl2 is increased the density of the medium increases in the tube so that density is low at the top and high at the bottom. Viral particle centrifuged through this medium will form a band at a position equal to their buoyant density. These are useful to separate viruses of different densities. Limitation of this method is that CsCl2 can permanently inactivate some viruses.

6.2.2. Other techniques for separation: Viruses can also be separated by electrophoresis and column chromatography but these are not the preferred way to separate virus while sometimes they are used to separate viral nucleic acids or proteins. Both the methods separate the virus on the basis of charge and/or size. Virus contains a variety of charged macromolecule on its surface which contributes to its electrophoretic mobility or ion-exchange characteristics. Viruses are sometimes ligated with the charged group to be separated by ion exchange chromatography. Molecular sieve chromatography can also be used to purify the viruses where large pores are formed with the help of special agarose through which virus particles can enter. 6.3. Purity of viruses: Many methods are used to assess the purity of virus. The ratio of UV absorption at 260 and 280 nm during a spectrophotometric analysis (260/280) is a characteristic feature to measure the purity of a virus sample and is dependent on the amount of nucleic acid and protein present in the virion. Serological methods such as enzyme-linked immunosorbent assay (ELISA), radioimmuno precipitation assay (RIPA), western blot, virus neutralization test (VNT), and complement fixation are also used to check the puirity of a virus sample. These methods require antibodies specific to viral proteins that may be monoclonal (single type of antibody specific to a single viral protein) or polyclonal (several different antibodies that may recognize several viral proteins or epitopes). Plaque assay is also performed in order to isolate the single colony from a pool of quasispecies viruses.

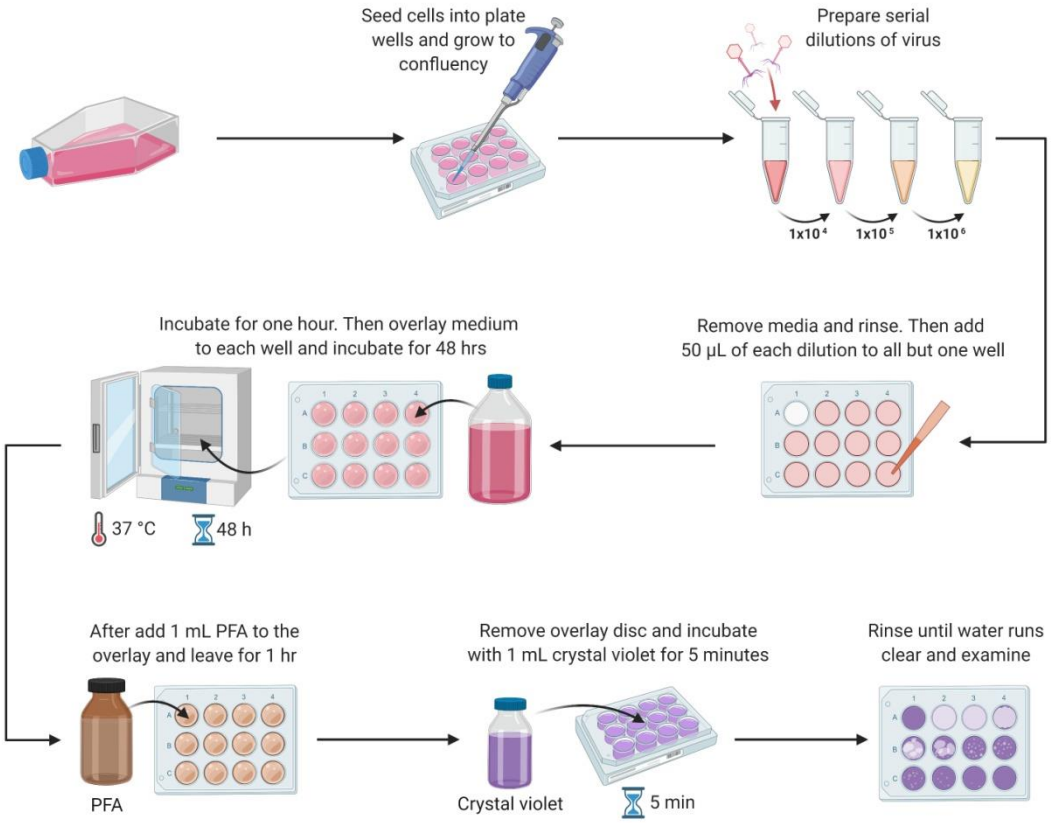
Viral quantification methods

Viral quantification is crucial for studying viral biology, replication, and infection mechanisms to accelerate antiviral and vaccine development. Accurate viral concentration determination enables exact normalization of infections and monitoring, optimization, and modification of viral propagation processes to maximize yields. In this article, we briefly describe some of the molecular and immunological approaches for the quantification of viruses along with their advantages and disadvantages.

1. Methods that measure viral infections/infectivity assays

Viral plaque assay is the most used quantitative viral assay. Plaque assays involve infecting a confluent monolayer of cells with a lytic virus at various dilutions. Infected monolayers are then immobilized with an overlay material. Plaques form as a result of limited infection and replication.

Plaques are then counted with neutral red or crystal violet. The virus titer is expressed in plaque-forming units (PFU) per milliliter (PFU/mL).

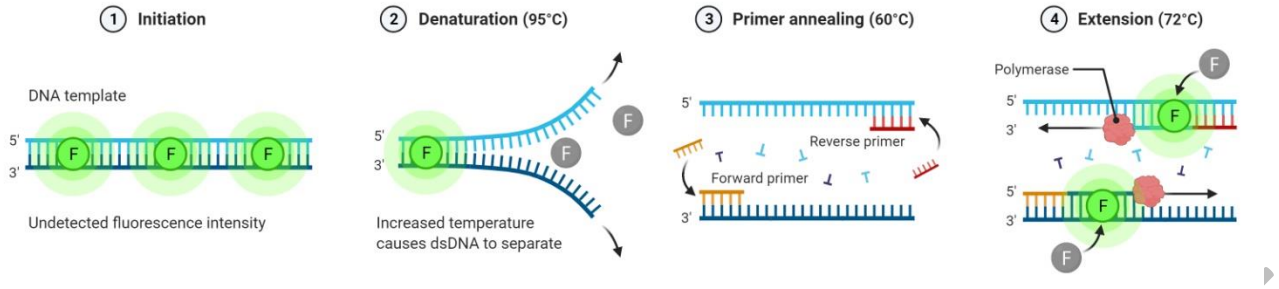


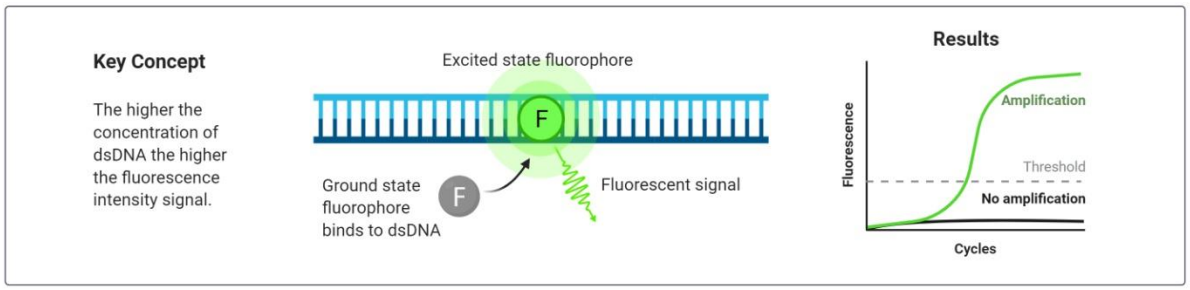
Plaque essay

Median Tissue Culture Infectious Dose (TCID50) is the virus dose required to infect 50% of inoculated cells. The virus is introduced to host cells in successive dilutions. A plate reader and a cell viability assay are then used to assess the proportion of dead cells after incubation.

2. Methods that measure viral protein antigens

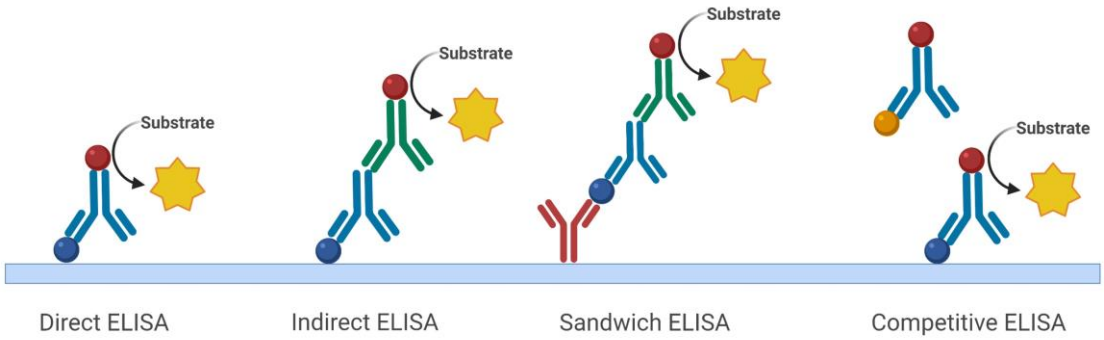
Quantitative Polymerase Chain Reaction (qPCR) qPCR measures the quantity of viral DNA or RNA in a sample. PCR is performed with a virus-specific primer in the presence of a dsDNA dye or a sequence-specific reporter. The signal from the reporting fluorophore grows as the DNA amplification proceeds. The number of cycles (Ct value) required to generate a specified fluorescence level is compared to the Ct value of the standard.



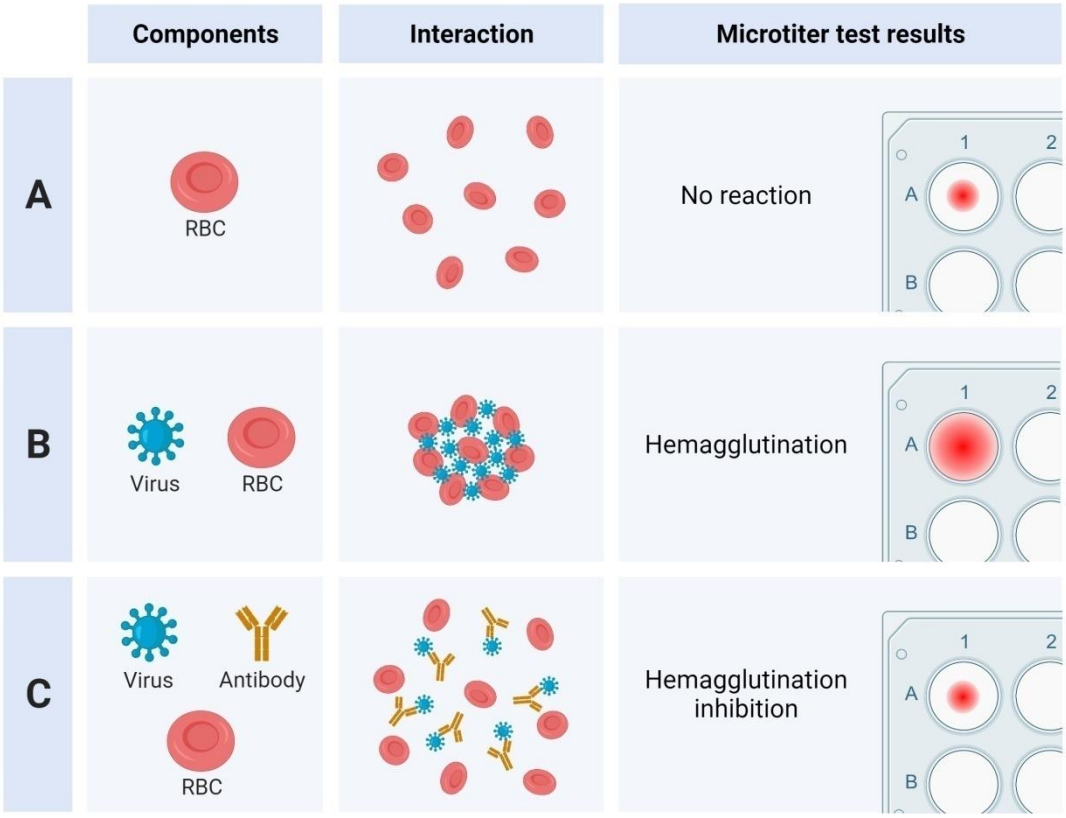


qPCR

Enzyme-Linked Immunosorbent Assay (ELISA) tests serological samples for the presence and/or concentration of antibodies generated by infection or viral antigens. A viral antigen or an antibody against the pathogen is first immobilized in a well which is then recognized by viral antibodies or virus-specific proteins introduced into the well. Antibodies that recognize the attached molecule generate a signal that is measured by a microplate reader. An antigen or antibody signal is proportional to the sample concentration.Figure 3 Different types of ELISA



Hemagglutination Assay (HA) assay is used to test influenza viruses' ability to aggregate red blood cells (RBCs). Viral inoculum is diluted and added to mammalian blood samples. Viruses with hemagglutinin protein cluster RBCs into foci of aggregated RBCs, which expand into networks incapable of normal precipitation. Wells are then scored based on whether RBCs precipitate into the wells or not, forming visible spots giving only a relative assessment of viral quantification.



Hemagglutination Assay

3. Methods that directly count viral particles

Viral Flow Cytometry counts intact virus particles in a sample by fluorescently detecting colocalized proteins and nucleic acids. A laser beam analyzes samples labeled with two dyes, one for proteins and one for nucleic acids. In addition to the measured sample flow rate, the number of particles creating simultaneous events on each of the two separate fluorescence channels is determined to calculate the concentration of virus particles per mL.

4. Methods that optically detect virions by microscopy:

Immunofluorescence assay is used for the detection of viral antigens or antiviral antibodies in clinical samples. The assay is conducted in two formats: direct immunofluorescence assay (DFA) that detects viral antigens, and indirect immunofluorescence assay (IFA) that detects antiviral antibodies. In the DFA, the antibody that recognizes the viral antigen is directly conjugated to a fluorescent dye. In the IFA, viral antigen-specific antibody is unlabeled and is detected with a second fluorescently labeled anti-human antibody

Viral Specimens collection

Viral Specimens collection plays a vital role in laboratory diagnosis. Proper sample collection leads to the proper diagnosis of disease. Sample should be collected aseptically. Appropriate samples

from the appropriate site and an adequate amount should be collected. Once collected samples are recommended to emulsify or mixed properly with viral transport medium (VTM).

Collection and Transport of Virological Specimens

- Specimen for detection of viruses should be collected as soon as possible after the appearance of the symptoms that is when the concentration of the virus is at its highest.
- Blood for antibody testing should be collected as early in the disease as possible and also 5- 10 days later. Two serum specimens (paired sera) are necessary to detect a rise in the titer of the Ab.
- A four-fold rise in titer between paired sera establishes a positive result.
- Central nervous system- (CSF)
- Respiratory system
    - **Upper respiratory- (**Throat swab, Nasopharyngeal swab, Ear discharge)
    - **Lower respiratory-** (Sputum, Bronchoscopy specimen, endotracheal aspirate, Lung biopsy/aspirates (Eye – (discharge)
    - **GIT-** (Stool, Rectal swab, Ascitic fluid)¬ Genitourinary system- (Urine, Vaginal/endocervical swab, Urethra¬ discharge) Body fluids- (Blood, Bile, Peritoneal effusion, Pleural effusion, Pericardial¬ effusion, Synovial fluid Surface specimen- (Skin, nails, hair)¬ Wound- (ulcers swabs)¬ Surgical specimen- (Tissue biopsy, Abscesses)

The following information is intended only as a general guide for viral sample collection

Feces

1. Place about 4-8 g of feces (small spoon) in a clean, dry, leak-proof container.

2. Deliver to the laboratory as soon as possible.

3. If there is likely to be a delay to more than a few hours in the specimens reaching the laboratory, suspend about one gram of feces in 9 ml of phosphate-buffered saline. If possible centrifuge at 2000 g for 15 minutes, and then transfer the supernatant fluid to a clean leak-proof container. 4. Label and keep at -20 °C. 5. Send to the virology laboratory in a cold box. 6. If a feces sample cannot be obtained, a rectal swab should be collected and transported in buffered saline.

Nasopharyngeal secretions

1. Collect a specimen by passing a sterile cotton wool swab, through the floor of the nasal cavity as far as the nasopharynx.

2. After few seconds, slowly withdraw the swab and immerse the swab in a container of sterile VTM. A specimen of nasopharyngeal secretion can also be obtained by using a sterile mucus extractor. Cerebrospinal fluid Collect 0.5- 1.0 ml of fluid in a dry, sterile, leak-proof container

- Refrigerated immediately at 4°C. Transport in an insulated cold box.
- When meninges are infected, the CSF will contain lymphocytes and the CSF total protein will be raised.

Skin and ulcer specimens

1. Collect skin scrapings in a dry sterile container.

2. Refrigerate immediately at 4°C, and then transport in a cold box.

Blood for serological tests

1. Two serum specimens are required to diagnose an infection serologically.

2. Collect the sample within 5 days of the onset of the symptoms, and the second sample 5-10 days later.

3. Collect 5-10 ml of venous blood in a dry sterile, screw-cup glass tube or bottle.

4. After the blood is collected, in a leak-proof container, refrigerate it at 4°C until transport in an ice box to the virology laboratory. Transporting of specimens to the Virology laboratory Most Viruses are unable to survive temperature over 50°C, freezing or fluctuating in temperature. Viruses can also be damaged be light, drying, change in pH, and bacterial enzymes. The usage of VTM will prevent specimens from drying out and help to preserve viral activity. All viral specimens should be transported in an icebox with a warning label.

Storage and Transportation of Viral specimens

Short term storage: +4°C Long term storage: -70°C/-190°C(liquid nitrogen)

- Use of Viral Transport medium Specimens are emulsified in the medium VTM (Viral Transport Medium) Composed of :

1. Buffered salt solution

2. Proteins (Bovine serum albumin)

3. Antimicrobial agents

4. Indicator

Function

1. Preserves viral infectivity within the specimen

2. Prevents specimen from drying

3. Prevents growth of bacteria and fungi

Criteria for rejecting Vial specimens

- Mismatch of information on the label and the request
- Inappropriate transport temperature
- Excessive delay in transportation
- Inappropriate transport medium
- specimen received in a fixative
- dry specimen
- Insufficient quantity
- Leakage

UNIT - II

Flaviviruses

The flaviviruses originated about 100,000 years ago in a common ancestor with a separation between mosquito-borne and tick-borne flaviviruses about 40,000 years ago [1]. They were originally considered as togaviruses on the basis of the similar structural constitution. Differences in gene sequence and replication strategy led the genus flavivirus to be classified into a member of the Flaviviridae, a family of positive, single-stranded, icosahedral enveloped RNA viruses that are found mainly in mosquitoes and ticks and can occasionally contract humans [2][3]. The name of flavivirus derives from the Latin flavus that means "yellow", consistent with the jaundice caused by yellow fever virus.

The genus Flavivirus is composed of more than 70 recognized arthropod-borne viruses (or arboviruses) serologically related and broadly distributed. However, individual species are restricted to certain geographical distribution, which is dependent on the ecology of their vertebrate and invertebrate hosts.

Structure

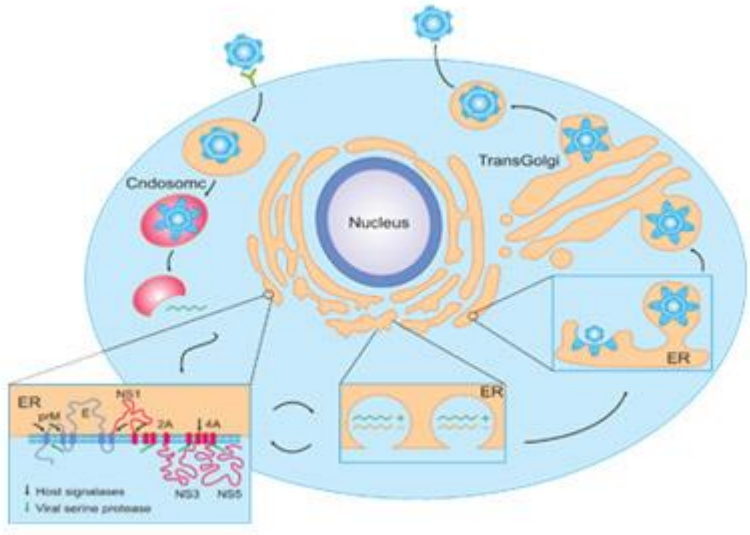
The flavivirus virion is spherical particle about 50 nm in diameter, with an approximately 11 kb genome. The viral genome has a type I cap structure at the 5'-terminal portion, which also possesses the conserved dinucleotide sequence 5'-AG-3'-CU. Most flaviviruses, except for a few tick-borne Encephalitis virus (TBEV) strains, lack a poly-A tail at the 3'-end of the genome and end up with the dinucleotide 5'-CU-3'. The genome comprises an about 11 kb large open reading frame (ORF) coding for a single polyprotein that is post-translationally cleaved into ten proteins. Among these proteins, three structural proteins, including capsid (C), pre-membrane (prM), and envelope (E), make up the viral particles. Seven nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) are required for viral replication & assembly and regulation of the host cell response.

Its RNA is contained within the capsid, constituting an isometric nucleocapsid. The nucleocapsid is further surrounded by a lipid bilayer from the host cell plasma membrane, and two virally encoded membrane proteins E and PrM are anchored on the lipid bilayer. The E protein is responsible for the receptor-mediated endocytic fusion and subsequent cell entry, as well as direct viral assembly and budding. Besides, the E protein of flaviviruses also harbors the antigenic determinants that induce neutralizing antibodies .The PrM can block premature fusion of the virus during its trafficking out of the cell.

Replication

Flaviviruses invade target cells via bites by an infected vector. They enter the host cells through receptor-mediated endocytosis and then are transported into endosomes, where the mild acidic conditions stimulate the fusion of the viral membrane with the endosomal membrane, resulting in the disassembly of nucleocapsid and the release of viral genomic RNA in the host cell

cytoplasm .In the host cell cytoplasm, the released RNA exploits host cell machinery and functions to encode three structural proteins and seven nonstructural proteins, finally completing the replication and assembly of progeny viruses Flaviviruses are initially synthesizd as immature particles containing a precursor form of the M protein (prM), which is cleaved by the cellular protease furin to produce mature virions .The mature infectious viral particles are released into extracellular medium by exocytosis.



Transmission

Although most pathogenetic flaviviruses are transmitted by infected mosquitoes or ticks bites, ZIKV can spread directly from person to person through sexual contact or vertically from mother to fetus .Besides, TBEV infections can also occur via the consumption of unpasteurized contaminated dairy products Most flaviviruses are zoonotic and depend upon non-human animal vectors for their survival, replication, and dispersal with the exception of DENV which propagates mainly in humans. Transmission by mosquitoes between people has only been reported for dengue virus, yellow fever virus, and West Nile virus that cause diseases in which human beings are usually dead-end hosts.

Prevention

Immunisation

Immunisation plays an important role in protecting against JEV. Two JEV vaccines with different modes of action are available for use. Imojev (live vaccine) is recommended for use in people aged ≥ 9 months and is given as a single dose. JEspect (inactivated vaccine) is recommended for use in people ≥ 2 months and is given as a two-dose schedule, 28 days apart. It can be used when live vaccine is contraindicated e.g. pregnancy and immunocompromised.

Personal protection

- Wearing loose-fitting, light-coloured clothing covering as much as the body as possible
- Using an insect repellent containing DEET (diethyl toluamide) or picaridin on uncovered skin
- Protecting rest and sleep areas with mosquito nets
- Avoiding times of peak mosquito activity – some mosquito species will bite during the day but many are particularly active for two to three hours around sunrise and sunset
- All people who work with potentially infected animals, work in areas in which infected mosquitoes may be present should wear appropriate personal protective equipment (PPE). The PPE should be chosen based on the assessed level of risk and the task.

Household protection

- Ensuring pot plant drip trays are emptied at least once a week or are filled with sand
- Ensuring all windows and openings of houses, boats, caravans and tents are fitted with fine (1mm) insect screens
- Ensuring rainwater and septic tank openings, wells or other large water containers are covered with wire mesh no coarser than 1mm
- Appropriate disposal of rubbish: emptying, then covering or puncturing containers that may hold water
- Stocking ornamental ponds and other man-made water bodies with small Australian native fish to eat any wrigglers
- Appropriately disinfecting swimming pools and ensuring unused swimming pools are emptied or stocked with small fish.
- Emptying wading pools at the end of each day
- Ensuring roof gutters are kept in good repair and that leaves and debris are removed regularly so that pools of water do not form
- Ensuring bird baths, stock troughs and pets' drinking water are emptied and refilled at least once a week

Control

There is no specific treatment for flaviviruses. Cases who become severely unwell may need supportive treatment in ICU.

Supportive care for flavivirus infections primarily involves managing symptoms and preventing complications, as there are no specific antiviral treatments available for most flaviviruses. This

care includes rest, adequate hydration to counteract potential fluid loss from vascular leakage (especially in dengue fever), pain relief for symptoms like fever, headaches, and joint pain.

Polio Virus

Poliovirus, the causative agent of polio (also known as poliomyelitis), is a serotype of the species Enterovirus C, in the family of Picornaviridae. There are three poliovirus serotypes, numbered 1, 2, and 3.Poliovirus is composed of an RNA genome and a protein capsid. The genome is a single-stranded positive-sense RNA (+ssRNA) genome that is about 7500 nucleotides long. The viral particle is about 30 nm in diameter with icosahedral symmetry. Because of its short genome and its simple composition—only a strand of RNA and a nonenveloped icosahedral protein coat encapsulating it—poliovirus is widely regarded as the simplest significant virus.Poliovirus is one of the most well-characterized viruses, and has become a useful model system for understanding the biology of RNA viruses.

Symptoms and risk

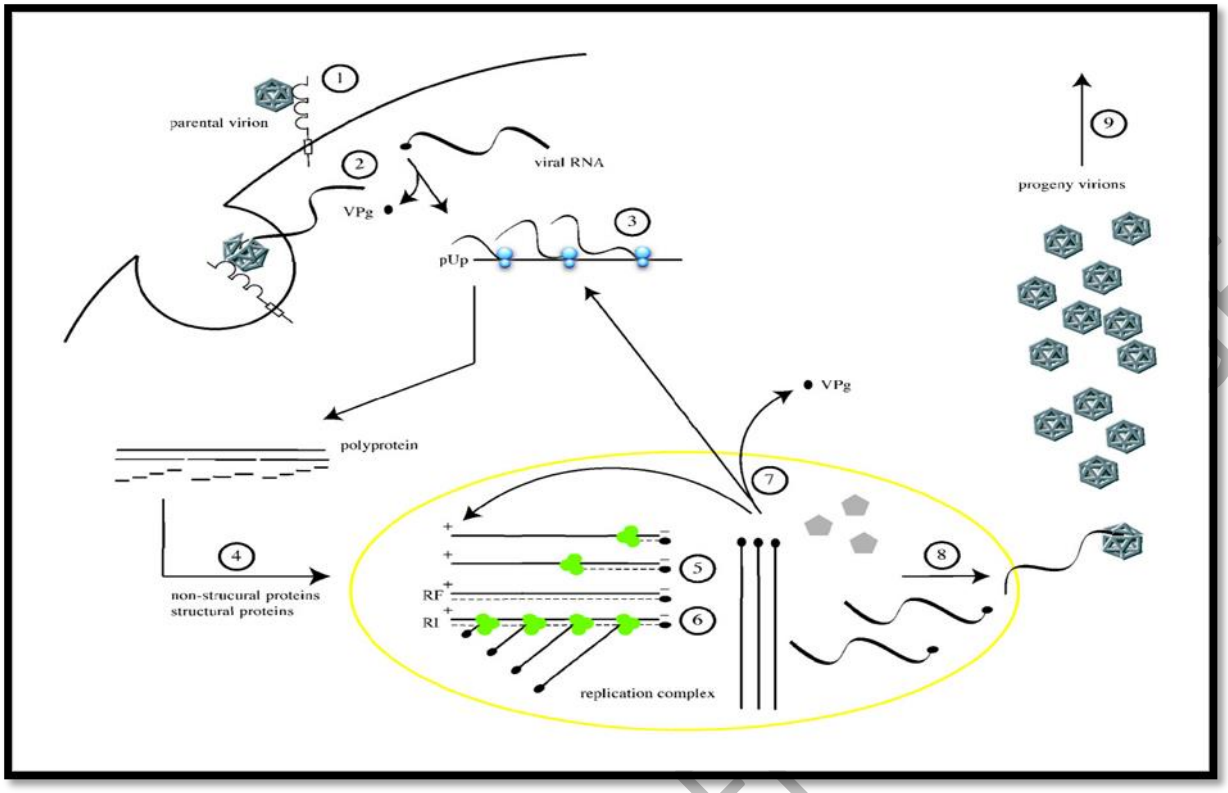
Polio is a highly infectious disease caused by a virus. It invades the nervous system and can cause total paralysis in a matter of hours. The virus is transmitted by person-to-person spread mainly through the faecal-oral route or, less frequently, by a common vehicle (for example, contaminated water or food) and multiplies in the intestine. Initial symptoms are fever, fatigue, headache, vomiting, stiffness of the neck and pain in the limbs. One in 200 infections leads to irreversible paralysis (usually in the legs). Among those paralysed, 5–10% die when their breathing muscles become immobilized.

Polio mainly affects children under 5 years of age. However, anyone of any age who is unvaccinated can contract the disease.

There is no cure for polio, it can only be prevented. Polio vaccine, given multiple times, can protect a child for life. There are two vaccines available: oral polio vaccine and inactivated polio vaccine. Both are effective and safe, and both are used in different combinations worldwide, depending on local epidemiological and programmatic circumstances, to ensure the best possible protection to populations can be provided.

Replication Cycle

Poliovirus infects human cells by binding to an immunoglobulin-like receptor, CD155 (also known as the poliovirus receptor or PVR) on the cell surface. Interaction of poliovirus and CD155 facilitates an irreversible conformational change of the viral particle necessary for viral entry. Following attachment to the host cell membrane, entry of the viral nucleic acid was thought to occur one of two ways: via the formation of a pore in the plasma membrane through which the RNA is then "injected" into the host cell cytoplasm, or via virus uptake by receptor-mediated endocytosis.



The replication cycle of poliovirus is initiated by binding to the cell surface receptor CD155.

(1). The virion forms a pore in the cell membrane through which viral RNA is released into the cytoplasm

(2). Translation of the viral RNA occurs by an IRES-mediated mechanism

(3). The polyprotein is cleaved, yielding mature viral proteins

(4). The positive-sense RNA serves as template for complementary negative-strand synthesis, producing double-stranded replicative form (RF) RNA

(5). Many positive strand RNA copies are produced from the single negative strand

(6). The newly synthesized positive-sense RNA molecules can serve as templates for translation of more viral proteins or can be enclosed in a capsid which ultimately generates progeny virions. Lysis of the infected cell results in release of infectious progeny virions.

Pathogenesis

Poliovirus is an enterovirus. Infection occurs via the fecal–oral route, meaning that one ingests the virus and viral replication occurs in the gastrointestinal tract. Virus is shed in the feces of infected individuals. In 95% of cases only a primary, transient presence of viremia (virus in the bloodstream) occurs, and the poliovirus infection is asymptomatic. In about 5% of cases, the virus spreads and replicates in other sites such as brown fat, reticuloendothelial tissue, and muscle. The sustained viral replication causes secondary viremia and leads to the development of minor

symptoms such as fever, headache, and sore throat. Paralytic poliomyelitis occurs in less than 1% of poliovirus infections. Paralytic disease occurs when the virus enters the central nervous system (CNS) and replicates in motor neurons within the spinal cord, brain stem, or motor cortex, resulting in the selective destruction of motor neurons leading to temporary or permanent paralysis.

This is a very rare event in babies, who still have anti-poliovirus antibodies acquired from their mothers. In rare cases, paralytic poliomyelitis leads to respiratory arrest and death. In cases of paralytic disease, muscle pain and spasms are frequently observed prior to onset of weakness and paralysis. Paralysis typically persists from days to weeks prior to recovery.

Prevention

Prevention of disease spread is accomplished by vaccination. There are two kinds of polio vaccine—oral polio vaccine (OPV), which uses weakened poliovirus, and inactivated polio vaccine (IPV), which is injected. OPV is less expensive and easier to administer, and can spread immunity beyond the person vaccinated, creating contact immunity. It has been the predominant vaccine used. However, under conditions of long-term vaccine virus circulation in under-vaccinated populations, mutations can reactivate the virus to produce a polio-inducing strain, while OPV can also, in rare circumstances, induce polio or persistent asymptomatic infection in vaccinated individuals, particularly those who are immunodeficient. IPV, being inactivated, does not carry these risks, but does not induce contact immunity. IPV is more costly and the logistics of its delivery are more challenging.

Treatment

Because no cure for polio exists, the focus is on increasing comfort, speeding recovery and preventing complications. Depending on the severity of disease, supportive treatments may include:

- Bed rest
- Pain relievers
- Hot moist packs to control muscle pain and spasms
- Portable ventilators to help with breathing
- Physical therapy exercises to prevent bone deformity and loss of muscle function
- Splints or other devices to encourage good position, or alignment, of the spine and limbs

Rhino virus

A cold is a contagious upper respiratory infection that affects your nose, throat, sinuses and windpipe (trachea). You may have heard that the common cold is a coronavirus. In fact, more than 200 different types of viruses can cause a cold. The most common cold virus is the rhinovirus.

We call colds “common” because as their name implies, they’re widespread. You’ll probably have more colds in your lifetime than any other illness. Adults catch two to three colds a year, while young children come down with a cold four or more times a year.

Common cold symptoms typically appear in stages. The common cold stages include early, active and late.

Stage 1: Early (Days 1 to 3)

Within one to three days of picking up a cold virus, you may notice a tickle in your throat. About half of all people with colds report a tickly or sore throat as their first symptom. Other common cold symptoms you may experience during this early stage include:

- Sneezing.
- Runny nose.
- Stuffy nose (nasal congestion).
- Cough.
- Hoarseness.

Stage 2: Active (Days 4 to 7)

Symptoms typically worsen or peak during this stage. In addition to the symptoms in stage 1, you may experience:

- Body aches.
- Headache.
- Runny eyes and nose.
- Fatigue.
- Fever (more common in children).

Stage 3: Late (Days 8 to 10)

Colds usually begin to wind down during this stage. You may be free and clear at this point. But some symptoms can persist. Some people develop a nagging cough that can last up to two months after a respiratory infection.

If your symptoms get worse and/or your fever returns, make a trip to a healthcare provider. You may have developed another infection or a complication, such as bronchitis, sinusitis or pneumonia.

Symptoms of colds in babies may include:

- Runny nose (the discharge may start clear; later, it becomes thicker and may be gray, yellow or green).
- Sneezing.
- Fever of 101 to 102 degrees Fahrenheit (38.3 to 38.9 degrees Celsius).
- Loss of appetite.
- Increased drooling because of sore throat and difficulty swallowing.
- Cough.
- Irritability.
- Slightly swollen glands.

Symptoms in infants and children

- Fever in an infant 2 months or younger.
- Difficulty breathing (especially if your baby's or child's nostrils widen with each breath).
- Fast or labored breathing.
- Wheezing.
- Ribs showing with each breath.
- Blue lips.
- Not eating or drinking, which could mean dehydration.
- Ear pain.
- Excessive crankiness or sleepiness.
- A cough that lasts more than 3 weeks.
- Your baby seems to be getting sicker.

Rhinoviruses cause up to 50% of common colds. There are more than 100 different rhinoviruses. But other types of viruses, such as coronaviruses, can also cause colds. More than 200 different viruses can cause a cold.

Pathogenesis of rhinovirus infection

The attachment of the virus to its receptors (ICAM-1, CHDR-3, low-density lipoprotein receptor [LDLR]) in susceptible individuals elicits an innate immune response leading to airway inflammation and remodeling.

Few cells are actually infected by rhinovirus, and the infection involves only a small portion of the epithelium. Symptoms develop 1-2 days after viral infection, peaking 2-4 days after inoculation, though reports have described symptoms as early as 2 hours after inoculation with primary symptoms 8-16 hours later. Viremia is uncommon.

A local inflammatory response to rhinovirus in the respiratory tract can lead to nasal discharge, nasal congestion, sneezing, and throat irritation. The nasal epithelium is not damaged. Various polymorphisms in cytokine genes have been shown to impact the severity of infection, suggesting a genetic predisposition. Detectable histopathology causing the associated nasal obstruction, rhinorrhea, and sneezing is lacking, which leads to the hypothesis that the host immune response plays a major role in the pathogenesis.

Infected cells release interleukin (IL)–8, which is a potent chemoattractant for polymorphonuclear (PMN) leukocytes. Concentrations of IL-8 in secretions correlate proportionally with the severity of common cold symptoms. Inflammatory mediators, such as kinins and prostaglandins, may cause vasodilatation, increased vascular permeability, and exocrine gland secretion. These, together with local parasympathetic nerve-ending stimulation, lead to cold symptoms.

Deficient production of interferon beta by asthmatic bronchial epithelial cells has been proposed as a mechanism for increased susceptibility to rhinovirus infections in individuals with asthma.

Viral clearance is associated with the host response and is due in part to the local production of nitric oxide. Rhinovirus is shed in large amounts, with as many as 1 million infectious virions present per milliliter of nasal washings. Viral shedding can occur a few days before cold symptoms are recognized by the patient, peaks on days 2-7 of the illness, and may last as long as 3-4 weeks.

Serotype-specific neutralizing antibodies are found 7-21 days after infection in 80% of patients. Although these antibodies persist for years, providing long-lasting immunity, recovery from illness is more likely related to cell-mediated immunity. Persistent protection from repeat infection by that serotype appears to be partially attributable to immunoglobulin A (IgA) antibodies in nasal secretions, serum immunoglobulin G (IgG), and, possibly, serum immunoglobulin M (IgM).

Clinical studies indicate sinus involvement in common colds. Abnormal computed tomography (CT) findings (eg, opacification, air-fluid levels, and mucosal thickening) are present in adults with common colds that resolve over 1-2 weeks without antibiotic therapy.

Despite what is reported in folklore, no good clinical evidence suggests that colds are acquired by exposure to cold weather, getting wet, or becoming chilled

Treatment

Medications that may relieve cold symptoms include:

- **Pain relievers:** Acetaminophen (Tylenol) and NSAIDs such as ibuprofen (Advil) may relieve headaches and fever.
- **Decongestants:** You can use medications like pseudoephedrine

- **Antihistamines:** Diphenhydramine (Benadryl) and other antihistamines may stop sneezing and a runny nose.
- **Cough suppressants:** Medications such as dextromethorphan (Robitussin and Vicks DayQuil Cough) and codeine can help reduce coughing. Providers don't routinely recommend these for children under 5 years of age.
- **Expectorants:** Guaifenesin (Mucinex) and other expectorants may help thin and loosen mucus.

Treatment of common cold in babies:

- Keep your baby comfortable.
- Give your baby fluids. For babies 6 months or younger, let them drink breast milk or formula. At 6 months, your baby can also have some water.
- Let your baby get plenty of rest.

Since most children can't blow their nose until about age 4, the following methods may help ease your baby's stuffy nose:

- **Saline and suction:** About 15 minutes before a feeding, use over-the-counter saline (salt water) drops to loosen up the mucus in your baby's nostrils. Suction out the liquid and mucus a few minutes later with a rubber bulb or oral suction device. This will clear the mucus out of your baby's nose and allow them to breathe and suck at the same time.
- **Petroleum jelly:** Dab petroleum jelly on the outside of your baby's nostrils to reduce irritation. Don't block the inside of your baby's nostrils. (Unless their provider recommends it, don't use nasal sprays on your baby. They may work for a bit but will make their congestion worse with continued use.)
- **Humidifier or vaporizer:** Moisten the air in your baby's room with a humidifier or vaporizer. The clean, cool mist will help moisten the air and decrease the drying of your baby's nasal passages and throat. Clean and dry the humidifier thoroughly before using it to get rid of bacteria or mold that may have collected in the device. Ensure you have the appropriate filter, and check if it needs to be replaced. Don't use hot water vaporizers because of the risk of burns.
- **Steam:** If you don't have a humidifier, take your baby into a steamy room such as a bathroom. Turn on the hot water, close the door and sit together in the steamy room for about 15 minutes. Don't leave your baby alone in the room. Be safe around water. Giving your baby a warm bath may also work.

Viral transmission

Rhinoviruses possess various transmission modes and can infect a huge population at any given time. Most commonly, they are transmitted to susceptible individuals through direct contact or via aerosol particles. The primary site of inoculation is the nasal mucosa, though the conjunctiva may be involved to a lesser extent. The virus attaches to respiratory epithelium and spreads locally. Rhinovirus species A and B bind to the major human rhinovirus receptor, ICAM-1 (found in high quantities in the posterior nasopharynx). Rhinovirus C (RV-C) binds to cadherin-related family member 3 receptor (CHDR-3). Viral particles usually are transmitted via inoculation into the eye or the nose from contact with the fingers that harbor the virus, especially since rhinoviruses are capable of surviving on hands for hours.

Highly contagious behavior includes nose blowing, sneezing, and physically transferring infected secretions onto environmental surfaces or paper tissue. Contrary to popular belief, behaviors such as kissing, talking, coughing, or even drooling do not contribute substantially to the spread of disease.

Infection rates approximate 50% within the household and range from 0% to 50% within schools, indicating that transmission requires long-term contact with infected individuals. Brief exposures to others in places such as movie theaters, shopping malls, friends' houses, or doctors' offices are associated with a low risk for transmission. Because children produce antibodies to fewer serotypes, those who attend school are the most common reservoirs of rhinovirus infection.

Indeed, a 2022 systematic review suggested the possibility that the major route of rhinovirus in many indoor settings is through airborne transmission although different studies were in disagreement. In the natural, "real life" setting, there appeared to be "low evidence" for transmission via hands and fomites, whereas the evidence for aerosol or airborne transmission was deemed better at "moderate."

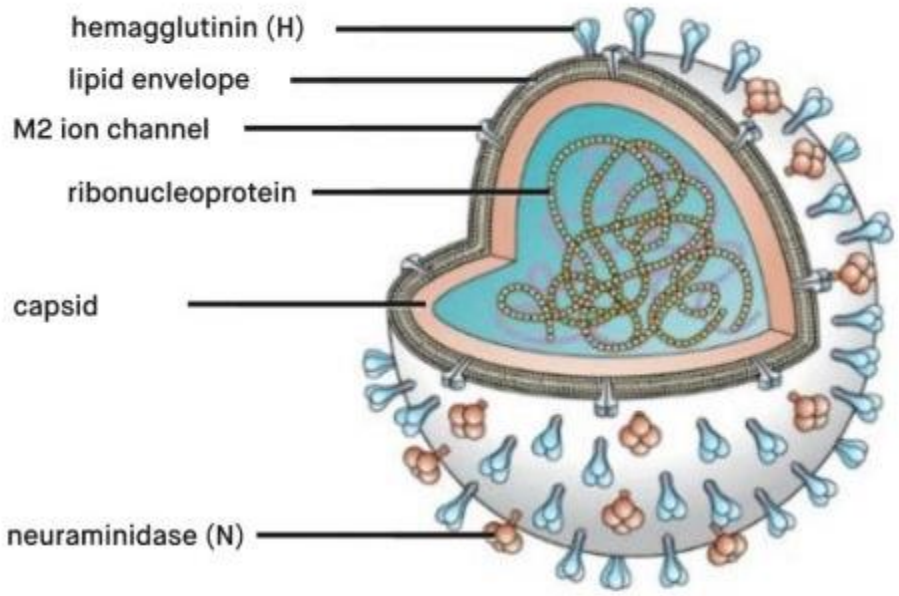
Control

There are several steps you can take to prevent a cold, including:

- **Washing your hands:** Wash your hands frequently, especially before eating or preparing food. Also, wash your hands after using the bathroom, wiping your nose or coming into contact with someone who has a cold.
- **Avoiding touching your face:** Cold viruses spread from your hands to your eyes, nose and mouth.
- **Cleaning frequently used surfaces:** Viruses can live on doorknobs and other places people often touch.
- **Using hand sanitizers:** When you can't wash your hands with soap and water, use alcohol-based hand sanitizer.

- **Strengthening your immune system:** Get enough sleep, eat a healthy diet and exercise so your body is ready to fight off germs.
- **Staying home:** To make sure you don't spread the cold to others, stay home when you're sick.

Structure of rhinovirus



Anatomy of Rhinoviruses

Hepatitis

Hepatitis is inflammation of the liver tissue. Some people or animals with hepatitis have no symptoms, whereas others develop yellow discoloration of the skin and whites of the eyes (jaundice), poor appetite, vomiting, tiredness, abdominal pain, and diarrhea. Hepatitis is acute if it resolves within six months, and chronic if it lasts longer than six months. Acute hepatitis can resolve on its own, progress to chronic hepatitis, or (rarely) result in acute liver failure. Chronic hepatitis may progress to scarring of the liver (cirrhosis), liver failure, and liver cancer.

Hepatitis is most commonly caused by the virus hepatovirus A, B, C, D, and E. Other viruses can also cause liver inflammation, including cytomegalovirus, Epstein–Barr virus, and yellow fever virus. Other common causes of hepatitis include heavy alcohol use, certain medications, toxins, other infections, autoimmune diseases, and non-alcoholic steatohepatitis (NASH). Hepatitis A and

E are mainly spread by contaminated food and water.[Hepatitis B is mainly sexually transmitted but may also be passed from mother to baby during pregnancy or childbirth and spread through infected bloodHepatitis C is commonly spread through infected blood; for example, during needle sharing by intravenous drug usersHepatitis D can only infect people already infected with hepatitis B.

Hepatitis A, B, and D are preventable with immunization Medications may be used to treat chronic viral hepatitisAntiviral medications are recommended in all with chronic hepatitis C, except those with conditions that limit their life expectancyThere is no specific treatment for NASH; physical activity, a healthy diet, and weight loss are recommended. Autoimmune hepatitis may be treated with medications to suppress the immune system. A liver transplant may be an option in both acute and chronic liver failure.

Symptoms

Hepatitis has a broad spectrum of presentations that range from a complete lack of symptoms to severe liver failure. The acute form of hepatitis, generally caused by viral infection, is characterized by constitutional symptoms that are typically self-limiting.[18][19] Chronic hepatitis presents similarly, but can manifest signs and symptoms specific to liver dysfunction with long-standing inflammation and damage to the organ.

Acute viral hepatitis follows three distinct phases:

1. The initial prodromal phase (preceding symptoms) involves non-specific and flu-like symptoms common to many acute viral infections. These include fatigue, nausea, vomiting, poor appetite, joint pain, and headaches. Fever, when present, is most common in cases of hepatitis A and E.[18] Late in this phase, people can experience liver-specific symptoms, including choluria (dark urine) and clay-colored stools.
2. Yellowing of the skin and whites of the eyes follow the prodrome after about 1–2 weeks and can last for up to 4 weeks. The non-specific symptoms seen in the prodromal typically resolve by this time, but people will develop an enlarged liver and right upper abdominal pain or discomfort.[] 10–20% of people will also experience an enlarged spleen, while some people will also experience a mild unintentional weight loss.
3. The recovery phase is characterized by resolution of the clinical symptoms of hepatitis with persistent elevations in liver lab values and potentially a persistently enlarged liver.[18] All cases of hepatitis A and E are expected to fully resolve after 1–2 months.[18] Most hepatitis B cases are also self-limiting and will resolve in 3–4 months. Few cases of hepatitis C will resolve completely.[18]

Occasionally, however, acute hepatitis can cause liver failure. Liver failure can be life threatening and may lead to a coma or even death.

Both drug-induced hepatitis and autoimmune hepatitis can present very similarly to acute viral hepatitis, with slight variations in symptoms depending on the cause.[23][24] Cases of drug-

induced hepatitis can manifest with systemic signs of an allergic reaction including rash, fever, serositis (inflammation of membranes lining certain organs), elevated eosinophils (a type of white blood cell), and suppression of bone marrow activity.

Fulminant hepatitis

Fulminant hepatitis, or massive hepatic cell death, is a rare and life-threatening complication of acute hepatitis that can occur in cases of hepatitis B, D, and E, in addition to drug-induced and autoimmune hepatitis. The complication more frequently occurs in instances of hepatitis B and D co-infection at a rate of 2–20% and in pregnant women with hepatitis E at rate of 15–20% of cases. In addition to the signs of acute hepatitis, people can also demonstrate signs of coagulopathy (abnormal coagulation studies with easy bruising and bleeding) and encephalopathy (confusion, disorientation, and sleepiness). Mortality due to fulminant hepatitis is typically the result of various complications including cerebral edema, gastrointestinal bleeding, sepsis, respiratory failure, or kidney failure.

Chronic hepatitis

Acute cases of hepatitis are seen to be resolved well within a six-month period. When hepatitis is continued for more than six months it is termed chronic hepatitis. Chronic hepatitis is often asymptomatic early in its course and is detected only by liver laboratory studies for screening purposes or to evaluate non-specific symptoms. As the inflammation progresses, patients can develop constitutional symptoms similar to acute hepatitis, including fatigue, nausea, vomiting, poor appetite, and joint pain. Jaundice can occur as well, but much later in the disease process and is typically a sign of advanced disease. Chronic hepatitis interferes with hormonal functions of the liver which can result in acne, hirsutism (abnormal hair growth), and amenorrhea (lack of menstrual period) in women. Extensive damage and scarring of the liver over time defines cirrhosis, a condition in which the liver's ability to function is permanently impeded. This results in jaundice, weight loss, coagulopathy, ascites (abdominal fluid collection), and peripheral edema (leg swelling). Cirrhosis can lead to other life-threatening complications such as hepatic encephalopathy, esophageal varices, hepatorenal syndrome, and liver cancer.

Causes

Causes of hepatitis can be divided into the following major categories: infectious, metabolic, ischemic, autoimmune, genetic, and other. Infectious agents include viruses, bacteria, and parasites. Metabolic causes include prescription medications, toxins (most notably alcohol), and non-alcoholic fatty liver disease. Autoimmune and genetic causes of hepatitis involve genetic predispositions and tend to affect characteristic populations

Viral hepatitis

Viral hepatitis is the most common type of hepatitis worldwide, especially in Asia and AfricaViral hepatitis is caused by five different viruses (hepatitis A, B, C, D, and E). Hepatitis A and hepatitis

E behave similarly: they are both transmitted by the fecal–oral route, are more common in developing countries, and are self-limiting illnesses that do not lead to chronic hepatitis.

Hepatitis B, hepatitis C, and hepatitis D are transmitted when blood or mucous membranes are exposed to infected blood and body fluids, such as semen and vaginal secretions Viral particles have also been found in saliva and breastmilk. Kissing, sharing utensils, and breastfeeding do not lead to transmission unless these fluids are introduced into open sores or cuts. Many families who do not have safe drinking water or live in unhygienic homes have contracted hepatitis because saliva and blood droplets are often carried through the water and blood-borne illnesses spread quickly in unsanitary settings

Hepatitis B and C can present either acutely or chronically. Hepatitis D is a defective virus that requires hepatitis B to replicate and is only found with hepatitis B co-infection. In adults, hepatitis B infection is most commonly self-limiting, with less than 5% progressing to chronic state, and 20 to 30% of those chronically infected developing cirrhosis or liver cancer. Infection in infants and children frequently leads to chronic infection.

Unlike hepatitis B, most cases of hepatitis C lead to chronic infection. Hepatitis C is the second most common cause of cirrhosis in the US (second to alcoholic hepatitis). In the 1970s and 1980s, blood transfusions were a major factor in spreading hepatitis C virus. Since widespread screening of blood products for hepatitis C began in 1992, the risk of acquiring hepatitis C from a blood transfusion has decreased from approximately 10% in the 1970s to 1 in 2 million currently.

Parasitic hepatitis

Parasites can also infect the liver and activate the immune response, resulting in symptoms of acute hepatitis with increased serum IgE (though chronic hepatitis is possible with chronic infections).[35] Of the protozoans, Trypanosoma cruzi, Leishmania species, and the malaria-causing Plasmodium species all can cause liver inflammation. Another protozoan, Entamoeba histolytica, causes hepatitis with distinct liver abscesses.

Of the worms, the cestode Echinococcus granulosus, also known as the dog tapeworm, infects the liver and forms characteristic hepatic hydatid cysts. The liver flukes Fasciola hepatica and Clonorchis sinensis live in the bile ducts and cause progressive hepatitis and liver fibrosis.

Diagnosis

Diagnosis of hepatitis is made on the basis of some or all of the following: a person's signs and symptoms, medical history including sexual and substance use history, blood tests, imaging, and liver biopsy. In general, for viral hepatitis and other acute causes of hepatitis, the person's blood tests and clinical picture are sufficient for diagnosis. For other causes of hepatitis, especially chronic causes, blood tests may not be useful.[In this case, liver biopsy is the gold standard for establishing the diagnosis: histopathologic analysis is able to reveal the precise extent and pattern of inflammation and fibrosis. Biopsy is typically not the initial diagnostic test because it is invasive

and is associated with a small but significant risk of bleeding that is increased in people with liver injury and cirrhosis.

Blood testing includes liver enzymes, serology (i.e. for autoantibodies), nucleic acid testing (i.e. for hepatitis virus DNA/RNA), blood chemistry, and complete blood count. Characteristic patterns of liver enzyme abnormalities can point to certain causes or stages of hepatitis. Generally, AST and ALT are elevated in most cases of hepatitis regardless of whether the person shows any symptoms. The degree of elevation (i.e. levels in the hundreds vs. in the thousands), the predominance for AST vs. ALT elevation, and the ratio between AST and ALT are informative of the diagnosis.

Ultrasound, CT, and MRI can all identify steatosis (fatty changes) of the liver tissue and nodularity of the liver surface suggestive of cirrhosis. CT and especially MRI are able to provide a higher level of detail, allowing visualization and characterize such structures as vessels and tumors within the liver. Unlike steatosis and cirrhosis, no imaging test is able to detect liver inflammation (i.e. hepatitis) or fibrosis. Liver biopsy is the only definitive diagnostic test that is able to assess inflammation and fibrosis of the liver.

Prevention

Hepatitis A

The CDC recommends the hepatitis A vaccine for all children beginning at age one, as well as for those who have not been previously immunized and are at high risk for contracting the disease.[82][83]

For children 12 months of age or older, the vaccination is given as a shot into the muscle in two doses 6–18 months apart and should be started before the age 24 months.[94] The dosing is slightly different for adults depending on the type of the vaccine. If the vaccine is for hepatitis A only, two doses are given 6–18 months apart depending on the manufacturer.[84] If the vaccine is combined hepatitis A and hepatitis B, up to 4 doses may be required.[84]

Hepatitis B

WHO-UNICEF estimates of hepatitis B vaccine (HepB-BD) coverage in countries from the European WHO region in the years 2000–2015

The CDC recommends the routine vaccination of all children under the age of 19 with the hepatitis B vaccine. They also recommend it for those who desire it or are at high risk.

Routine vaccination for hepatitis B starts with the first dose administered as a shot into the muscle before the newborn is discharged from the hospital. An additional two doses should be administered before the child is 18 months.

For babies born to a mother with hepatitis B surface antigen positivity, the first dose is unique – in addition to the vaccine, the hepatitis immune globulin should also be administered, both within

12 hours of birth. These newborns should also be regularly tested for infection for at least the first year of life.

There is also a combination formulation that includes both hepatitis A and B vaccines.

Other

There are currently no vaccines available in the United States for hepatitis C or E. In 2015, a group in China published an article regarding the development of a vaccine for hepatitis E. As of March 2016, the United States government was in the process of recruiting participants for the phase IV trial of the hepatitis E vaccine.

Hepatitis A

Because hepatitis A is transmitted primarily through the oral-fecal route, the mainstay of prevention aside from vaccination is good hygiene, access to clean water and proper handling of sewage.

Hepatitis B and C

As hepatitis B and C are transmitted through blood and multiple bodily fluids, prevention is aimed at screening blood prior to transfusion, abstaining from the use of injection drugs, safe needle and sharps practices in healthcare settings, and safe sex practices.

Hepatitis D

The hepatitis D virus requires that a person first be infected with hepatitis B virus, so prevention efforts should focus on limiting the spread of hepatitis B. In people who have chronic hepatitis B infection and are at risk for superinfection with the hepatitis D virus, the preventive strategies are the same as for hepatitis B.

Hepatitis E

Hepatitis E is spread primarily through the oral-fecal route but may also be spread by blood and from mother to fetus. The mainstay of hepatitis E prevention is similar to that for hepatitis A (namely, good hygiene and clean water practices).

Treatment

Hepatitis A

Hepatitis A usually does not progress to a chronic state, and rarely requires hospitalization. Treatment is supportive and includes such measures as providing intravenous (IV) hydration and maintaining adequate nutrition.

Rarely, people with the hepatitis A virus can rapidly develop liver failure, termed fulminant hepatic failure, especially the elderly and those who had a pre-existing liver disease, especially hepatitis C. Mortality risk factors include greater age and chronic hepatitis C. In these cases, more aggressive supportive therapy and liver transplant may be necessary.

Hepatitis B

In healthy patients, 95–99% recover with no long-lasting effects, and antiviral treatment is not warranted.Age and comorbid conditions can result in a more prolonged and severe illness. Certain patients warrant hospitalization, especially those who present with clinical signs of ascites, peripheral edema, and hepatic encephalopathy, and laboratory signs of hypoglycemia, prolonged prothrombin time, low serum albumin, and very high serum bilirubin.

In more severe acute cases, patients have been successfully treated with antiviral therapy similar to that used in cases of chronic hepatitis B, with nucleoside analogues such as entecavir or tenofovir. As there is a dearth of clinical trial data and the drugs used to treat are prone to developing resistance, experts recommend reserving treatment for severe acute cases, not mild to moderate.

HIV-AIDS

Introduction

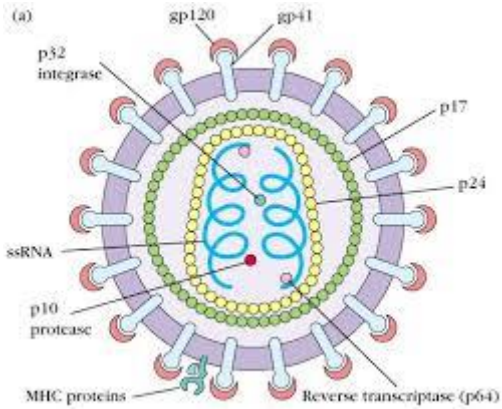
As the name AIDS implies it is a condition where there is a deficiency in the Body's Natural Defense mechanisms or the Immune System. It is acquired because it is not a hereditary or due to long-term use of some medicines such as those for the treatment of cancer, because of certain behavioral pattern. Syndrome is a group of symptoms. When one gets AIDS there can be wide range of symptoms all due to the bodies diminished ability to fight disease. It is important to remember that every one who has AIDS over a period of time depending upon their general health and natural defense mechanism of the body. AIDS is one of the Sexually Transmitted Disease, worldwide distributionand epidemic disease.

It was first described in USA in 1981. The disease appears to have begun in Central Africa early as the 1950s. Montagnier and his colleagues first reported isolation of an etiological agent in 1983 from the Pasteur Institute, Paris. Prevalence Rate of AIDS WHO estimates that 8-10 million adults & I million children worldly are infected with AIDS virus. By the year 2010 – 2025, 2 crores and 5 lakhs infected with the virus. On the basis of report 73.3% of AIDS is acquired through by Sexual contact, 7.4% AIDS acquired through Transfusion process. 0.7% through Homosexual contact, 9% through Intravenous drug abusers. Upto 1995 about 295,473 deaths have been reported in US. Mortality rate from AIDS is extremely high. The case fatality rate averages about 92% for those adults diagnosed with AIDS before 1987. By the year 2010 AIDS will become the major killer of children with an estimated 250 million infections worldwide.

The first AIDS case in India was reported in 1986 from Chennai. Since there has been rapid spread of HIV infection all over the country. By March 1998, the National AIDS Control Organization had reported that a total of 71400 people were having HIV infection from among 3.2 million people who were tested for it. Of these about 10% of AIDS cases are available only in India. About 80% were males & 20% females. Almost 89% people with AIDS were in the age group of 15-44 years, which are the most economically productive year for any individual.

Causative Agent

Primarily the HIV-1 virus causes AIDS. This virus is a Retrovirus and closely related to Human T-cell Leukemia virus. Sometimes HIV-2 virus also causes it. HIV is an enveloped virus with cylindrical core inside. The core contains two copies of ssRNA and several enzymes. Ten virus specific proteins have been discovered. One of them the gp 120 envelope protein, participates in attachment to CD4+ cells. Virus is spherical in shape about 90-120nm in size the name HIV was given by International Committee on Virus Nomenclature in 1986.

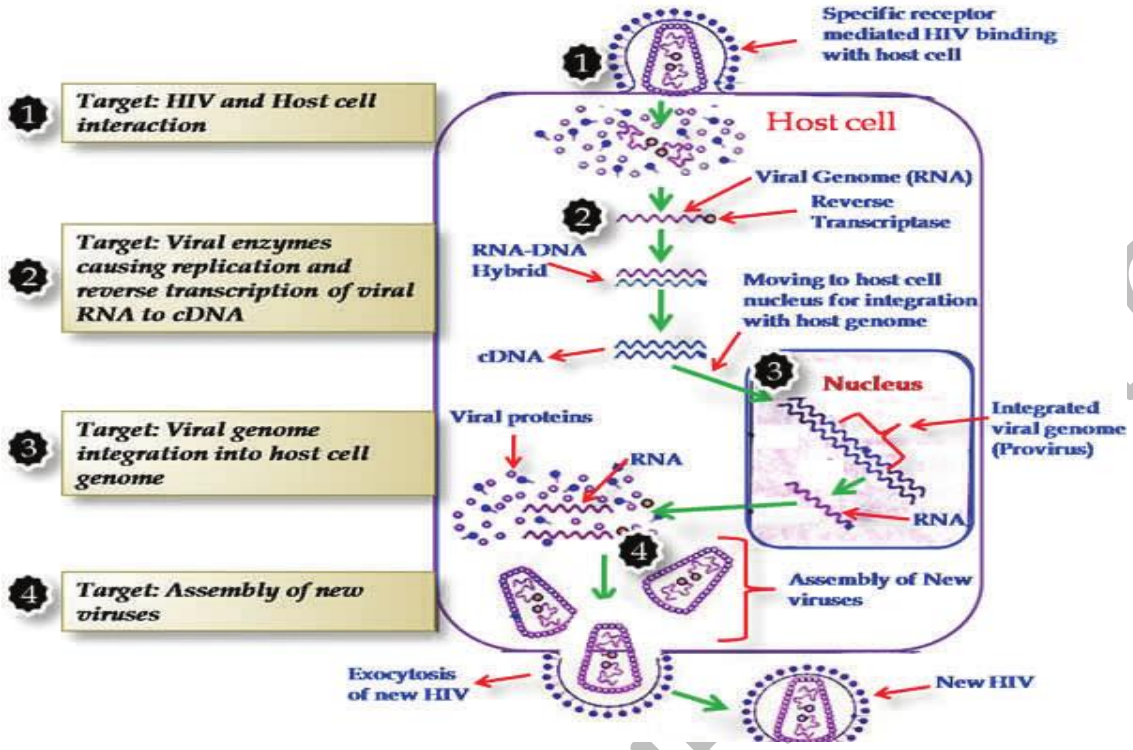


HIV 1 virion is a pleomorphic structure containing 72 external spikes. The two major viral envelope proteins, gp 120 & gp41 form these spikes. The core of HIV 1 contains 4 nucleocapsid proteins. The phosphorylated p25 polypeptide forms the chief component of the inner shelf of the nucleocapsid, where as the p17 contains 2 copies of single stranded RNA that is associated with the various preformed virus enzymes, including Reverse Transcriptase, Integrase, Ribonuclease and protease.

HIV is a thermolabile, being inactivated in 10 minutes at 50°C & in seconds at 100°C. At room temperature, in dried blood it may survive for upto 7 days. HIV is inactivated within 10 minutes by the treatment with 50% Ethanol, 3.5% Isopropanol, .5% Lysol, 0.5% Paraformaldehyde, 0.3% H2O2, 10% Household bleach. For treatment of contaminated medical instruments 2% solution of glutaraldehyde is useful.

Transmission

HIV is believed to have originated in Central Africa. From here it is spread to the rest of the world. HIV is primarily transmitted by Sexual contact (Homo and Hetero), Direct exposure of a person's bloodstream to body fluids, Mother to child through placenta, Skin erosion, Intravenous drug abuse, Transfusion process, Invasive medical procedure, Drug abuse with needle sharing and New born can infected through Breast breeding.



Pathogenesis

Once virus enters inside of the body the virus gp 120- envelope protein binds to the CD4 glycoprotein plasma membrane receptor on CD4+ T cell macrophages, dendrite cell, monocytes. After the envelope has fused with the plasma membrane, the virus releases its core protein and 2 RNA strand in the cytoplasm. Inside the infected cell, the core protein remains associated with the RNA as it is copied into single stranded DNA by the RNA/DNA dependent DNA polymerase activity of the Reverse Transcriptase enzyme,

Ribonuclease H and the DNA is duplicated to from a dsDNA copy of the original RNA genome. The viral dsDNA is then translocated to the nucleus and integrated into the host chromosomal DNA by the viral Integrase enzyme. This integrated viral DNA and chromosomal DNA is called Provirus. Then transcriptional factors stimulate transcription of proviral DNA into genomic ssRNA and after processing several mRNAs are formed. Then viral RNA is exported to cytoplasm. After completion of this process, host cell enzymes catalyses the synthesis of viral protein. HIV ssRNA and proteins assemble beneath the host cell membrane, into which gp41 and gp 120 are inserted. The cell enlarges and form bud. Bud forms a new virus. Eventually host cell lyses.

Laboratory diagosis

HIV infection can be detected by,

a) Specific tests

- Virus isolation
- Detection of HIV specific antibodies (ELISA)
- Western blot
- Polymerase chain reaction

b) Non specific test

- Total and Differential WBC count
- Assay of T cell Platelet count
- Estimation of IgG and IgA level
- Skin test for CMI

Prevention

- Avoid sexual contact with HIV infected individuals.
- Don't share shaving materials.
- Avoid drug abuse.
- Follow Tamil culture.
- Screen blood before transfusion.
- Use condoms during sexual contact.

Treatment

Everyone diagnosed with HIV should take antiretroviral therapy medicines, also called ART. This is true no matter what stage the disease is in or what the complications are.

ART is usually a mix of two or more medicines from several classes. This approach has the best chance of lowering the amount of HIV in the blood. There are many ART options that mix more than one HIV medicine into a single pill, taken once daily.

Each class of medicines blocks the virus in different ways. Treatment involves mixing medicines from different classes to:

- Account for medicine resistance, called viral genotype.
- Keep from creating new medicine-resistant strains of HIV.
- Suppress the virus in the blood as much as possible.

Two medicines from one class, plus a third medicine from another class, are most often used.

The classes of anti-HIV medicines include the following:

- **Non-nucleoside reverse transcriptase inhibitors (NNRTIs)** turn off a protein needed by HIV to make copies of itself.

  Examples include efavirenz, rilpivirine (Edurant) and doravirine (Pifeltro).

- **Nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs)** are faulty versions of the building blocks that HIV needs to make copies of itself.

  Examples include abacavir (Ziagen), tenofovir disoproxil fumarate (Viread), emtricitabine (Emtriva), lamivudine (Epivir) and zidovudine (Retrovir). Retrovir is no longer suggested for routine use in the U.S. because of high rates of toxic effects.

  Mixes of medicines also are available, such as emtricitabine-tenofovir disoproxil fumarate (Truvada) and emtricitabine-tenofovir alafenamide fumarate (Descovy).

- **Protease inhibitors (PIs)** make HIV protease inactive. HIV protease is another protein that HIV needs to make copies of itself.

  Examples include atazanavir (Reyataz), darunavir (Prezista) and lopinavir-ritonavir (Kaletra).

- **Integrase inhibitors** stop the action of a protein called integrase. HIV uses integrase to put its genetic material into CD4 T cells.

  Examples include bictegravir sodium-emtricitabine-tenofovir alafenamide fumarate (Biktarvy), raltegravir (Isentress), dolutegravir (Tivicay) and cabotegravir (Vocabria).

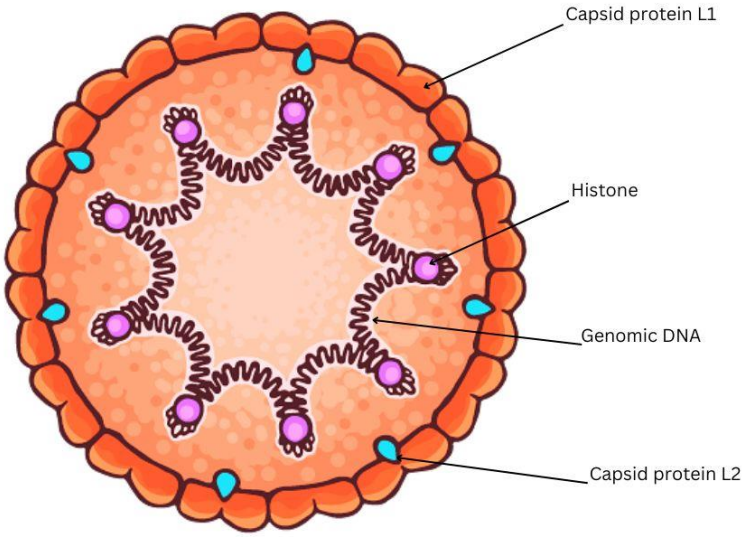
- **Entry or fusion inhibitors** block HIV's entry into CD4 T cells.

  Examples include enfuvirtide (Fuzeon) and maraviroc (Selzentry). Newer medicines include ibalizumab-uiyk (Trogarzo) and fostemsavir (Rukobia).

Human papillomavirus

Human papillomavirus (HPV) causes wart in human. According to CDC, it is a most common sexually transmitted infection. It is passed between people through skin-to-skin contact. There are over 100 varieties of HPV. This virus can affect your genitals, mouth or throat. Some cases of genital HPV infection may not cause any health problems. However, some types of HPV can lead to the development of genital warts and even cancers of the cervix, anus and throat.

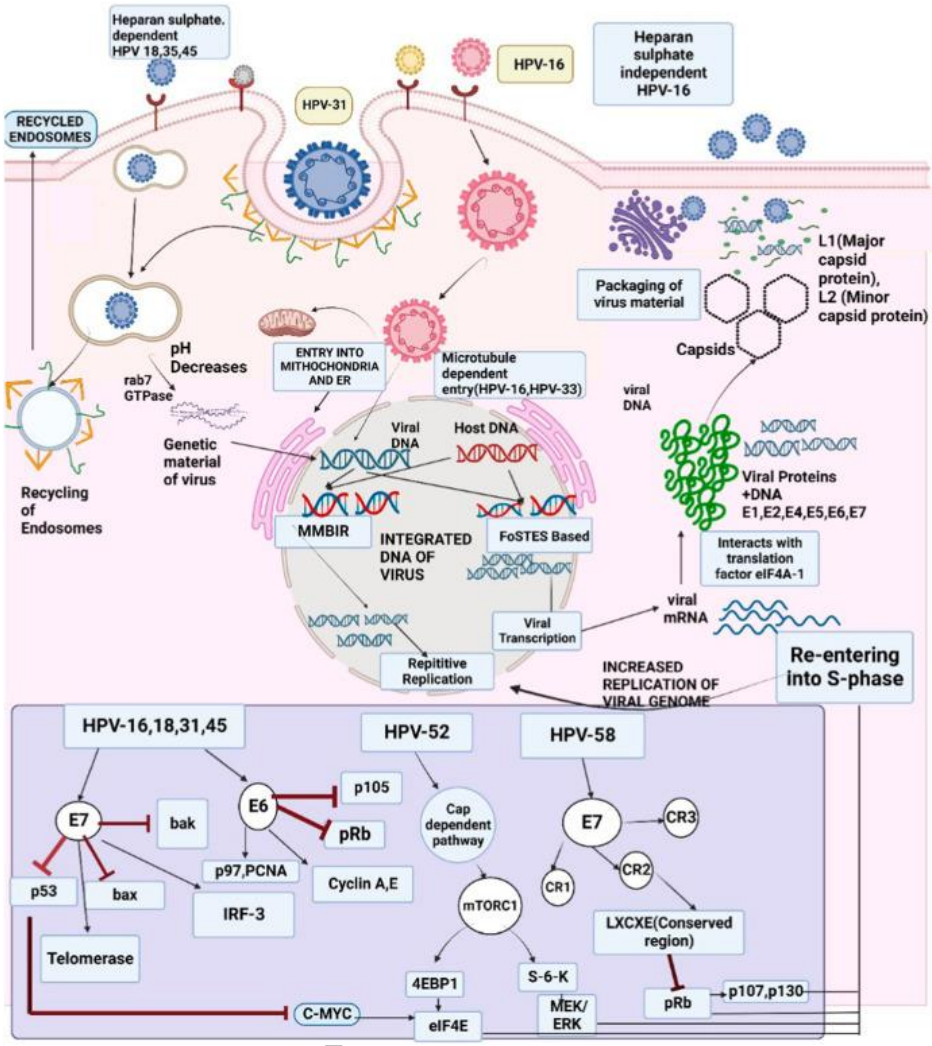
Causative agent



HPV is a small naked, icosahedral virus with double stranded DNA as genome. It belongs to the family papova viridae. It is a non enveloped virus with 55nm diameter. It has 72 capsomeres. Papilloma viruses are a family called Papillomaviridae, which consists of a total of 170 members known as human papillomaviruses. The members are grouped into 16 genera, which are named with a Greek letter as prefix and the ending papillomavirus. For example: Alphapapillomavirus, Betapapillomavirus, etc. From a clinical point of view, the human papillomaviruses that infect the mucosa of the genital tract (which are located in genus Alphapapillomavirus) have been divided into two groups: low-risk, mainly associated with benign genital wartsand highrisk, which have a high oncogenic potential and are the causative agents of cervical cancer. All papillomaviruses associated with cancer are located in the genus Alpha papilloma virus.

Viral genome and proteins

Molecular weight of the DNA is 5.2 million Daltons. The papillomavirus genome is between 6800 and 8400 base pairs (bp) and is associated with host histone proteins like H2A, H2B, H3 and H4. The virus contains a non-coding regulatory region, which is called the long control region (LCR) and a region containing late expression genes, giving rise to two structural proteins. In total there are 9 or 10 open reading frames. The LCR contains response elements for cellular transcription factors such as AP1, SP1, OCT1, etc., as well as viral proteins E1 and E2, which control replication and expression of the viral genome. Reading frames are grouped into two sets called early expression genes (E) and late expression genes (L). In the first group are E1, E2, E4, E5, E6and E7, while in the second are L1 and L2. Two additional reading frames can be identified in some papillomavirus, which are designated E3 and E8.



Multiplication and Pathogenesis

Papillomaviruses infects only squamous epithelial cells of skin and mucous membrane. Virus multiplied in the basal layer of the skin. The replicative cycle of papillomaviruses is commonly divided into two stages. They are early and late. The establishment of the virus in tissue requires infection of the basal keratinocytes. The introduction of virions into the cell is initiated by the interaction of L1 protein with heparan sulfate and syndecan 3 on the cellular surface. Most papillomaviruses appear to enter the cell by clathrin-dependent receptor-mediated endocytosis.

The stripping of the virion and the output of the viral genome occur in the endosome. Subsequently, the L2 protein and the genome migrate to the nucleus. Once inside the nucleus, the genome is transcribed in a series of complex processes involving the presence of multiple promoters, different mRNA modification patterns (splicing)and a differentiated production of these between different cells. E1 and E2 are the first proteins to be expressed, which generates a control in the number of copies of the episomal viral genome. These proteins are maintained at

20 to 100 copies per cell. In the suprabasal layer, expression of E1, E2, E5, E6and E7 genes contributes to the maintenance of the viral genome and induces cell proliferation, increasing the number of cells able to be infected, which results in increased viral production.

Introduction

Rabies is acute, fulminant, fatal encephalitis. It is a madman disease that has instilled terror in human society. The reason is that, with rare exceptions, all of the people who are bitten by a rabid animal, got rabies. Rabies is an important zoonotic infection in which man is dead end of the infection and hence doesn't play any role in its spread to new host. In most of the developing countries, dogs are the principal reservoir of rabies (canine rabies) where as sylvatic rabies involving animal such as foxes, raccoons, cats, fats and coyotes. Rabies has been recognized from very ancient times. The word Rabies derived from the Latin word Rabidus, which means mad. It is an epidemic disease. 8.11.2. Causative Agent Rabies virus caused rabies infection. It belongs to the family Rhabdovirus and genus Lyssa virus. In Greek Lyssa means Rabies. Rabies virus is bullet shaped Size is about 180 x 75 nm. Genome is negative sense single stranded RNA. It is nucleo capsid in nature Two layers, matrix layer and outer envelope cover the genome. Matrix is made up of M protein. Outer 133 Self-Instructional Material NOTES Viral Infections envelope is made up of lipid bilayer as like plasma membrane. External envelope having spike like projections. It is made up of glycoproteins. Spikes are responsible for pathogenic property of the virus. RNA dependent RNA polymerase is responsible for genome replication. L and P proteins control its activity. Rabies viruses of man and animals all over the world appears to be of a single antigenic type. Antigens of Rabies viruses are G protein, M protein, N protein, Hemaglutinin Chemical compositions of the viruses are 4% RNA, 67% protein, 26% lipid and 3% carbohydrate Susceptibility of virus towards physical and chemical agents Rabies virus is highly resistant against cold, dryness and decay. The virus is highly thermolabile with a half-life of approximately 4 hours at 40°C and 35 seconds at 60°C. The virus cannot withstand pH less than 4 or more than 10. It is also susceptible to oxidizing agents, most organic solvents, surface acting agents and quaternary ammonium compounds. Proteolytic enzymes, ultraviolet rays and X-rays rapidly inactivate the Rabies virus. Soaps and detergents are effective against the Rabies virus because of their lipid eliminating property, which destroys the outer covering of the virus.

Important feature usually associated with the high-risk virus is that the viral genome is integrated into the genome of the cell, while in the low-risk virus the genome remains episomal. This integration process has been associated with the move from a high-grade lesion to invasive cancer. HPV infected cells have large perinuclear vacuoles surrounded by dense cytoplasm and form a special structure called Koilocytosis. It usually takes 3-4 months for the development of benign outgrowth of cells in to warts.

Many warts are resolved spontaneously and are benign. Certain types of papilloma are associated with dysplasia that may become cancerous. HPV causes different types of warts depends on the type of virus, location of infection and effectiveness of host immune response.

Symptoms

Many men that are infected with HPV have no symptoms, although some may develop genital warts. Some women may notice that they have genital warts, which can appear inside the vagina, in or around the anusand on the cervix or vulva. Skin lesions are plantar wart, common wart, flat wart, and epidermodysplasia verrucafermis. Mucous lesions are laryngeal papilloma, oral papilloma, conjuctival papilloma, carcinoma, condyloma, high grade dysplasia and focal epithelial hyperplasia. HPV can also cause cervical cancer and other cancers of the genitals, head, and neckand throat. Some strains of HPV can cause penile, analand throat cancer in men.

Lab Diagnosis

HPV does not grown in conventional tissue culture methods. Histological appearance of hyperplasia of pricle cells and excess production of keratin confirms HPV infection. Testing for HPV is different in men and women. PCR amplification tests confirm HPV in both men and women. Regular Pap tests help to identify abnormal cells in women. This can signal cervical cancer or other HPV-related problems.

Treatments

Most cases of HPV go away spontaneously on their own, so there's no treatment required. Genital warts can be treated with antiviral drugs, burning with an electrical current or freezing with liquid nitrogen.

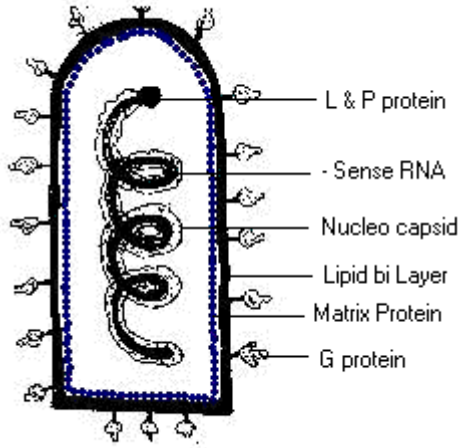
Prevention

The easiest ways to prevent HPV are to use "condoms and to practice safe sex. The Gardasil 9 vaccine is available for the prevention of genital warts and cancers caused by HPV. The vaccine can protect against nine types of HPV. The CDC recommends the HPV vaccine for boys and girls ages 11 or 12.

Rabies

Rabies is acute, fulminant, fatal encephalitis. It is a madman disease that has instilled terror in human society. The reason is that, with rare exceptions, all of the people who are bitten by a rabid animal, got rabies. Rabies is an important zoonotic infection in which man is dead end of the infection and hence doesn't play any role in its spread to new host. In most of the developing countries, dogs are the principal reservoir of rabies (canine rabies) where as sylvatic rabies involving animal such as foxes, raccoons, cats, fats and coyotes. Rabies has been recognized from very ancient times. The word Rabies derived from the Latin word Rabidus, which means mad. It is an epidemic disease.

Structure of Rabies Virus -bullet shape



Causative Agent

Rabies virus caused rabies infection. It belongs to the family Rhabdovirus and genus Lyssa virus. In Greek Lyssa means Rabies. Rabies virus is bullet shaped

Size is about 180 x 75 nm. Genome is negative sense single stranded RNA. It is nucleo capsid in nature

Two layers, matrix layer and outer envelope cover the genome. Matrix is made up of M protein. Outer envelope is made up of lipid bilayer as like plasma membrane. External envelope having spike like projections. It is made up of glycoproteins. Spikes are responsible for pathogenic property of the virus. RNA dependent RNA polymerase is responsible for genome replication. L and P proteins control its activity. Rabies viruses of man and animals all over the world appears to be of a single antigenic type.

Antigens of Rabies viruses are G protein, M protein, N protein, Hemaglutinin

Chemical compositions of the viruses are 4% RNA, 67% protein, 26% lipid and 3% carbohydrate

Susceptibility of virus towards physical and chemical agents

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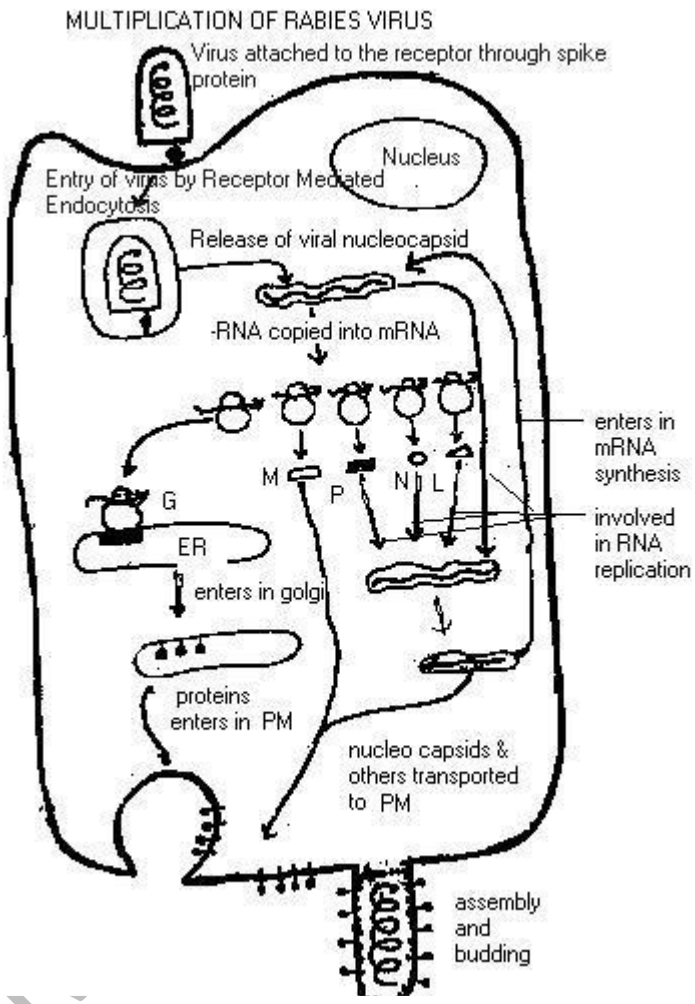
Symptoms

Incubation period is about 30-60 days. Symptoms are Hydrophobia, Lock jaw, Encephalitis, Hysteria, Acute polyneuritis, Polio myelitis, Fever, Vomiting, Excessive salivation, Paralysis of lower jaw, Restlessness, Convolescence, Paralysis leads to death .



Multiplication

Virus binds to the cellular receptor and enters the cell via receptor mediated endocytosis.

The viral membrane fuses with the membrane of the vesicle releasing viral nucleocapsid.

This structure comprises negative sense RNA coated with nucleocapsid and a small number of L and P proteins, which catalyze RNA replication Negative sense RNA is copied into 5 subgenomic mRNA by L and P proteins.

The N,P,M and L mRNAs are translated by free cytoplasmic mRNA

G mRNA is translated by ribosome's bound to the endoplasmic reticulum.

Newly synthesized P, N and L proteins involved in RNA replication. This process begins with positive sense RNA synthesis.

Positive sense RNA of the host serves as the template.

Some of the negative sense RNA enters to viral protein synthesis

G mRNA transcribes and synthesis glycoproteins.

G proteins travel to the plasma membrane.

Progeny nucleocapsid and M proteins are transported to the adjacent area of plasma membrane.

Assembly takes place and new viruses are released through budding process and infect new cells.

Pathogenesis

The virus present in the saliva of the Rabid animal is deposited in the biting site. The virus attaches cells via spikes to nicotinic acetylcholine receptors of tissue cells. The virus appears to multiply in the muscles, connective tissues or nerves at the site of deposition. It penetrates the nerve endings either immediately or after a varying interval and travels in the axoplasm towards the spinal cord and brain. The movement of the virus in the axons is passive at a speed of about 3mm per hour. The virus then multiplies extensively in brain tissue, causing the symptoms of encephalitis. Characteristic inclusion bodies called negri bodies, form at the site of viral replication in the brain but the cells are not lysed.

The virus spreads outward from the brain via the nerves to various body tissues, notably the salivary glands, eye and fatty tissue under the skin as well as heart and other vital organs.

The immune response of the host probably plays an important role in pathogenesis since viral antigens are expressed on the surface of the infected cells.

Labdiagnosis

Specimen - Saliva / sputum, Skin biopsy, Hair follicle, Cerebrospinal fluid, Blood, Corneal swab, Urine, Brain

Laboratory tests

Negri body examination, Fluorescent antibody test, Mouse inoculation, Serum virus neutralization test, Complement fixation test, Counter immuno electrophoresis test, Enzyme Linked Immuno Sorbent Assay, Immuno peroxidase test, Hemagglutination test, Hemagglutination inhibition test, Passive hemagglutination test, Passive diffusion, Electron microscopy, Tissue culture techniques, Polymerase Chain Reaction.

Negri body examination

Sellers staining procedure is followed. It is an Intracytoplasmic inclusion bodies. Cut and open the brain. Expose hippocampus region. Cut small piece and place it on a filter paper with cut surface

facing upwards. Place the filter paper on a glass slide. Lightly sponge cut surface with the edge of filter paper to remove blood. Press clean microscopic slide on the tissue piece. While the smears are wet, flood the smear with working stain. Stain for 2-3 seconds. Wash with water. Air dry and Examine under 100 X.

Observation

Nerve cells: Blue cytoplasm and dark blue nucleus

Stroma: Pink

Erythrocytes: Copper colored

Negri bodies: Magenta to dark red with dark blue or black inner granules.

Freshly isolated strains of Rabies virus are called street virus.

Prevention and Treatment

This section was categorized into three, all three carry equal importance and one should not be given undue importance, or utter neglect, at the cost of other two components. These components are Management of wound, Post exposure immunization and Pre exposure immunization.

Management of wounds - Since the Rabies virus enters the human body through a bite or scratch, it is imperative to remove as much as saliva with soap. After the removal of soap, any quaternary ammonium compound (1- % cetrimonium bromide) may be applied as antiseptic along with antirabies serum.

Post exposure immunization -

Because of long incubation period of rabies it is possible to institute prophylactic post exposure immunization. Immunization must be started at the earliest to ensure that the individual will be protected before the rabies virus invades central nervous system.

Two types of agents are employed to confer immunity to an individual who has been exposed to the Rabies virus: antirabies serum/ rabies immunoglobulin and anti rabies vaccine. Antirabies serum provides passive immunity in the form of readymade antirabies antibody to tide over the initial phase of infection.

Antirabies vaccines available in India

Semples Sheep Brain Vaccine

Human Diploid Cell Vaccine (HDCV)

Primary Chick Embryo Cell Vaccine (PCECV)

Purified Vero Cell Rabies Vaccine (PVRV)

Site of vaccination - The ideal site for vaccination is the anterior abdominal wall, this area offers enough space to accommodate 10 injections at 10 different sites and cause least discomfort to the patient.

Dose schedule - The 6-dose schedule spread over a period of three months was recommended. It is also called Essen schedule (proposed by international conference on Rabies, held at Essen, Germany). Days of vaccination is 1st, 3rd, 7th, 14th, 30th and 90th days.

Pre exposure immunization

There is no vaccines for mass pre exposure vaccination. The pre exposure prophylaxis, hence, is recommended for definite group of individuals who because of their profession or hobby are at higher risk of getting exposed to rabies virus.

Control of Rabies

Any strategy for control of rabies in developing countries shall have following components. They are Epidemiological surveillance, Mass vaccination, Dog population management, Community participation.

INFLUENZA

Introduction

It is a viral infection of the respiratory tract cause fever, headache, muscle ache and weakness. Orthomyxo virus family member Influenza virus causes this disease. The name myxo virus was proposed originally for a group of enveloped RNA viruses characterized by their ability to absorb into mucoprotien receptors on erythrocytes, causing hemagglutination.

History

Influenza is an acute infectious disease of the respiratory tract, which occurs in sporadic, epidemic and pandemic form. Italians gave the name influenza during the year 1358. In 1933, Smith isolated the causative agent (Influenza A). Burner (1935) developed chick embryo technique for propagation of virus. Francis and Magill (1940) isolated a serotype of the influenza and named influenza B. Taylor (1949) isolated the third serotype of influenza virus, type C.

Causative Agent

Influenza Virus causes Influenza. It belongs to Orthomyxo virus family.

It consist of three species, they are A, B and C. Type A usually responsible for the large outbreaks and is a constantly changing virus. New strains of type A virus develop regularly and results in a new epidemic every few years.

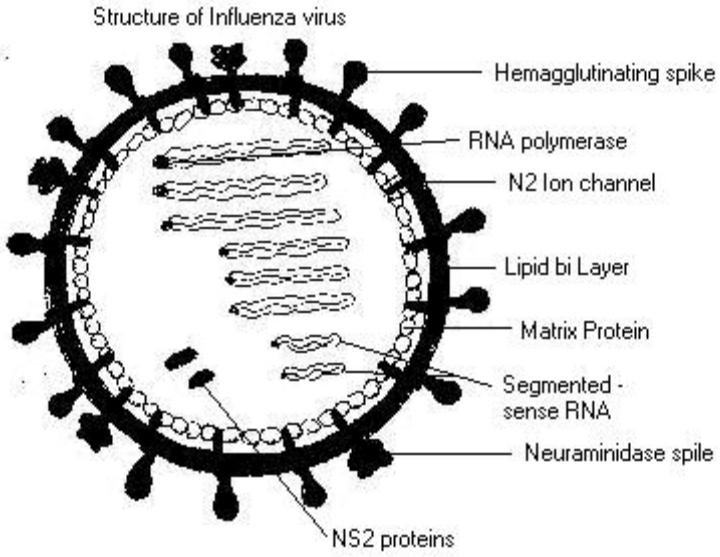
Type B and C is fairly stable virus.

Type B causes smaller out breaks. Type C usually cause mild illness like common cold. Influenza virus are spherical and 80-120nm in diameter. Antisense RNA genome occurs in 8 separate

segments containing 10 genes. The segments are complexed with nucleoprotein to form nucleocapsid with helical symmetry. Nucleocapsid is enclosed in an envelope consisting of a lipid bilayer and two surface glycoproteins, a hemagglutinin and neuraminidase. Readily inactivated by non-polar solvents and by surface-active agents. Influenza C virus having 7 segments of RNA and only one surface protein. The virus inactivated by heating at 50°C for 30 minutes. It remains viable at 0-4° for about a week. Infectivity lost rapidly at 20°C. Preserved at –70° or by freeze - drying. Membrane protein is known as matrix protein or M protein. M2 protein projects through the envelope to form ion channel. It is responsible for gene transfer. H gene is responsible for hemagglutinin spike and N gene responsible for Neuraminidase. Spikes measures about 10mm in length and molecular weight of 225000 Dalton.

Gene responsible for viral protein

10 genes from 8 segments of antisense RNA responsible for synthesis.



Symptoms

Fever	Malaise
Cough with or without mucous	Stuffy and congested nose
Nasal discharge	Sore throat
Headache	Clammy skin
Muscle ache and stiffness	Nosebleed
Shortness of breath	Vomiting
Chillness	Joint Stiffness
Sweating	Elbow pain
Fatigue	Loss of appetite
Abnormal taste	

Pathogenesis

Influenza virus is transmitted from person to person primarily in droplets released by sneezing and coughing. It is highly contagious disease. The incubation period for influenza is 1-4 days. Alveoli is the primary target for virus. Infected cells will sloughs, allowing extravagation of fluid and secondary submucosal inflammation. During initial stage it liquefies mucous and attaches other cells. The infection in mucosal cell results in cellular destruction and desquamation of the superficial mucosa. The resulting odema and mononuclear cell infiltration of the involved areas are accompanied by symptoms like cough, sore throat and nasal discharge. Most of the symptoms are because of interferons. Current evidence indicates that the extent of virus induced cellular destruction is the prime factor determining the occurrence. In an uncomplicated case, virus can be recovered from respiratory secretions for 3-8 days. The disease may extensively involve the alveoli, resulting in interstitial pneumonia, sometimes with marked accumulation of lung hemorrhage and odema.

Multiplication

Virus replication takes about 6 hours and kills the host cell. The virus enters permissive cells via the hemaggultinin subunit, which binds to cell membrane glycolipids or glycoprotein contain-ing sialic acid or N-acetylneuraminic acid, the receptor for virus adsorption. The virus is then engulfed by pinocytosis into endosomes. The acid environment of the endosome, uncoating the nucleocapsid and releasing it into the cytoplasm. A transmembrane protein derived from the matrix gene forms an ion channel for protons to enter the virion and destabilize protein binding allowing the nucleocapsid to be transported to the nucleus, where the genome is transcibed by viral enzymes to viral mRNA. Unlike replication of other RNA viruses, orthomyxo virus replication depends on the presence of active host cell DNA. The synthesized viral mRNA are transported to the cytoplasm, where it translated by host ribosome.

mRNA's specifying viral membrane proteins (HA,NA,M) are translated by ribosome bound to endoplasmic reticulum and they undergoes glycosylation. The nucleocapsid is assembled in the nucleus. After the attachment of M1 protein to newly synthesized RNA, viral RNA synthesis is stopped and nucleocapsids are transported out. HA and NA proteins are transported to the cell surface and are incorporated into the plasma membrane. Virion nucleocapsids along with NS2 associate with regions of plasma membrane containing HA and NA proteins. After acquiring envelop and undergo maturation as they bud through the host cell membrane.

Epidemiology

Influenza viruses are classified as types A,B,C on the basis of antigenicity of their nucleoproteins and matrix protein. Rainy season is a peak time for influenza. Influenza epidemic is of two types. Both type A and type B viruses cause yearly epidemics. Type A caused influenza pandemics. Two different mechanisms of antigenic change are responsible for producing the strains that cause these two types of epidemic. Some of the Influenza strains were transmitted from animal to humans. Mostly it is transmitted through person-to-person contact and also droplet spread.

Laboratory Diagnosis

The most commonly employed method for laboratory diagnosis is recovery of the virus from specimens containing respiratory secretions, such as nasal wash and throat swab or sputum.

Methods Include Isolation of virus, Cold agglutination, Influenza complement fixation, Immunoflourescent technique

Isolation of Virus

Respiratory secretions are treated with antibiotics and inoculated into amniotic cavity of 10-11 day old egg or monkey kidney cells. Incubate at 35°C for 3 days. Then eggs were chilled and harvest amniotic fluid. The presence of viral antigens are demonstrated by using hemadsorption test at 4°C. Type B Agglutinate both Guinea and Fowl cells. Type C agglutinate only fowl cells.

ELISA also useful for demonstration of antigens. Serological examination is by specific antigen and antibody reaction.

Prevention

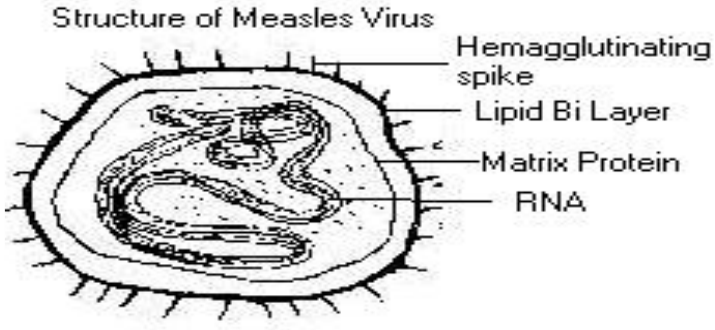
Inactivated influenza virus vaccines have been used for old age people. The virus for the vaccine are grown in chick embryo, inactivated by formalin, purified to some extent and adjusted to a dosage known to elicit an antibody response in most individuals.

Treatment

The synthetic drugs Amantadine and Rimantadine hydrochloride effectively used to prevent infection and illness caused by type A and but not by type B viruses. The drugs interfere with virus uncoating and transport by blocking the trans membrane M2 ion channel. Drugs prevent about 50-67% of infection. Drug resistance also occurs.

MEASLES

Measles is a highly contagious skin disease that is epidemic throughout the world. Measles virus, a member of the genus Morbili virus and the family Paramyxoviridae. Thomas Sydenham in 1690 gave the first clear and accurate description about Measles. In 1846 an outbreak of Measles occurred in remote areas of Islands. Gold Berger and Anderson established the viral etiology of Measles in 1911 by transmitting the disease to Monkey through the inoculation of filtrates of blood and nasopharyngeal secretions.



Causative agent

Measles is caused by a pleomorphic, medium sized (120-200 nm in dia) virus. It comes under Paramyxoviridae family. Its genome is RNA. It is an enveloped virus. It has two biologically active projections one 'H' is responsible for viral attachment to host cells and causes hemagglutinations. The outer 'M' is responsible for fusion of the viral outer membrane with the host cell. The M antigen also responsible for producing multi nucleated giant cells. It has tightly coiled nucleic acid surrounded by the lipo protein envelope. The virus grows well on human or monkey kidney and human amnion culture, which are the preferred cells for primary isolation. The other name for multinucleated giant cells is war thin-finkeldey cells. The virus is heat labile and readily inactivated by heat. UV rays, ether and formaldehyde.

Symptoms

Incubation period is about 10-12 days. It begins with fever, runny nose, cough and swollen weepy eyes. Within a few days, a fine red rash appears on the fore head and spread outward over the rest of the body. Unless complications occur, symptoms disappear within one week.

Unfortunately many cases are complicated by secondary infections caused by bacterial pathogens, mainly Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes and Haemophilus influenzae. Very rarely, Measles reactivation is observed after two to ten years and forms a disease called Subacute Sclerosing Pan Encephalitis (SSPE), which is marked by slow progressive degeneration of the brain, resulting in death within two years. Measles occurs during pregnancy results in an increased risk of miscarriage, premature labor and low birth weight. Protracted diarrhoea is often seen as a complication in children in poor nations.

Pathogenesis

The respiratory route and conjunctiva acquire Rubeola virus. It primarily replicates in the upper respiratory epithelium then spreads to lymphoid tissues and following further replication eventually spreads throughout the body. Mucous membrane involvement is responsible for an important diagnostic sign Koplicks spot (small bluish white ulceration on the buccal mucosa). Damage to the respiratory mucous membrane partly explains the markedly increased susceptibility of Measles patients to secondary bacterial infections, especially infection of the middle ear and lung.

The skin rash of measles results from the cytopathic effect of Rubeola virus replication in skin vascular endothelial cells and cellular immune response against the viral antigen in the skin. It is not known why the rash characteristically outward, often clearing on the face before it reaches the lower parts. The measles virus temporally suppress the cellular immunity, which can cause reactivation of Herpes Simplex Virus

Laboratory Diagnosis

Primary diagnosis is with the help of Kopliks spot formation.

Sample - Throat/Nasopharyngeal swab, Urine, Whole blood

Turn around time: 14 days

Cytologic Diagnosis- Specimens should be fixed with formalin and stained with Hematoxylin and Eosin. Characteristic giant cells containing eosinophilic intranuclear and intracytoplasmic inclusions are observed for the first 2 or 3 days of the specimen.

Antigen Detection- It is with Immunofluorescence technique, immuno enzyme staining increase the sensitivity of the test.

Virus Isolation - Isolated by cell culture technique from respiratory secretions and other samples. Primary cultures of human embryonic kidney cell and monkey kidney cells are more sensitive for viral isolation.

Nucleic Acid Detection - This technique was done in immunocompromised patients who maynot be capable of antibody response. Viral nucleic acid is detected by using reverse transcriptase, PCR insitu hybridization or reverse transcriptase, PCR and Amplification of RNA extracted from specimens.

Epidemiology

Humans are the only natural host for Rubeolla virus. It is eradicated from US but occasionally epidemics were observed.

Control

Children too young to be vaccinated. MMR vaccine is used.

Preschool children also vaccinated.

MUMPS

Introduction

It results from an acute viral infection. Target of Mumps is parotid gland, is located just below and in front of the ear. Mumps means mumble. Mumps begins with painful swelling of one or both parotid gland.

Causative Agent

Mumps virus causes Mumps. It is an enveloped virus included under the family Paramyxoviridae. It is a ssRNA containing negative sense virus. It is a helical shaped virus. Viral etiology was demonstrated by Johnson and Goodpastuer in 1934. Hebel cultivated it in embroyonated eggs. In 1955, Henle and Deinhardt grew it in tissue culture. Virus posses hemagglutinin, neuraminidase and fusion protein. It is a heat labile and chemically sensitive virus.

Symptoms

Incubation period – 16 to 18 days. Parotid swelling with pain is the first sign. Fever, extreme pain during swallowing.

Pathogenesis

Virus is transmitted in saliva and respiratory secretionsand its portal of entry is respiratory tract. This virus multiplies in the respiratory tract and local lymphnode in the neck. Virus spreads throughout the body by the blood stream and produces symptoms only after infecting other tissues such as the parotid glands, meninges. In the salivary gland, the virus multiplies in the epithelium of ducts that convey saliva to the mouth. This destroys the epithelium. The body inflammatory response to the infection is responsible for the severe swelling and pain. In adults it infect tubule and cause death of testicular tissue. The immune system of the host eliminates the infection. Response to the infection is responsible for the severe swelling and pain. In adults it infect tubule and cause death of testicular tissue. The immune system of the host eliminates the infection.

Epidemiology

Humans are the only natural host of Mumps and natural infection confers life long immunity.

Lab Diagnosis

Generally serological diagnosis is not necessary. This virus can be identified with hemagglutination inhibition test. Embryonated eggs and cell culture techniques are used for culturing.

Control

An effective vaccine is a variable and is often administered as part of the trivalent Measles, Mumps and Rubella (MMR) vaccine. It provides protection for at least 10 years.

CHICKEN POX

Introduction

Chicken pox is a common childhood infection. It is a mild, highly contagious disease, chiefly occurs in children, characterized by a generalized vesicular eruption of the skin and mucous membrane. Chicken pox is caused by Varicella virus. It belongs to herpes viridae family.

Causative agent

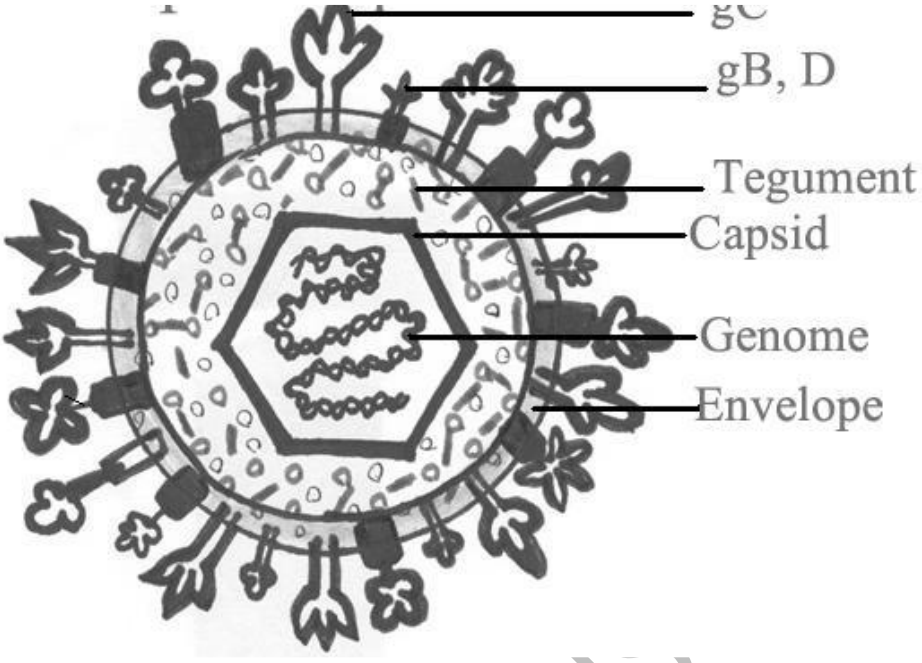
Varicella viruses are large viruses.Virion is spherical shaped enveloped virus. Capsid is Icosahedral in shape. Genome is linear double standard DNA, 124-235kbp. More than 35 proteins are available in virion. Virion Replicates is in the nucleus. Genome is large enough to code for at least 100 proteins.

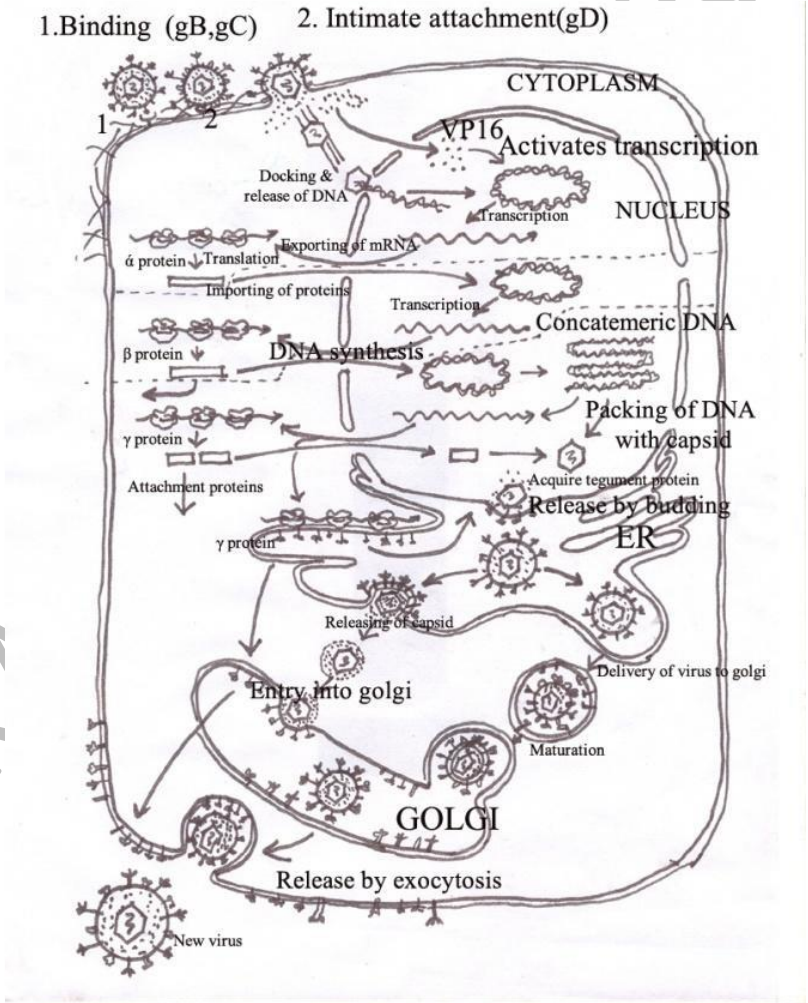
Replication

- Virion binds to the extracellular protein through gB and gC receptor.
- Another viral protein gD interacts with a second cellular receptor.
- This interaction mediates fusion of virus with host plasma membranes.
- The virus is uncoated, liberating tegument proteins and nucleocapsid into the cytoplasm.

Viral nucleocapsid docks at the nuclear pore and release viral DNA into the nucleus, where the DNA circularizes.

- VP16 enhance transcription of viral genome and stimulate transcription of immediate early genes by host cell RNA polymerase II.
- Immediate early mRNAs are spliced and transported to the cytoplasm, where they are translated.
- Immediate early proteins (α proteins) are imported into the nucleus, where they activate the transcription of early genes.
- β protein genes are transported to the cytoplasm after transcription and are translated. β proteins are imported to the nucleus where they induce DNA replication and synthesis substrate for DNA synthesis.
- DNA replication produces long concatameric DNA molecules, the templates for late gene expression.
- Late mRNAs are transported to the cytoplasm and synthesis of gamma protein. These proteins are structural proteins and are needed for viral assembly.
- Some late proteins are inserted to ER and are transported to Golgi apparatus for glycosylation.
- Mature glycoproteins are transported to plasma membrane of the infected cell.
- Some gamma proteins are transported to the nucleus for assembly of nucleocapsid and DNA packaging.
- Newly replicated viral DNA is packaged into preformed capsids.
- These capsids, together with some tegument proteins bud from the inner nuclear membrane into the lumen of ER and acquire envelope.
- Enveloped virus then transported to the PM for release by exocytosis.
- Latent infection occurs primarily in neurons found in sensory and autonomic ganglia. During this infection Latency Associated Transcript (LTT) promoter is synthesized and are involved in protein synthesis.


gC
gB, D
Tegument
Capsid
Genome
Envelope


1.Binding (gB,gC)
2. Intimate attachment(gD)
CYTOPLASM
1
2
VP16
Activates transcription
Docking &
release of DNA
Transcription
NUCLEUS
Exporting of mRNA
α protein
Translation
Importing of proteins
Transcription
Concatemeric DNA
β protein
DNA synthesis
Packing of DNA
with capsid
γ protein
Acquire tegument protein
Attachment proteins
Release by budding
ER
γ protein
Releasing of capsid
Delivery of virus to golgi
Entry into golgi
Maturation
GOLGI
Release by exocytosis
New virus

Pathogenesis

The route of infection is the mucosa of the URT or the conjunctiva. The virus circulates in the blood and undergoes multiple cycles of replication and eventually localizes in the skin. Lesions of varicella infection are associated with cutaneous and mucosal endothelial cells. Swelling of epithelial cells, ballooning degeneration and the accumulation of tissue fluids results in vesicle formation. Eosinophilic inclusion bodies are found in the nuclei of infected cells. Multinucleated giant cells are common.

Zoster lesions are histopathologically similar to varicella. There is also an acute inflammation of the sensory nerves and ganglia. It is not clear what triggers reactivation of latent Varicella-Zoster virus infection in ganglia. It is believed that warning immunity allows viral replication to occur in a ganglion, causing intense inflammation and pain.

Symptoms

Incubation period is of 10-23 days. Malaise and fever are earliest symptoms. Followed by rash characteristically begins on the scalp and trunk and spreads. Macules evolve in 2-3mm vesicles, evolve in successive crops. Lesions on mucous membranes are easily transmitted and may appear as ulcers. Lesions appear on the Mouth, Rectum and Vagina. Other symptoms include Headache, Sore throat, Loss of appetite and Irritability.

Zoster infection usually starts with severe pain in the area of skin or mucosa supplied by one or more groups of sensory nerves and ganglia. The most common complication of Zoster is Post Herpatic Neuralgia (PHN). Pain may be characterized by burning, itching or tingling sensations.

Lab Diagnosis

Demonstration of multinucleated giant cells and type A intranuclear inclusion bodies. CF, Neutralization test are used a serological test

Treatment

Vidarabine and Acyclovir are useful for treatment. Zoster Immuno Globulin (ZIG) also useful.

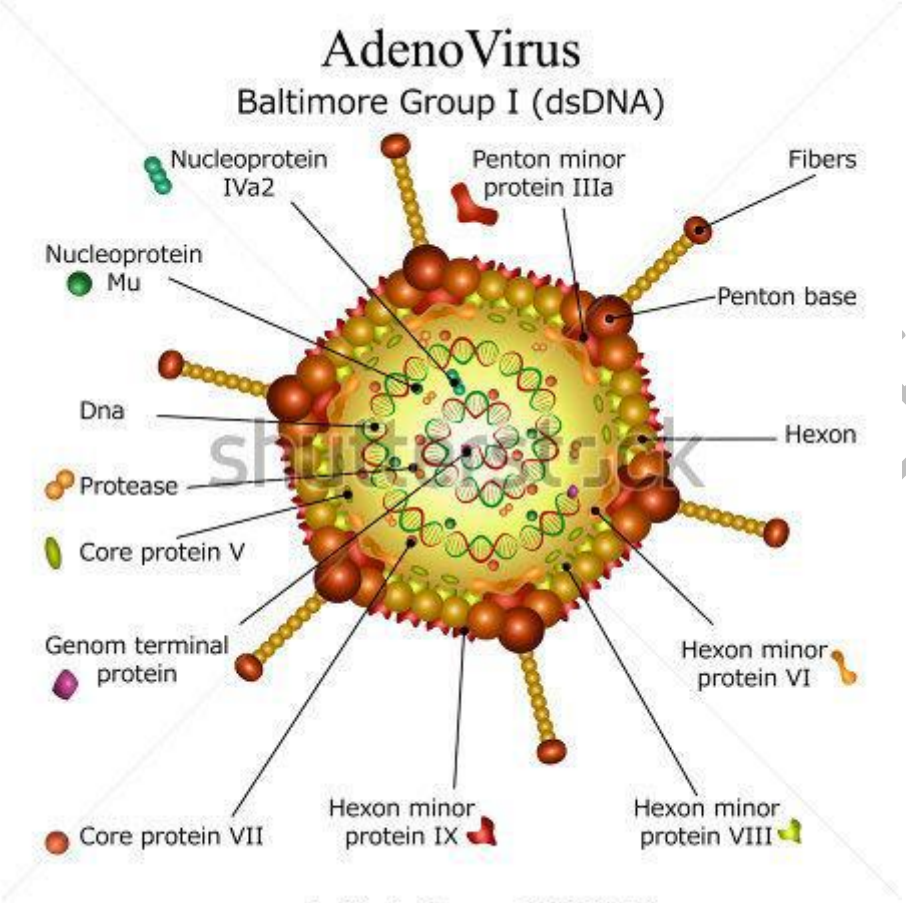
ADENOVIRUS

I. Structure

Adenoviruses are the group of medium sized, non enveloped ds DNA virus that share common complement fixing antigen.

Size: 70-90 nm in diameter

Shape: Icosahedral



Genome:

▪ Linear ds DNA molecule of 26-45 kbp long and DNA have inverted terminal repeats of approximately 100bp at both ends.

▪ Each DNA strand is covalently attached to virus encoded protein at 5’ end

Capsid:

▪ Capsid is Icosahedral in shape and is composed of 292 capsomeres with 20 triangular facets and 12 vertices.

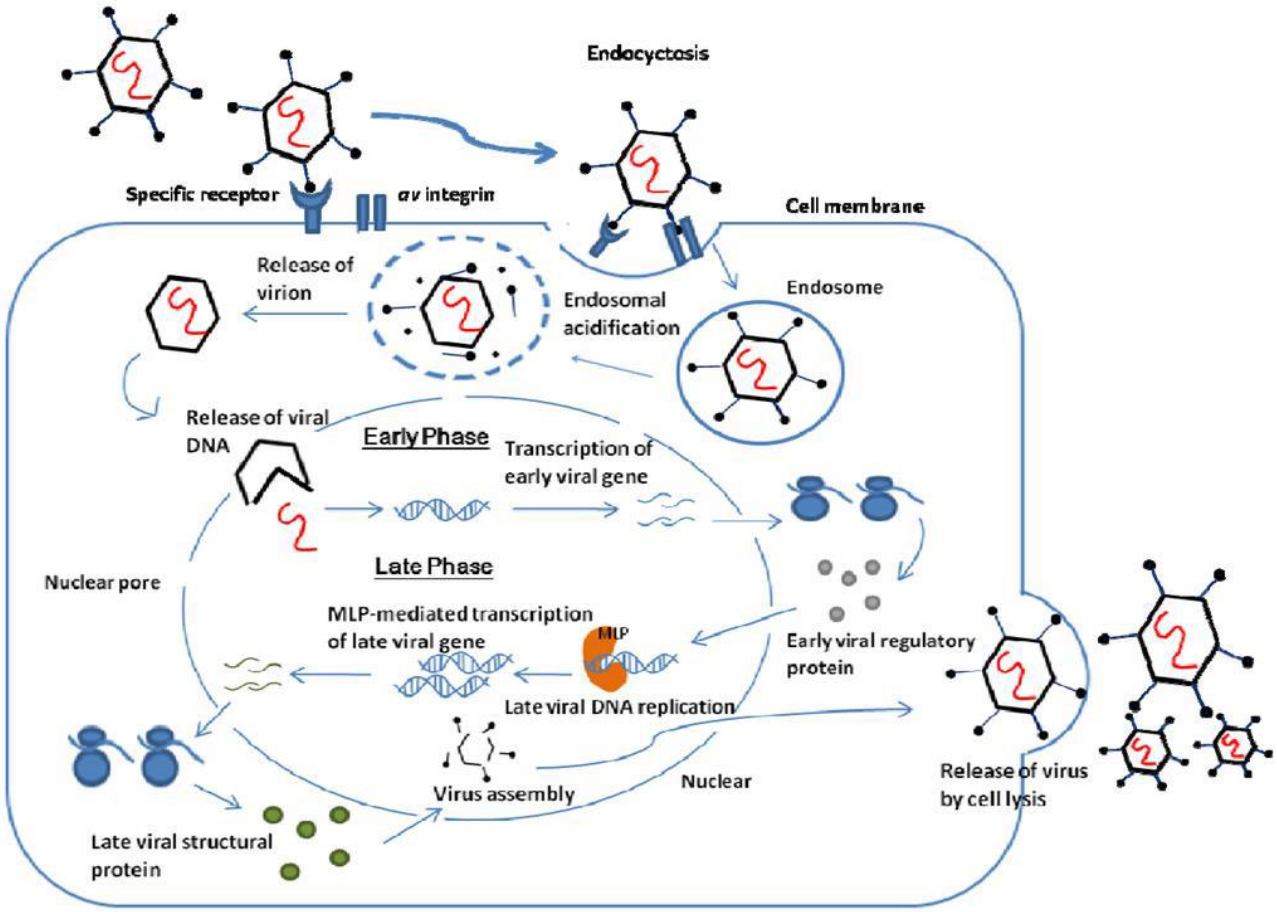
▪ The capsid consists of 240 hexons and 12 pentons

Each pentons unit consists of a penton base anchored in capsid and a projection (fiber or knob) with a knot at distal end. Thus the virion looks like space vehicle.

▪ The projection or fiber helps to bind Adenovirus to host cell.

▪ The fiber contains viral attachment protein which acts as hemagglutinin.

▪ Penton base carries a toxin like activity that causes cytopathic effect (CPE) on host cell.



Step I: Attachment and entry

▪ Adenovirus attaches to the host cell via its fiber structure to Coxsackie and Adenovirus receptor (CAR) receptor on host cell.

▪ The attachment of fiber to its receptor on host cell is followed by interaction of penton base with cellular integrin which promote receptor mediated internalization.

Step II: Uncoating

▪ The virus is internalized into clathrin coated endosome and the high pH of endosome helps in uncoating of virus

▪ Transport of viral DNA into nucleus

▪ The viral nucleocapsid is transported from cytosol to nucleus by the help of microtubules.

Step III: Early Transcription

▪ It is the early event in viral replication, and occurs before viral DNA synthesis begins.

▪ It is the preparatory phase in which transcription of viral DNA occurs to mRNA (early transcript).

▪ Early transcript undergoes translation to produce about 20 different early proteins. These early proteins induces host cell to enter into S-phase of cell cycle and to create condition favorable for viral replication.

Step IV: DNA replication

▪ Viral DNA replication takes place in nucleus.

▪ The viral encoded protein at 5' end of viral DNA strand acts as primer for initiation of viral DNA synthesis.

▪ Late event begins concomitantly with onset of viral DNA synthesis.

Step V: Late Transcription and translation

▪ A large single primary transcript is synthesized from virus DNA which spliced into 18 fragments and each fragment acts as mRNA and are transported to cytoplasm.

▪ In the cytoplasm, translation occurs and viral structural proteins are synthesized.

Step VI: Viral morphogenesis and release

▪ Morphogenesis of Adenovirus occurs inside nucleus.

▪ Viral DNA then gets packaged into preformed capsid forming mature virus particle.

▪ Mature virus particles are stable, infectious and resistant to nuclease enzyme of host cell.

▪ Adenovirus infection does not lyse the host cell.

▪ Mature virus is then release from host cell by budding.

III. Mode of transmission of Adenovirus

▪ Adenovirus infection transmits from person to person directly by-

▪ Aerosol droplets (respiratory route)

▪ Faeco-oral route

▪ Contaminated fingers to infects conjunctiva

▪ Contaminates fomites

IV. Pathogenesis

▪ Adenovirus can infects and replicates in epithelial cells of respiratory tracts, gastro-intestinal tracts, urinary bladder and eyes.

▪ After entry of Adenovirus inside human body, it can multiply in epithelial cells of conjunctiva, pharynx or small intestine according to mode of entry and then spreads to regional lymph nodes.

▪ Usually Adenovirus does not spread beyond regional lymph node.

▪ Adenovirus produces three types of infections:

1. Lytic infection

▪ Virus actively replicates inside host cell producing lytic effects causing cell death and releases progeny viruses.

After local infection viruses may spread to visceral organs.

2. Latent infection:

▪ Adenovirus has a property to become latent in lymphoid organs such as tonsil and payer's patches.

▪ Latent infection can be reactivated in person with underlying immunity.

3. Oncogenic transformation:

▪ Certain Adenovirus (group A and B) can transform host cell into cancerous cell by integrating viral DNA into host DNA.

▪ Although the oncogenicity has not been seen in Human infection.

V. Adenovirus infection and diseases

▪ Adenovirus primarily infects children and accounts less in adults.

▪ Most Adenovirus infections are mild and self limiting.

1. Respiratory diseases:

▪ Pharyngitis: Adenovirus is major causes of non-bacterial pharyngitis.

▪ Pneumonia: Adenovirus 3 and 7 are associated with pneumonia.

▪ Acute respiratory disease: fever, rhinorrhoea, cough, sore throat that lasts for 3-5 days.

▪ Pharyngoconjunctival fever: Fever, red eyes, sore throat that occurs primarily in school going children.

2. Eye infection:

▪ Keratoconjunctivitis: It is highly contagious and characterized by photophobia, tearing, pain and inflammation of conjunctiva.

▪ Gastro-intestinal infection

▪ Intangible gastroenteritis:I It is characterized by fever and watery diarrhoea.

3. Other infections:

▪ Pertussis like symptoms in children

▪ Musculoskeletal disorder

▪ Genital infection

▪ Skin infection

VI. Lab diagnosis

1. Specimens:

▪ Depends upon nature of infections.

▪ Throat swab, nasopharyngeal aspirates, conjunctival aspirates, conjunctival swab, urine, stool, blood, body fluids

2. Microscopy: Electron microscope

3. Antigen detection: ELISA, DFA test

4. Serology or antibody detection

5. Molecular technique: PCR, DNA probe

6. Virus culture: cell line culture

VII. Prevention from Adenovirus infection

- Maintain personal hygiene
- Wash hands often with soap and water
- Avoid direct personal contact with diseased person
- Cover mouth and nose while coughing and sneezing
- Avoid touching eye, nose or mouth without washing hands
- Visit Hospital in case of any symptoms

Treatments

▪ Not specific

▪ Some antiviral drugs such as Ganciclovir, Vidarabine, Ribavirin, Cidofovir

ROTAVIRUS

- Family: Reoviridae
- Genus: Rotavirus

Classification of Rotavirus:

▪ Classified into seven distinct groups (A to G) based on structural antigen VP6.

▪ Group A, B, and C Rotaviruses are found in Human infection as well as animal infection

▪ Group A Rotaviruses are most frequent Human pathogen

Structure, composition and properties of Rotavirus

1. Structure:

Characteristics wheel like appearance (Rota-means wheel)

▪ Size: 65nm-100nm in diameter

▪ Shape: Spherical shape

▪ Symmetry: Icosahedral

2. Genome composition:

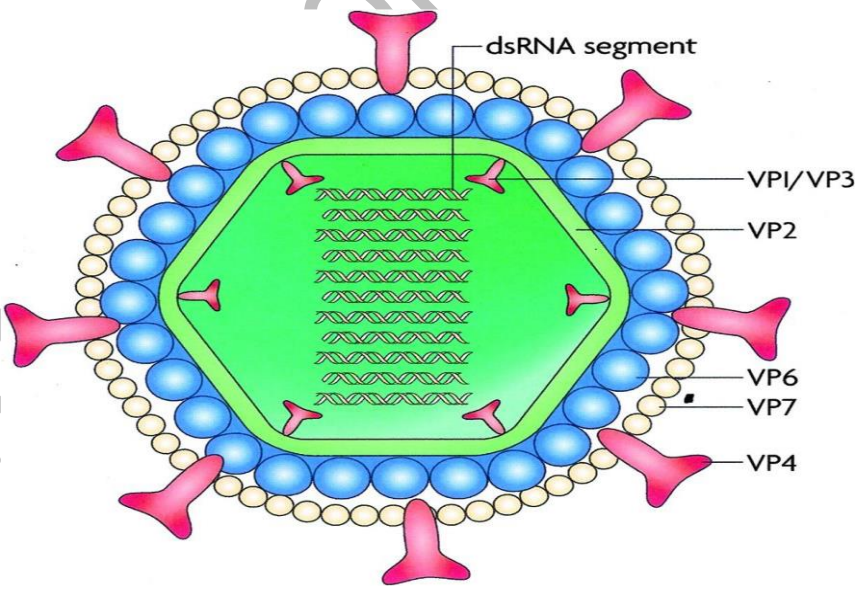
▪ Genome: 11 segments of double stranded RNA (ds RNA)

▪ Protein: 6 structural protein (VP) and 6 Non-structural protein (NSP)

▪ Envelope: Absent

▪ Nucleic acid is surrounded by two layer of capsid- inner capsid (VP6) and outer capsid (VP7)

▪ VP4 is the spike protein, it is a cell surface receptor



3. Other properties

▪ Replication: Occurs in cytoplasm of infected cell.

▪ Rota virus contain an RNA-dependent RNA polymerase and other enzymes capable of producing capped RNA transcripts

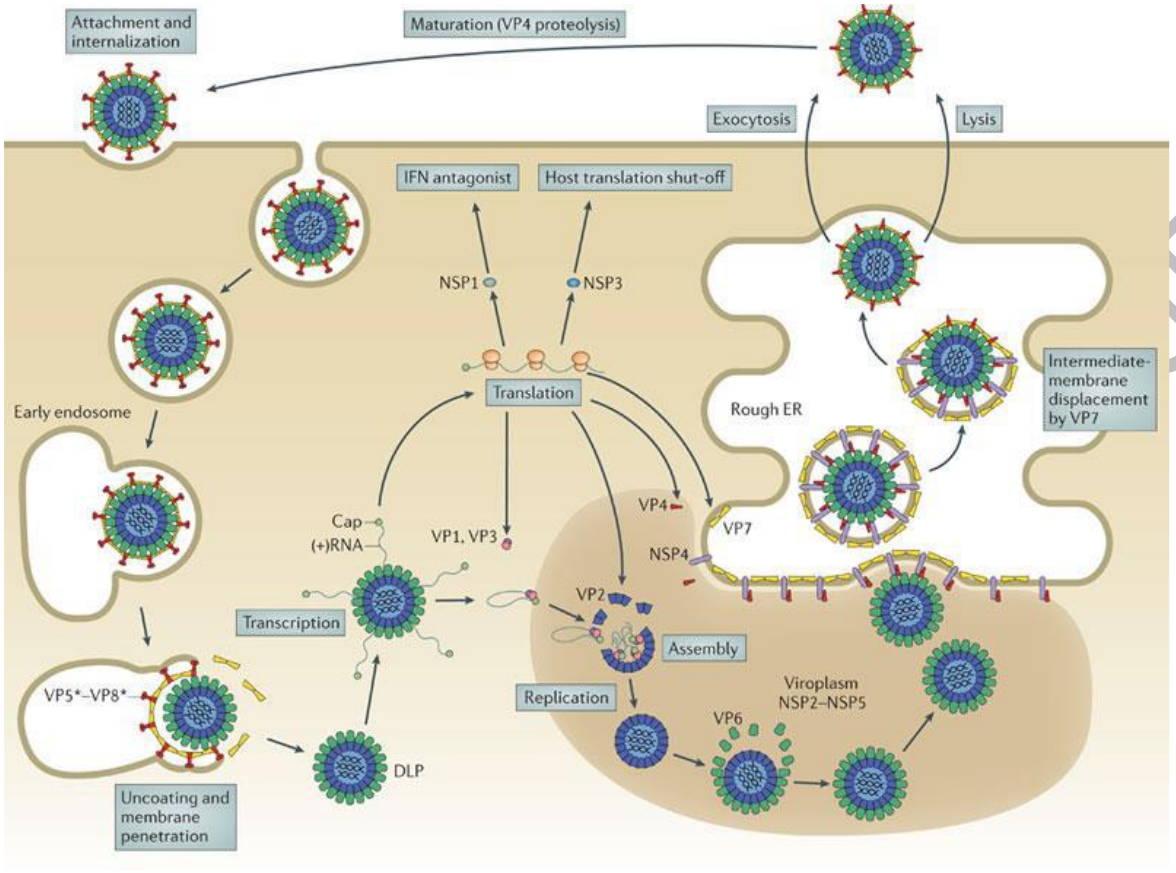
- Rota virus do not grow in cell line culture

Rotaviruses are inactivated by-

- Heating to 100°C
- Treatment with acid (pH<3),
- Glutaraldehyde (3%),
- Phenol,
- Formalin,
- Chlorine
- Alcohol (70%),

Replication of Rota virus:

1. Attachment: by VP4 on cell surface receptor
2. Penetration: receptor mediated endocytosis
3. Un-coating in lysosome
4. Transcription is mediated by endogenous virus dependent RNA polymerase (transcriptase)
5. Translation to produce viral structural protein
6. Synthesis of full length transcript
7. Some of the full length transcript are encapsidate
8. Synthesis of –ve sense RNA strand with capsid to form ds RNA
9. Formation of inner capsid
10. Morphogenesis: budding of single shelled virus into RER acquiring pseudo envelope
11. Removal of pseudo envelope and replaced by outer capsid in RER
12. Maturation
13. Cell lysis and Release



Mode of Transmission:

- Ingestion of contaminated food and water
- Directly from faeces contaminated fingers
- Occasionally by droplet infection
- Children below 5 years are mostly affected
- Adults are infected by contact with pediatric cases Incubation period: 2-3 days

Pathogenesis:

- Rota virus replicates in enterocyte near the tip of villi destroying enterocytes
- Viral encoded toxin: early profuse, secretory diarrhea is caused by enterotoxin, NSP4.
- Disruption of intestinal epithelium due to virus replication
- Histologic changes of enterocytes that triggers enteric nervous system, intestinal secretion and immune response

▪ The acute infection and diarrhoea normally resolves within 7 days in immunocompetent hosts.

Clinical symptoms:

1. Local infection:

▪ Acute Gastroenteritis, severe in case of infants aged 6-24 months.

▪ Infected Infants are unable to digest milk due to lactase deficiency caused by destruction of enterocytes

▪ Malabsorption of Na+, water, and disaccharides

▪ Symptoms of Dehydration: decrease in urination, dry mouth and throat and feeling dizzy when standing up.

2. Systemic infection:

High grade Fever

▪ Lymphocytosis and transient neutropenia

3. Complication:

▪ Febrile Convulsion in small children

▪ Severe dehydration, hypotonia and shock

Laboratory diagnosis:

Specimen: faeces in early infection,

▪ Viral antigen detection: solid phase agglutination, ELISA

▪ Electron microscopy

▪ EIA (enzyme immune assay): it is sensitive to detect virus in stool

▪ Dip stick/ rapid test

▪ PCR: For genotyping of Rotavirus

▪ Virus culture: No cell line culture

Treatment:

▪ Oral rehydration

▪ Other supportive rehydration therapy to control loss of water and electrolytes

▪ Vaccine: Two Oral rotavirus vaccines are currently licensed for use in infants 1. RotaTeq (RV5) is given in 3 doses at ages 2 months, 4 months, and 6 months

2. Rotarix® (RV1) is given in 2 doses at ages 2 months and 4 months

Prevention and control:

- Sanitation
- Waste water treatment
- Health professional should wash their hands with soap and water before and after patient contact

UNIT-III

SARS (SEVERE ACUTE RESPIRATORY SYNDROME)

Cause

SARS coronavirus (SARS-CoV) – virus identified in 2003. SARS-CoV is thought to be an animal virus from an as-yet-uncertain animal reservoir, perhaps bats, that spread to other animals (civet cats) and first infected humans in the Guangdong province of southern China in 2002.

Transmission

- An epidemic of SARS affected 26 countries and resulted in more than 8000 cases in 2003. Since then, a small number of cases have occurred as a result of laboratory accidents or, possibly, through animal-to-human transmission (Guangdong, China).
- Transmission of SARS-CoV is primarily from person to person.
- It appears to have occurred mainly during the second week of illness, which corresponds to the peak of virus excretion in respiratory secretions and stool, and when cases with severe disease start to deteriorate clinically.
- Most cases of human-to-human transmission occurred in the health care setting, in the absence of adequate infection control precautions. Implementation of appropriate infection control practices brought the global outbreak to an end.

SYMPTOMS

• Symptoms are influenza-like and include fever, malaise, myalgia, headache, diarrhoea, and shivering (rigors). No individual symptom or cluster of symptoms has proved to be specific for a diagnosis of SARS.

• Although fever is the most frequently reported symptom, it is sometimes absent on initial measurement, especially in elderly and immunosuppressed patients.

• Cough (initially dry), shortness of breath, and diarrhoea are present in the first and/or second week of illness. Severe cases often evolve rapidly, progressing to respiratory distress and requiring intensive care.

Geographical distribution

• The distribution is based on the 2002–2003 epidemic. The disease appeared in November 2002 in the Guangdong province of southern China.

• This area is considered as a potential zone of re-emergence of SARS-CoV.

• Other countries/areas in which chains of human-to-human transmission occurred after early importation of cases were Toronto in Canada, Hong Kong Special Administrative Region of China, Chinese Taipei, Singapore, and Hanoi in Viet Nam.

Risk for travellers

• Currently, no areas of the world are reporting transmission of SARS. Since the end of the global epidemic in July 2003, SARS has reappeared four times – three times from laboratory accidents (Singapore and Chinese Taipei), and once in southern China where the source of infection remains undetermined although there is circumstantial evidence of animal-to-human transmission.

• Should SARS re-emerge in epidemic form, WHO will provide guidance on the risk of travel to affected areas

• Travellers should stay informed about current travel recommendations. However, even during the height of the 2003 epidemic, the overall risk of SARS-CoV transmission to travellers was low.

Prophylaxis

• None. Experimental vaccines are under development.

Precautions

• Follow any travel recommendations and health advice issued by WHO.

H1N1 FLU VIRUS (SWINE FLU)

• H1N1 flu is also known as swine flu. It's called swine flu because in the past, the people who caught it had direct contact with pigs. That changed several years ago, when a new virus emerged that spread among people who hadn't been near pigs.

• In 2009, H1N1 was spreading fast around the world, so the World Health Organization called it a pandemic. Since then, people have continued to get sick from swine flu, but not as many.

• While swine flu isn't as scary as it seemed a few years ago, it's still important to protect yourself from getting it. Like seasonal flu, it can cause more serious health problems for some people. The best bet is to get a flu vaccine, or flu shot, every year. Swine flu is one of the viruses included in the vaccine.

How Do You Catch It?

The same way as the seasonal flu. When people who have it cough or sneeze, they spray tiny drops of the virus into the air. If you come in contact with these drops, touch a surface (like a doorknob or sink) where the drops landed, or touch something an infected person has recently touched, you can catch H1N1 swine flu.

What Is Age-Related Macular Degeneration?

Age-related macular degeneration is an eye disease that may get worse over time. It’s the leading cause of severe vision loss in people over age 60. Learn more about the symptoms,

Causes of Polycythemia Vera

You can have polycythemia vera for years without knowing it. Find out more about the symptoms of polycythemia vera, how it affects your body, and what causes it.

SWINE FLU SYMPTOMS

These, too, are pretty much the same as seasonal flu. They can include:

- Cough
- Fever
- Sore throat
- Stuffy or runny nose
- Body aches
- Headache
- Chills
- Fatigue

The people who really need to be tested are those in the hospital or those at high risk for life-threatening problems from swine flu, such as:

- Children under 5 years old
- People 65 or older
- Children and teens (under age 18) who are getting long-term aspirin therapy and who might be at risk for Reye's syndrome after being infected with swine flu. Reye's syndrome is a life-threatening illness linked to aspirin use in children.
- Pregnant women
- Adults and children with chronic lung, heart, liver, blood, nervous system, neuromuscular, or metabolic problems
- Adults and children who have weakened immune systems (including those who take medications to suppress their immune systems or who have HIV)
- People i in nursing homes and other long-term care facilities

Treatment

Some of the same antiviral drugs that are used to treat seasonal flu also work against H1N1 swine flu. Oseltamivir (Tamiflu), peramivir (Rapivab), and zanamivir (Relenza) seem to work best, although some kinds of swine flu don’t respond to oseltamivir.

These drugs can help you get well faster. They can also make you feel better. They work best when you take them within 48 hours of the first flu symptoms, but they can help even if you get them later on.

Antibiotics won't do anything for you. That's because flu is caused by a virus, not bacteria.

Over-the-counter pain remedies and cold and flumedications can help relieve aches, pains, and fever. Don't give aspirin to children under age 18 because of the risk of Reye's syndrome. Make sure that over-the-counter cold medications do not have aspirin before giving them to children

DENGUE

Dengue is one of the most important human infection. It is caused by Dengue virus. This virus is included under the family Flaviviridae, genus Flavi virus. In its serious form Dengue causes two important manifestations. Dengue hemorrhagic fever and Dengue Shock Syndrome. Dengue virus is transmitted from person to person by Aedes aegypt mosquitoes. It is in Arbo virus group as it is transmitted through Arthropod vector Mosquito. It is also called Break bone fever.

Causative agent

Dengue virus causes this disease. It is an enveloped virus. This virus is closely related Yellow fever virus. It is a positive sense single-stranded RNA genome. Genome is packaged in the capsid. The outer envelope is formed by envelope protein and is the protective antigen. It aids in entry of the virus into the inside of the cell. The genome also encodes several nonstructural proteins (NS1, NS2a, NS2b, NS3, N4a, NS4b, NS5). NS1 is produced as secretory form also. NS3 is a putative helicaseand NS5 is the viral polymerase.

Signs and symptoms

The most common symptom of dengue is fever with any of the following. They are Nausea, vomiting, Rash, Aches and pains (eye pain, typically behind the eyes, muscle, joint, or bone pain). Symptoms of dengue typically last 2–7 days. Most people will recover after about a week.

Warning signs of severe dengue

Stomach or belly pain, tenderness, Vomiting (at least 3 times in 24 hours), Bleeding from the nose or mouth, Muscle and Joint pain, rash, diarrhea, hypertension, Pleural effusion, Vomiting blood, or blood in the stool, intestinal bleeding.

Pathogenesis

The virus enters the blood through mosquito bite. After entry, it attachs WBC, reproduces inside the cells and move throughout the body. The white blood cells produce cytokines and interferons, which are responsible for many of the symptoms of dengue. In severe infection, the virus production inside the body is greatly increased and it affects liver and bone marrow. Fluid from the bloodstream leaks through the wall of small blood vessels into body cavities due to capillary permeability. As a result, less blood circulates in the blood vesselsand the blood pressure

becomes so low that it cannot supply sufficient blood to vital organs. Furthermore, dysfunction of the bone marrow due to infection of the stromal cells leads to reduced numbers of platelets, this increases the risk of bleeding, the other major complication of dengue fever. In a small proportion of cases, the disease develops into severe dengue, also known as dengue hemorrhagic fever, resulting in bleeding, low levels of blood platelets and blood plasma leakage, or into dengue shock syndrome, where dangerously low blood pressure occurs.

Replication

The virus enters the blood through mosquito bite. After entry, it attachs langergans cells. The virus enters cells through mannose receptor. The dendritic cell moves to the nearest lymph node. Meanwhile, the virus genome is translated in membrane-bound vesicles on the cell's endoplasmic reticulum, where the cell's protein synthesis apparatus produces new viral proteins that replicate the viral RNA and begin to form viral particles. Immature virus particles are transported to the Golgi apparatus, the part of the cell where some of the proteins receive necessary sugar chains (glycoproteins). The now mature new viruses are released by exocytosis. They are then able to enter other white blood cells, such as monocytes and macrophages.

Lab Diagnosis

The earliest change detectable on laboratory investigations is a low white blood cell count, which may then be followed by low platelets. Elevated levels of SGOT and SGPT along with low platelet count indicate dengue. In severe disease, plasma leakage results in hemoconcentration and hypoalbuminemia. Diagnosis can be done by virus isolation in cell cultures, nucleic acid detection by PCR, viral antigen detection (such as for NS1) or specific antibodies (serology).

Prevention

The primary method of controlling A. aegypti is by eliminating its habitats. This is done by getting rid of open sources of water, Generalized spraying of organophosphae insecticides. People can prevent mosquito bites by wearing clothing that fully covers the skin, using mosquito netting while restingand/or the application of insect repellent (DEET being the most effective). The vaccine is produced by Sanofi and goes by the brand name Dengvaxia. It is based on a weakened combination of the yellow fever virus and each of the four dengue serotypes.

Treatment

There is no specific medication to treat dengue. Treat the symptoms of dengue and see your healthcare provider. Rest as much as possible. Take acetaminophen or paracetamol to control fever and relieve pain. Do not take aspirin or ibuprofen. Drink plenty of fluids such as water or drinks with added electrolytes to stay hydrated.

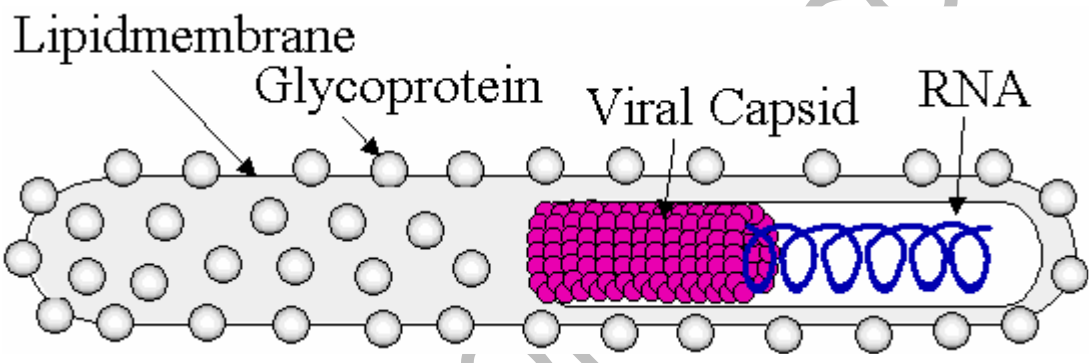
EBOLA VIRUS

The Ebola virus causes an acute, serious illness which is often fatal. It is first appeared in 1976. Ebola virus disease (EVD), formerly known as Ebola haemorrhagic fever. The virus is transmitted

to people from wild animals and spreads in the human population through human-to-human transmission. The average EVD case fatality rate is around 50%. Community engagement is key to successfully controlling outbreaks. Early supportive care with rehydration, symptomatic treatment improves survival. There is no licensed treatment proven to neutralize the virus but a range of blood, immunological and drug therapies are under development. The disease occurred first in a village near Ebola river, hence the name of the diseases Ebola.

Causative agent

Ebola virus is belongs to filo virus group. It is pleomorphic in nature. They are enveloped, filomentous or shorter U shaped of Circular thread like virus. Virus have helical nucleocapsid. It contains single stranded negative sense RNA as a genome. This virus is inactivated by heating at 60°C for 30 minutes. UV rays, gamma rays, lipid solvents, phenolic group of disinfectants are able to inactivate the virus. Ebola is a highly virulent virus.



Transmission

It is thought that fruit bats of the Pteropodidae family are natural Ebola virus hosts. Ebola is introduced into the human population through close contact with the blood, secretions, organs or other bodily fluids of infected animals such as fruit bats, chimpanzees, gorillas, monkeys, forest antelope or porcupines found ill or dead or in the rainforest. Ebola then spreads through human-to-human transmission via direct contact

Multiplication

Ebola viruses replicate efficiently in all cell types including endothelial cells, macrophages and parenchymal cells. Platelet dysfunction appears to be the major pathogenic factor of this disease. After incubation period vius induce a specific symptoms.

Symptoms

The incubation period is from 2 to 21 days. A person infected with Ebola cannot spread the disease until they develop symptoms. Symptoms of ebola are fever, fatigue, muscle pain, headache, sore throat, vomiting, diarrhoea, rash. In some cases, both internal and external bleeding.

Laboratory diagnosis

Low white blood cell and platelet counts, Elevated liver enzymes.

Antibody-capture enzyme-linked immunosorbent assay (ELISA), Direct immunofluorescence test detects eosinophilic cytoplasmic inclusion bodies. Serum neutralization test. Reverse transcriptase polymerase chain reaction (RT-PCR) assay. Electron microscopy and ·virus isolation by cell culture.

Treatment

Supportive care - rehydration with oral or intravenous fluids - and treatment of specific symptoms improves survival. There is as yet no proven treatment available for EVD. However, a range of potential treatments including blood products, immune therapies and drug therapies are currently being evaluated.

Vaccines

An experimental Ebola vaccine proved highly protection against Ebola Virus Disease. The vaccine, called rVSV-ZEBOV is effective for ebola viral disease. Community involvement is a key factor in controlling the disease. .

HIKUNGUNYA

Chikungunya is a viral disease. Chikungunya was first identified in Tanzania in the early 1952 and has caused periodic outbreaks in Asia and Africa since the 1960s. In India first major outbreakis noted in 1963 followed by in 2013. This disease is transmitted to humans by infected Aedes aegypti and Aedes albopictus, hence it is in Arbo viruses.

Causative agent

It is caued by chikunguny virus, which is the member of the genus Alphavirus, family togaviridae. It is an enveloped virus with icosahedral capsid. It contains positive sense single stranded RNA virus. The virus consists of four nonstructural proteins and three structural proteins. The structural proteins are the capsid and envelope spike proteins namely E1 and E2.

Replication

Chikungunya virus is able to multiply in epithelial as well as endothelial cells, fibroblasts, macrophages. Viruses are highly cytopathic innature but susceptible to type-I and –II Interferons. The replication and propagation of viruses is dependent on entry into permissive cells. Alphavirus entry into cells is initiated by receptor-binding, followed by clathrin-mediated endocytosis. Fusion to endosomal membranes transports nucleocapsid (NC) into the cytoplasm, where RNA is released after disassembly. Genomic RNA is used for both translation of proteins from genomic and subgenomic (26S) RNAand transcription of nascent (+) RNA via a (−)RNA template. ncoating of alphavirus nucleocapsids occurs almost immediately (~1 minute) after their penetration into the cytoplasm. 60S ribosomal RNA interacts with the C protein, facilitating uncoating of the nucleocapsid and release of viral RNA for initiation of protein synthesis. Upon synthesis, the E2

glycoprotein precursor, PE2 (p62 in SFV) and E1 glycoproteins interact with each other (preferentially in cis) to form heterodimers. These heterodimer complexes are then transported from the endoplasmic reticulum to the cell surface via the Golgi complex. At a late stage of transport, the PE2 precursor is cleaved in its lumenal domain by host furin-like protease to generate mature E2 and E3 proteins. This cleavage induces a conformational change that weakens the E1-E2 interaction in the spike heterodimer, priming the fusion peptide for activation upon exposure to low pH. Interactions between the C protein and the cytoplasmic domain of the E2 protein drive the budding process, with E1-E2 heterodimers forming an envelope around nucleocapsid-like particles. Upon release from cells, virions acquire a membrane bilayer derived from the host cell plasma membrane.

Pathogenesis

Susceptible cells to chikungunia virus are human epithelial and endothelial cells, primary fibroblastsand monocyte-derived macrophages. After the first round of replication there is a host immune response, but the virus goes to the lymph nodes and then to other tissues via the circulatory system. Replication at other tissues leads to the viremic phase of the disease. Upon infection, CHIKV indirectly stimulates production of type I interferon (IFN) via activation of non-hematopoietic cells, including primarily fibroblasts, an action which is essential for clearing CHIKV from the body. CHIKV also appears to induce a signaling cascade by activating interferon promoter stimulator 1 (IPS-1), leading to the buildup of IRF3-dependent mRNAs while also blocking these mRNAs from encoding proteins.

CD8+ lymphocytes are found in skin rashes of acute patients, while CD4+ T-cells comprise the majority in synovial effusions of chronic patients. There is evidence that inflammation stemming from CHIKV infection has consequences for osteoblast and osteoclast proliferation and function, which may contribute to the effects of chronic CHIKV. Several of the cytokines associated with infection, such as TNF-α, IL-6and IL-1, also promote osteoclast activity and have been associated with steoclastogenesis.

Symptoms

Symptoms usually begin 3–7 days after being bitten by an infected mosquito. The most common symptoms are fever and joint pain. Other symptoms may include headache, muscle pain, joint swelling, or rash. Chikungunya disease does not often result in death, but the symptoms can be severe and disabling. Most patients feel better within a week. In some people, the joint pain may persist for months.

Lab Diagnosis

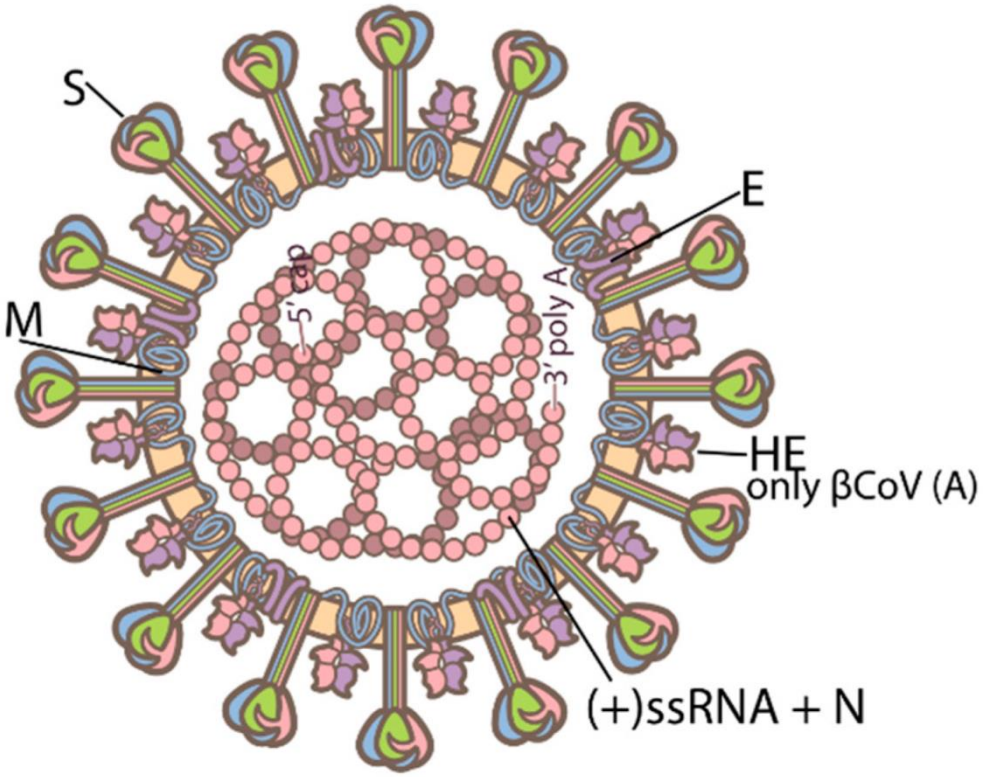
The symptoms of chikungunya are similar to those of dengue and Zika. Definitive laboratory diagnosis can be accomplished through viral isolation, RT-PCR or serological diagnosis. RT-PCR using nested primer pairs is used to amplify several chikungunya-specific genes from whole blood, generating thousands to millions of copies of the genes in order to identify them. RT-PCR can also be used to quantify the viral load in the blood. Serological diagnosis requires a larger amount of

blood than the other methodsand uses an ELISA assay to measure chikungunya-specific IgM levels in the blood serum.

CORONA VIRUS

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019 in Wuhan, a city in Hubei Province of China, and subsequently spread worldwide, causing a major global pandemic. Prior to the emergence of SARS-CoV-2, coronaviruses were well known infectious agents in many species, including humans. Four common cold coronaviruses (HCoV-229E, HCoV-HKU1, HCoV-NL63, and HCoV-OC43) were estimated to cause 15 to 30 percent of mild upper respiratory infections, with significant seasonal variation Two other pathogenic coronaviruses had previously caused severe disease in humans: severe acute respiratory syndrome coronavirus (SARS-CoV), which circulated from 2002 to 2004 following a spillover event from an animal host , and Middle East Respiratory Syndrome (MERS), which emerged in 2012 and causes ongoing infection associated with repeated spillover events from camel reservoirs and occasional clusters of human-to-human transmission

Before the World Health Organization (WHO) declared an end to the coronavirus disease 2019 (COVID-19) global health emergency in May 2023, SARS-CoV-2 infection resulted in an estimated 15 million excess deaths in 2020 and 2021 alone. As SARS-CoV-2 transitions to endemicity, it remains an important cause of illness around the world. In the United States from 2023 to 2024, COVID-19-related hospitalization rates among infants <6 months of age (320 per 100,000) and adults ≥75 years of age (940 per 100,000) remained higher than influenza-related hospitalization rates had been for those high-risk age groups over the prior 10 years.



Structure of a coronavirus

Structure

Coronaviruses are large, roughly spherical particles with unique surface projections. Their size is highly variable with average diameters of 80 to 120 nm. Extreme sizes are known from 50 to 200 nm in diameter. The total molecular mass is on average 40,000 kDa. They are enclosed in an envelope embedded with a number of protein molecules. The lipid bilayer envelope, membrane proteins, and nucleocapsid protect the virus when it is outside the host cell.

The viral envelope is made up of a lipid bilayer in which the membrane (M), envelope (E) and spike (S) structural proteins are anchored. The molar ratio of E:S:M in the lipid bilayer is approximately 1:20:300. The E and M protein are the structural proteins that combined with the lipid bilayer to shape the viral envelope and maintain its size. S proteins are needed for interaction with the host cells. But human coronavirus NL63 is peculiar in that its M protein has the binding site for the host cell, and not its S protein. The diameter of the envelope is 85 nm. The envelope of the virus in electron micrographs appears as a distinct pair of electron-dense shells (shells that are relatively opaque to the electron beam used to scan the virus particle).

The M protein is the main structural protein of the envelope that provides the overall shape and is a type III membrane protein. It consists of 218 to 263 amino acid residues and forms a layer 7.8 nm thick. It has three domains, a short N-terminal ectodomain, a triple-spanning transmembrane domain, and a C-terminal endodomain. The C-terminal domain forms a matrix-like lattice that adds to the extra-thickness of the envelope. Different species can have either N- or O-linked glycans in their protein amino-terminal domain. The M protein is crucial during the assembly, budding, envelope formation, and pathogenesis stages of the virus lifecycle.

The E proteins are minor structural proteins and highly variable in different species. There are only about 20 copies of the E protein molecule in a coronavirus particle. They are 8.4 to 12 kDa in size and are composed of 76 to 109 amino acids. They are integral proteins (i.e. embedded in the lipid layer) and have two domains namely a transmembrane domain and an extramembrane C-terminal domain. They are almost fully α-helical, with a single α-helical transmembrane domain, and form pentameric (five-molecular) ion channels in the lipid bilayer. They are responsible for virion assembly, intracellular trafficking and morphogenesis (budding).

The spikes are the most distinguishing feature of coronaviruses and are responsible for the corona- or halo-like surface. On average a coronavirus particle has 74 surface spikes.[53] Each spike is about 20 nm long and is composed of a trimer of the S protein. The S protein is in turn composed of an S1 and S2 subunit. The homotrimeric S protein is a class I fusion protein which mediates the receptor binding and membrane fusion between the virus and host cell. The S1 subunit forms the head of the spike and has the receptor-binding domain (RBD). The S2 subunit forms the stem which anchors the spike in the viral envelope and on protease activation enables fusion. The two subunits remain noncovalently linked as they are exposed on the viral surface until they attach to the host cell membrane. In a functionally active state, three

S1 are attached to two S2 subunits. The subunit complex is split into individual subunits when the virus binds and fuses with the host cell under the action of proteases such as cathepsin family and transmembrane protease serine 2 (TMPRSS2) of the host cell.

After binding of the ACE2 receptor, SARS-CoV spike is activated and cleaved at the S1/S2 level.

S1 proteins are the most critical components in terms of infection. They are also the most variable components as they are responsible for host cell specificity. They possess two major domains named N-terminal domain (S1-NTD) and C-terminal domain (S1-CTD), both of which serve as the receptor-binding domains. The NTDs recognize and bind sugars on the surface of the host cell. An exception is the MHV NTD that binds to a protein receptor carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1). S1-CTDs are responsible for recognizing different protein receptors such as angiotensin-converting enzyme 2 (ACE2), aminopeptidase N (APN), and dipeptidyl peptidase 4 (DPP4).

A subset of coronaviruses (specifically the members of betacoronavirus subgroup A) also has a shorter spike-like surface protein called hemagglutinin esterase (HE). The HE proteins occur as homodimers composed of about 400 amino acid residues and are 40 to 50 kDa in size. They appear as tiny surface projections of 5 to 7 nm long embedded in between the spikes. They help in the attachment to and detachment from the host cell.

Inside the envelope, there is the nucleocapsid, which is formed from multiple copies of the nucleocapsid (N) protein, which are bound to the positive-sense single-stranded RNA genome in a continuous beads-on-a-string type conformation. N protein is a phosphoprotein of 43 to 50 kDa in size, and is divided into three conserved domains. The majority of the protein is made up of domains 1 and 2, which are typically rich in arginines and lysines. Domain 3 has a short carboxy terminal end and has a net negative charge due to excess of acidic over basic amino acid residues.

Genome

Coronaviruses contain a positive-sense, single-stranded RNA genome. The genome size for coronaviruses ranges from 26.4 to 31.7 kilobases. The genome size is one of the largest among RNA viruses. The genome has a 5′ methylated cap and a 3′ polyadenylated tail.

The genome organization for a coronavirus is 5′-leader-UTR-replicase (ORF1ab)-spike (S)-envelope (E)-membrane (M)-nucleocapsid (N)-3′UTR-poly (A) tail. The open reading frames 1a and 1b, which occupy the first two-thirds of the genome, encode the replicase polyprotein (pp1ab). The replicase polyprotein self cleaves to form 16 nonstructural proteins (nsp1–nsp16).

The later reading frames encode the four major structural proteins: spike, envelope, membrane, and nucleocapsid.[Interspersed between these reading frames are the reading frames for the accessory proteins. The number of accessory proteins and their function is unique depending on the specific coronavirus.

The life cycle of a coronavirus

Infection begins when the viral spike protein attaches to its complementary host cell receptor. After attachment, a protease of the host cell cleaves and activates the receptor-attached spike protein. Depending on the host cell protease available, cleavage and activation allows the virus to enter the host cell by endocytosis or direct fusion of the viral envelope with the host membrane.

Coronaviruses can enter cells by either fusing to their lipid envelope with the cell membrane on the cell surface or by internalization via endocytosis.

Insertion of a cleavage site can boost viral entry in different cell types by enabling promiscuous cleavage of the spike when contacting different proteases.

Genome translation

On entry into the host cell, the virus particle is uncoated, and its genome enters the cell cytoplasm. The coronavirus RNA genome has a 5’ methylated cap and a 3’ polyadenylated tail, which allows it to act like a messenger RNA and be directly translated by the host cell's ribosomes. The host ribosomes translate the initial overlapping open reading frames ORF1a and ORF1b of the virus genome into two large overlapping polyproteins, pp1a and pp1ab.

The larger polyprotein pp1ab is a result of a -1 ribosomal frameshift caused by a slippery sequence (UUUAAAC) and a downstream RNA pseudoknot at the end of open reading frame ORF1a. The ribosomal frameshift allows for the continuous translation of ORF1a followed by ORF1b.

The polyproteins have their own proteases, PLpro (nsp3) and 3CLpro (nsp5), which cleave the polyproteins at different specific sites. The cleavage of polyprotein pp1ab yields 16 nonstructural proteins (nsp1 to nsp16). Product proteins include various replication proteins such as RNA-dependent RNA polymerase (nsp12), RNA helicase (nsp13), and exoribonuclease (nsp14).

Replicase-transcriptase

Replicase-transcriptase complex

A number of the nonstructural proteins coalesce to form a multi-protein replicase-transcriptase complex (RTC). The main replicase-transcriptase protein is the RNA-dependent RNA polymerase (RdRp). It is directly involved in the replication and transcription of RNA from an RNA strand. The other nonstructural proteins in the complex assist in the replication and transcription process. The exoribonuclease nonstructural protein, for instance, provides extra fidelity to replication by providing a proofreading function which the RNA-dependent RNA polymerase lacks.

Replication – One of the main functions of the complex is to replicate the viral genome. RdRp directly mediates the synthesis of negative-sense genomic RNA from the positive-sense genomic RNA. This is followed by the replication of positive-sense genomic RNA from the negative-sense genomic RNA.

Transcription – The other important function of the complex is to transcribe the viral genome. RdRp directly mediates the synthesis of negative-sense subgenomic RNA molecules from the positive-sense genomic RNA. This process is followed by the transcription of these negative-sense subgenomic RNA molecules to their corresponding positive-sense mRNAs. The subgenomic mRNAs form a "nested set" which have a common 5'-head and partially duplicate 3'-end.

Recombination – The replicase-transcriptase complex is also capable of genetic recombination when at least two viral genomes are present in the same infected cell. RNA recombination appears to be a major driving force in determining genetic variability within a coronavirus species, the capability of a coronavirus species to jump from one host to another and, infrequently, in determining the emergence of novel coronaviruses.[64] The exact mechanism of recombination in coronaviruses is unclear, but likely involves template switching during genome replication.

Assembly and release

The replicated positive-sense genomic RNA becomes the genome of the progeny viruses. The mRNAs are gene transcripts of the last third of the virus genome after the initial overlapping reading frame. These mRNAs are translated by the host's ribosomes into the structural proteins and many accessory proteins. RNA translation occurs inside the endoplasmic reticulum. The viral structural proteins S, E, and M move along the secretory pathway into the Golgi intermediate compartment. There, the M proteins direct most protein-protein interactions required for the assembly of viruses following its binding to the nucleocapsid. Progeny viruses are then released from the host cell by exocytosis through secretory vesicles. Once released the viruses can infect other host cells.

Transmission

Infected carriers are able to shed viruses into the environment. The interaction of the coronavirus spike protein with its complementary cell receptor is central in determining the tissue tropism, infectivity, and species range of the released virus. Coronaviruses mainly target epithelial cells. They are transmitted from one host to another host, depending on the coronavirus species, by either an aerosol, fomite, or fecal-oral route.

Human coronaviruses infect the epithelial cells of the respiratory tract, while animal coronaviruses generally infect the epithelial cells of the digestive tract. SARS coronavirus, for example, infects the human epithelial cells of the lungs via an aerosol route by binding to the angiotensin-converting enzyme 2 (ACE2) receptor. Transmissible gastroenteritis coronavirus (TGEV) infects the pig epithelial cells of the digestive tract via a fecal–oral route by binding to the alanine aminopeptidase (APN) receptor.

Classification

Phylogenetic tree of coronaviruses

Coronaviruses form the subfamily Orthocoronavirinae, which is one of two subfamilies in the family Coronaviridae, order Nidovirales, and realm Riboviria. They are divided into the four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus.
Alphacoronaviruses and betacoronaviruses infect mammals, while gammacoronaviruses and deltacoronaviruses primarily infect birds.

Genus: Alphacoronavirus;

Species: Alphacoronavirus 1 (TGEV, Feline coronavirus, Canine coronavirus), Human coronavirus 229E, Human coronavirus NL63, Miniopterus bat coronavirus 1, Miniopterus bat coronavirus HKU8, Porcine epidemic diarrhea virus, Rhinolophus bat coronavirus HKU2, Scotophilus bat coronavirus 512

Genus Betacoronavirus;

Species: Betacoronavirus 1 (Bovine Coronavirus, Human coronavirus OC43), Hedgehog coronavirus 1, Human coronavirus HKU1, Middle East respiratory syndrome-related coronavirus, Murine coronavirus, Pipistrellus bat coronavirus HKU5, Rousettus bat coronavirus HKU9, Severe acute respiratory syndrome–related coronavirus (SARS-CoV-1, SARS-CoV-2), Tylonycteris bat coronavirus HKU4

Genus Gammacoronavirus:

Species: Avian coronavirus, Beluga whale coronavirus SW1

Genus Deltacoronavirus

Species: Bulbul coronavirus HKU11, Porcine coronavirus HKU15

Origin

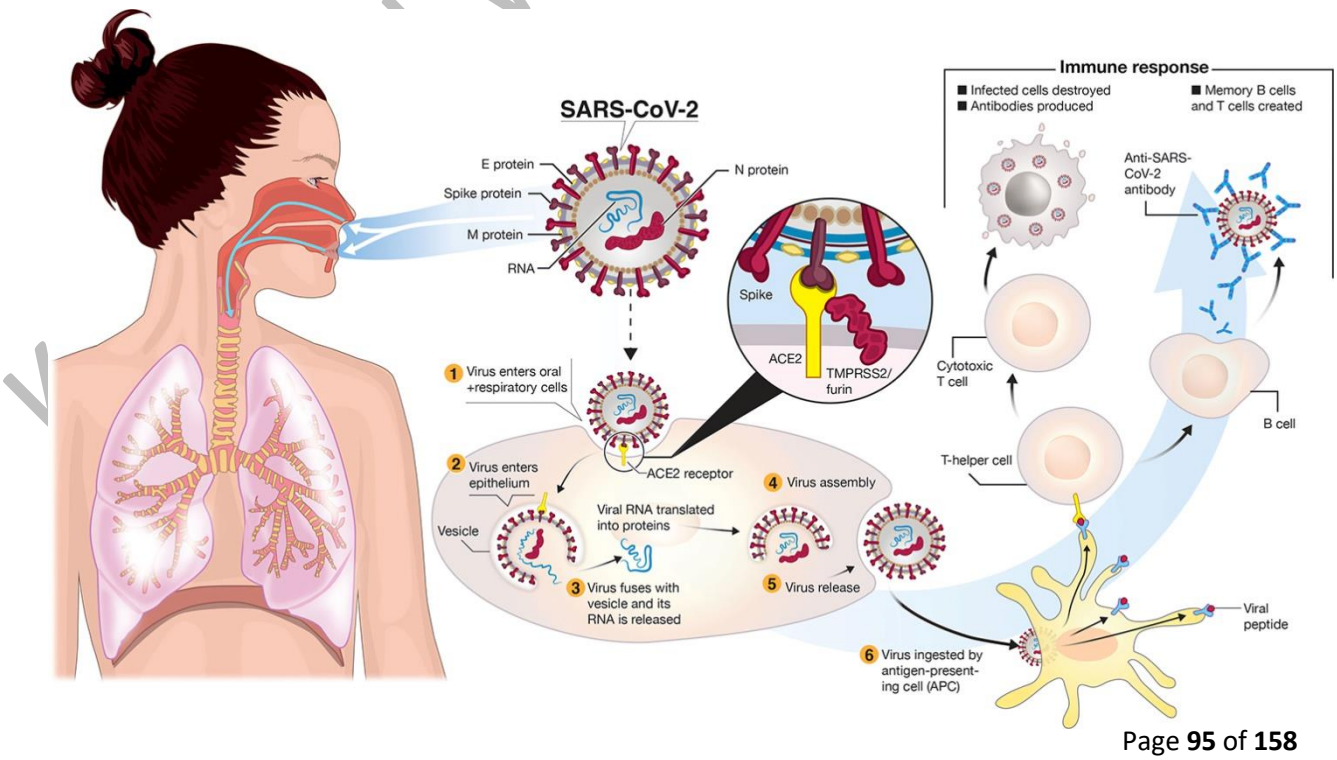
Origins of human coronaviruses with possible intermediate hosts

The most recent common ancestor (MRCA) of all coronaviruses is estimated to have existed as recently as 8000 BCE, although some models place the common ancestor as far back as 55 million years or more, implying long term coevolution with bat and avian species. The most recent common ancestor of the alphacoronavirus line has been placed at about 2400 BCE, of the betacoronavirus line at 3300 BCE, of the gammacoronavirus line at 2800 BCE, and the deltacoronavirus line at about 3000 BCE. Bats and birds, as warm-blooded flying vertebrates, are an ideal natural reservoir for the coronavirus gene pool (with bats the reservoir for alphacoronaviruses and betacoronavirus – and birds the reservoir for gammacoronaviruses and deltacoronaviruses). The large number and global range of bat and avian species that host viruses have enabled extensive evolution and dissemination of coronaviruses.

Many human coronaviruses have their origin in bats. The human coronavirus NL63 shared a common ancestor with a bat coronavirus (ARCoV.2) between 1190 and 1449 CE. The human coronavirus 229E shared a common ancestor with a bat coronavirus (GhanaGrp1 Bt CoV) between 1686 and 1800 CE. More recently, alpaca coronavirus and human coronavirus 229E diverged sometime before 1960. MERS-CoV emerged in humans from bats through the intermediate host of camels. MERS-CoV, although related to several bat coronavirus species, appears to have diverged from these several centuries ago. The most closely related bat coronavirus and SARS-CoV diverged in 1986. The ancestors of SARS-CoV first infected leaf-nose bats of the genus Hipposideridae; subsequently, they spread to horseshoe bats in the species Rhinolophidae, then to Asian palm civets, and finally to humans.

Unlike other betacoronaviruses, bovine coronavirus of the species Betacoronavirus 1 and subgenus Embecovirus is thought to have originated in rodents and not in bats. In the 1790s, equine coronavirus diverged from the bovine coronavirus after a cross-species jump.[86] Later in the 1890s, human coronavirus OC43 diverged from bovine coronavirus after another cross-species spillover event. It is speculated that the flu pandemic of 1890 may have been caused by this spillover event, and not by the influenza virus, because of the related timing, neurological symptoms, and unknown causative agent of the pandemic. Besides causing respiratory infections, human coronavirus OC43 is also suspected of playing a role in neurological diseases. In the 1950s, the human coronavirus OC43 began to diverge into its present genotypes. Phylogenetically, mouse hepatitis virus (Murine coronavirus), which infects the mouse's liver and central nervous system, is related to human coronavirus OC43 and bovine coronavirus. Human coronavirus HKU1, like the aforementioned viruses, also has its origins in rodents.

Infection in humans



Transmission and life-cycle of SARS-CoV-2 causing COVID-19

Coronaviruses vary significantly in risk factor. Some can kill more than 30% of those infected, such as MERS-CoV, and some are relatively harmless, such as the common cold. Coronaviruses can cause colds with major symptoms, such as fever, and a sore throat from swollen adenoids. Coronaviruses can cause pneumonia (either direct viral pneumonia or secondary bacterial pneumonia) and bronchitis (either direct viral bronchitis or secondary bacterial bronchitis). The human coronavirus discovered in 2003, SARS-CoV, which causes severe acute respiratory syndrome (SARS), has a unique pathogenesis because it causes both upper and lower respiratory tract infections.

Six species of human coronaviruses are known, with one species subdivided into two different strains, making seven strains of human coronaviruses altogether.

Seasonal distribution of HCoV-NL63 in Germany shows a preferential detection from November to March.

Four human coronaviruses produce symptoms that are generally mild, even though it is contended they might have been more aggressive in the past:[94]

1. Human coronavirus OC43 (HCoV-OC43), β-CoV
2. Human coronavirus HKU1 (HCoV-HKU1), β-CoV
3. Human coronavirus 229E (HCoV-229E), α-CoV
4. Human coronavirus NL63 (HCoV-NL63), α-CoV–

Three human coronaviruses produce potentially severe symptoms:

1. Severe acute respiratory syndrome coronavirus (SARS-CoV), β-CoV (identified in 2003)
2. Middle East respiratory syndrome-related coronavirus (MERS-CoV), β-CoV (identified in 2012)
3. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), β-CoV (identified in 2019)

These cause the diseases commonly called SARS, MERS, and COVID-19 respectively.

Common cold

Although the common cold is usually caused by rhinoviruses, in about 15% of cases the cause is a coronavirus. The human coronaviruses HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoV-NL63 continually circulate in the human population in adults and children worldwide and produce the generally mild symptoms of the common cold. The four mild coronaviruses have a seasonal incidence occurring in the winter months in temperate climates. There is no preponderance in any season in tropical climates

INFECTION IN ANIMALS

Coronaviruses have been recognized as causing pathological conditions in veterinary medicine since the 1930s. They infect a range of animals including swine, cattle, horses, camels, cats, dogs, rodents, birds and bats. The majority of animal related coronaviruses infect the intestinal tract and are transmitted by a fecal-oral route. Significant research efforts have been focused on elucidating the viral pathogenesis of these animal coronaviruses, especially by virologists interested in veterinary and zoonotic diseases

PREVENTION AND TREATMENT

A number of vaccines using different methods have been developed against human coronavirus SARS-CoV-2. Antiviral targets against human coronaviruses have also been identified such as viral proteases, polymerases, and entry proteins. Drugs are in development which target these proteins and the different steps of viral replication

Vaccines are available for animal coronaviruses IBV, TGEV, and Canine CoV, although their effectiveness is limited. In the case of outbreaks of highly contagious animal coronaviruses, such as PEDV, measures such as destruction of entire herds of pigs may be used to prevent transmission to other herds.

ELECTRON TECHNIQUES FOR DETECTION OF A VIRUS

Immuno-electron microscopy (IEM) is based on the same serological principles as ELISA, and can be used for further virus identification during routine TEM diagnosis. IEM has the advantage that it works directly with raw serum, so no further purification of immunoglobulins or conjugation steps are required. Due to the small reaction volumes required, antibody consumption is low. Most TEM laboratories keep comprehensive collections of antisera specific to a broad spectrum of virus species and isolates. Antisera can be stored long term at 4°C in the presence of 0.05% sodium azide without significant loss of activity. Depending on the composition of antigens, as well as the epitopes present in the original virus purification used for immunization, polyclonal antisera can be heterogeneous in their reaction. Therefore, antisera can serve different objectives during routine diagnosis

Homogenized tissues with high virus concentrations, as well as purified virus preparations, are suitable for determination of particle size. If suitable antibodies are available, low virus titer in the original material can be enriched by pre-incubation of the grid. For unknown viruses, data gained from virion measurements and morphology provides information on genome organization and genome length as well as particle stability. This is also applicable to validation of artificially generated virus genomes, infectious full-length clones or derived virus mutants. Particle length distribution discloses valuable data on the multi-component nature of a virus and the encapsidation of subgenomic or satellite sequences. IEM was seminal in demonstrating the bipolar structure of some helical viruses

When using embedded material, EM facilitates single cell analyses as well as direct comparison of adjacent cells from distinct tissues, e.g., leaf parenchyma with vasculature. Modified organelles, membranes as well as generated structures harboring viral replication complexes can be correlated with the infection process the ultrastructural localization, function and interaction of viral proteins as well as dsRNA molecules with the host have been investigated in artificial expression systems. These methods were also applied to study systemic spread in the host including intracellular and intercellular movement with cell-to-cell movement and long distance movement. Recent studies have revealed the function of host susceptibility factors comprising membrane-acting ESCRT and SNARE proteins during replication of tombusviruses and production of budded baculovirions, respectively). A process akin to autophagy was shown to be beneficial for replication of coccolithoviruses in algae.

Immunogold labeling enhances imaging of antibody binding furthermore, as well as facilitating localization and quantification, it enables multiple labeling on the same object by using gold particles of different size. In metal-tagging TEM (METTEM) viral proteins are fused with metallothionein, which, after incubation with gold salts, leads to the production of electron-dense gold nanoclusters). This technology has been used in combination with immunogold labeling and for three-dimensional (3D) imaging of the interaction of viral replicase with viral RNA Accuracy in virus quantification can be improved by using a scanning transmission electron microscopy detector (STEM, in a scanning electron microscope (SEM).

Combined use of TEM and SEM improves characterization of larger objects like baculovirus occlusion bodies. TEM enables high resolution of virion structure, localization, and measurement of particles and ultrastructural details within embedded occlusion body preparations. On the other hand, SEM analyses are ideal for high throughput screening of samples, quality control of preparations, and measurements for comparison of different isolates.

Incorporation of pressure-limiting apertures and gaseous detection devices allows direct investigation of hydrated biological samples using SEM Variable pressure can be adjusted to object sensitivity This technique enables high-throughput screening of material for virus-transmitting arthropod vectors. Furthermore, live imaging of developmental stages and vector interaction with the host surface is now possible.

The use of back scattered electron detectors in field emission SEM permits an enlarged field of view. Thus, large cellular volumes embedded in resin sections can be visualized at high resolution

SEM works well in direct combination with light microscopy, and datasets for 3D reconstruction can be obtained easily The correlation of light and electron microscopy (CLEM) combines the advantages of both methods—the ability to simultaneously locate the target in a comparatively large volume and determine its ultrastructure . With the increasing speed of developments in the field of microscopy, CLEM offers a broad spectrum of applications depending on the specific question. Good knowledge of viral replication is mandatory for designing antiviral strategies and therapies. Here, by employing fluorescent-tagged molecules, CLEM can be very helpful in finding cells of interest within layers of tissue from living samples or derived cultures

Cryo-Electron Microscopy

The introduction of direct electron detectors (DEDs) and advances in image processing have extended the resolution limit of electron cryo-microscopy (cryo-EM) into the atomic range, allowing ab initio atomic model building. Cryo-EM is ideally suited to exploring the 3D structure of macromolecular assemblies, and elucidation of the 3D arrangement of such complexes helps understand their function in living cells. These technological developments have always involved analyses of viruses, particularly plant viruses, because their symmetrical capsids, as well as the availability of highly pure samples, greatly facilitates reconstruction. Tobacco mosaic virus (TMV)—one of the very first objects to be seen in an electron microscope has been used to evaluate 3D reconstructions from data recorded on different DEDs illustrating improvements in resolution into the 3 Å range compared to the 4–5 Å obtained with CCD cameras under optimal conditions.

Encapsidation of the viral genome is an essential step of virus particle assembly and, more generally, of the viral life cycle. Cryo-EM now paves the way to elucidating mechanisms of capsid assembly and genome encapsidation, and to understanding the mechanisms that ensure only the viral genome is specifically packaged from among a background of myriad host DNAs/RNAs. Cowpea mosaic virus (CPMV)—a positive-sense, single-stranded RNA plant virus—and other members of the order Picornavirales have been investigated intensively in recent decades. Very recently, high-resolution cryo-EM structures of wild type and empty virus-like particles have been determined, implicating the C-terminal region of the small coat protein (CP) subunit as being required for virus assembly. The wild-type structure reveals the dense nature of the RNA inside the capsid shell, with an arrangement suggesting extensive base-pairing during encapsidation.

Viral diagnosis techniques

Cytopathic effect:

- Many viruses can be detected and initially identified by observation of the morphological changes in the cultured cells in which they replicate.
- The CPE produced by different types of viruses are characteristic and help in the initial identification of virus isolates.
- Nuclear shrinking, vacuoles in the cytoplasm, syncytia formation, rounding up, and detachment are the examples ofalteration of morphology of the cells.
- Most CPEs can be demonstrated in unfixed and unstained monolayer of cells under low power of microscope.
- For example, adenoviruses produce large granular changes resembling bunches of grapes, SV-14 produces well-defined cytoplasmic vacuolation, measles virus produces syncytium formation, herpes virus produces discrete focal degeneration, and enteroviruses cause crenation of cells and degeneration of the entire cell sheet.

Hemadsorption:

- Hemadsorption is the process of adsorption of erythrocytes to the surfaces of infected cells which serves as an indirect measurement of viral protein synthesis.
- This property is made use of to detect infection with noncytocidal viruses as well as the early stage of cytocidal viruses.
- Viruses, such as influenza virus, parainfluenza virus, mumps virus, and togavirus, when infect cell lines code for the expression of red cell agglutinins, which are expressed on the infected cell membrane during infections.
- These hemagglutinins bind some erythrocytes to the infected cell surface.
- Sometimes, viruses can be detected by agglutination of erythrocytes in the culture medium.

Heterologous interference:

- This property is used to detect viruses that do not produce classic CPEs in the cell lines.
- In this method, the growth of non-CPE-producing virus in cell culture can be tested by subsequent challenge with a virus known to produce CPEs.
- The growth of the first virus will inhibit infection by the cytopathic challenge virus by interference.
- For example, rubella virus usually does not produce any CPE, but prevents the replication of picornaviruses, which is inoculated as a cytopathic challenge virus.

Transformation:

- Oncogenic viruses that are associated with formation of tumors induce cell transformation and loss of contact inhibition in the infected cell lines.
- This leads to surface growth that appears in a piled-up fashion producing microtumors.
- Examples of such oncogenic viruses that produce transformation in cell lines are some herpes viruses, adenoviruses, hepadanoviruses, papovavirus, and retroviruses.

Light microscopy:

- Viral antigens in infected cell cultures are demonstrated by staining virus-infected cells of tissue sections with specific viral antibody conjugated with horseradish peroxidase.
- This is followed by addition of hydrogen peroxide along with a benzidine derivative substance.
- In a positive reaction, a red insoluble precipitate is deposited on the cell line, which is demonstrated by examination under ordinary light microscope.

Immunofluorescence:

- Direct immunofluorescence using specific antibodies is frequently used to detect viral antigens in inoculated cell lines for identification of viruses.

Electron microscopy:

- The viruses can also be demonstrated in infected cell lines by EM.

Diagnostic virology is concerned with identifying the virus associated with clinical signs and symptoms. Procedures most commonly used include:

1. Detection of a meaningful immune response to the virus (antibody or cell-mediated) by immunologic assay(s)

2. Identification of the agent by staining of specimens or sections of tissue (light and electron microscopy)

3. Isolation and identification of the agent (cell culture or fertile eggs)

4. Detection of viral nucleic acid (probes or amplification). Detection of Immune Response Often, it is difficult to identify a virus in relation to the disease observed, or when conducting a retrospective study of a population to determine exposure to a virus, or when measuring the response of an individual to a vaccine. In these cases, indirect methods of measure are needed, such as measuring antibody response to the virus of interest.

Several methods exist for this purpose. A few of the most commonly used methods include:

MOLECULAR METHODS OF VIRAL DIAGNOSIS

• Virus neutralization (VN)

• Hemagglutination inhibition (HI)

• Enzyme linked immunosorbent assay (ELISA)

• Indirect fluorescent antibody (IFA)

• Complement fixation (CF)

• Agar-gel immunodiffusion (AGID)

• Agar-gel precipitin (AGP)

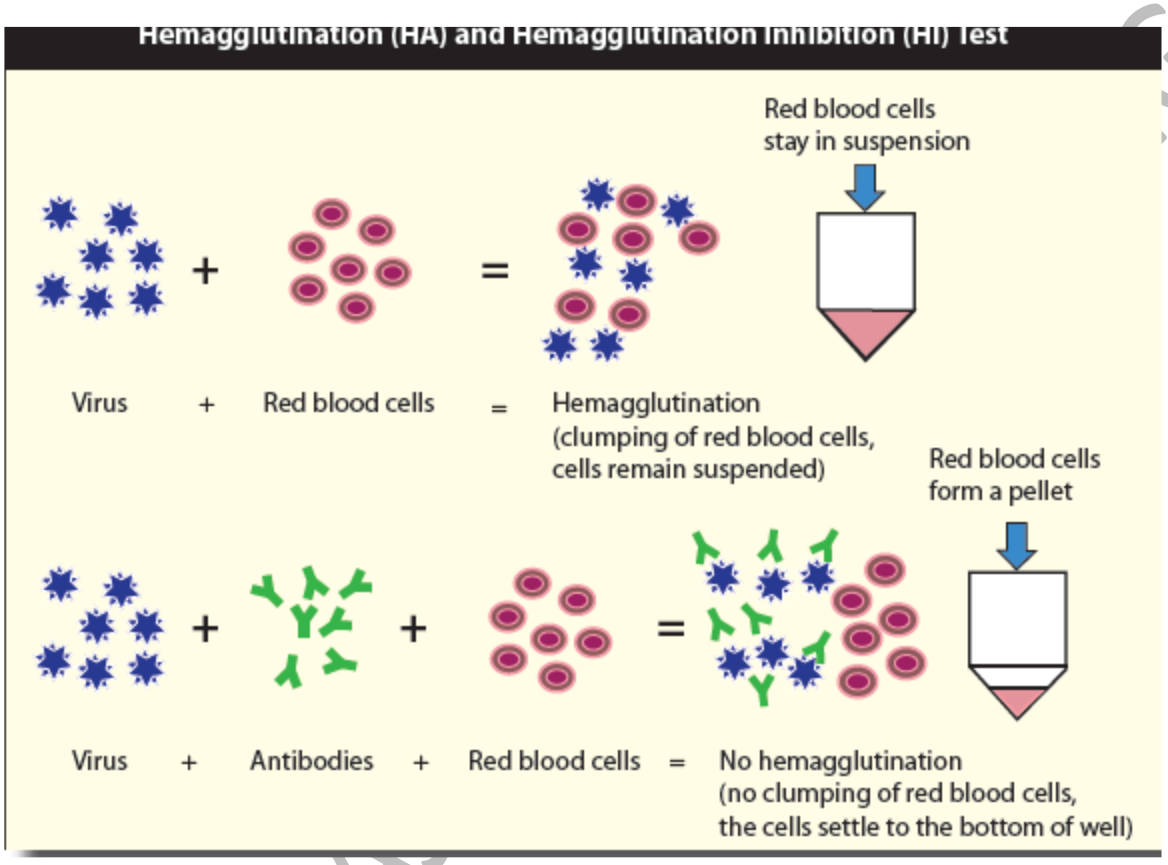
• Latex agglutination (LA)

The principles of these assays are fundamentally the same, they depend upon antibody-antigen interactions and consist of a known virus or viral protein, a patient sample (usually serum), and an indicator. If antibodies are present in the patient's serum, they will bind to the virus. If no antibodies are present, no binding will occur. The indicator is observed to determine whether the sample is positive or negative for antibodies.

Virus Neutralization

In the virus neutralization (VN) test, the sample of interest is incubated with the target virus and changes in cell culture are observed (called cytopathic effect, CPE). If the sample contains antibodies, it will prevent the virus from growing in the cell culture and no CPE will be observed. If no antibodies are present in the sample, the virus will grow and CPE will be observed.

Hemagglutination Inhibition



Certain viruses have a protein on their surface that interacts with red blood cells and is able to attach to them. This property is called hemagglutination and the surface protein of the virus is hemagglutinin. The inhibition or blocking of this activity is the basis of the hemagglutination inhibition (HI) test. The most well known virus with this property is the influenza virus. Like the virus neutralization (VN) test, the patient's serum sample is incubated with the virus of interest but instead of growing the virus in cells, red blood cells are added to the virus-serum mix. If antibodies are present, the hemagglutination activity will be blocked; if no antibodies are present the virus will agglutinate (bind together). In this case the red blood cells are the indicator.

Enzyme-Linked Immunosorbent Assay

The enzyme-linked immunosorbent assay ELISA is a very popular technique due to the ease of use and low cost. The ELISA consists of plastic wells coated with either the antigen (virus) of interest

or a protein specific to the antigen (virus) of interest. The unknown sample (serum) is allowed to bind to the coated well, an antibody labeled with an enzyme is applied, an indicator is added, and then a color change is observed. The presence of color indicates the presence of antibodies and the absence of color indicates the absence of antibodies.

Agar-Gel Immunodiffusions (AGID)

The agar-gel immunodiffusion (AGID), also referred to as an agar gel precipitin (AGP) test, involves the diffusion of virus and antibody through an agar (gelatin-like substance), which will form a line of identity where the antigen-antibody complexes form.

Complement Fixation Test

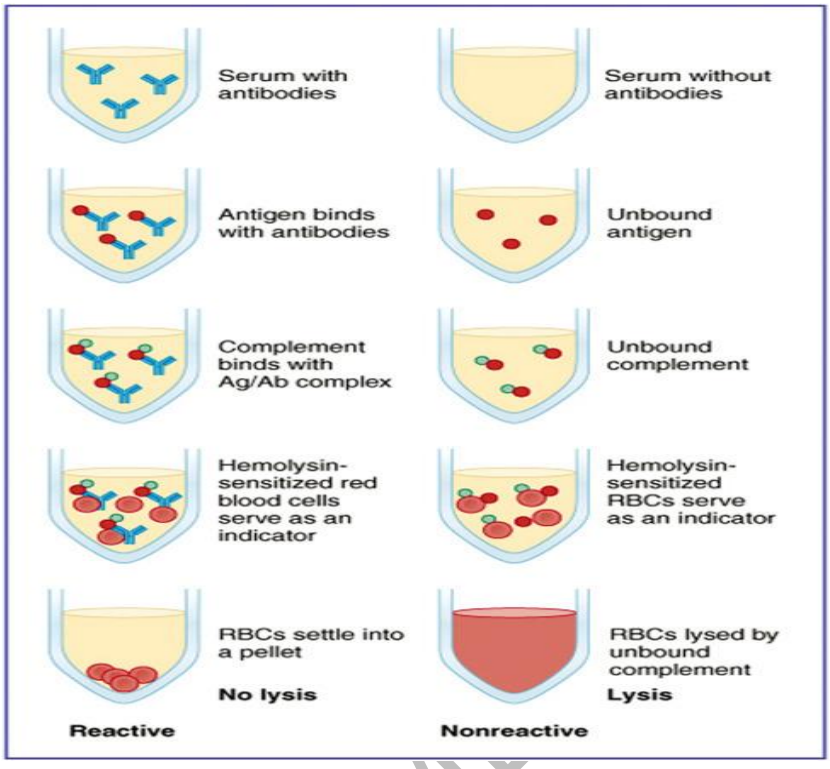
Procedure

1. A serum sample is taken.
2. It is then heated at about 56 °C to remove the complement proteins already present in the sample.
3. The serum is then adsorbed with washed sheep RBC. It prevents interference in the test by anti-RBC antibodies which are cross-reactive.
4. Then the antigen and complement are added to the sample.
5. It is then subjected to incubation at a temperature of 37 °C for 30 minutes. It provides conditions and time for the formation of the Ag-Ab complex.
6. And the indicator system is then added and the sample is observed for change due to occurrence or non-occurrence of hemolysis.

If the sample contains the specific antibody or antigen of interest, there will be no change in the solution after the test and it will be considered a positive test. The non- hemolyzed sensitized RBC will remain as it is and settle down in the sample.

If there is some change in the appearance of the sample or solution during the test due to hemolysis, it will be considered a negative test.

COMPLEMENT FIXATION TEST



Antiviral Agents

Since viruses lack the structures and metabolic processes that are altered by common antibiotics, antibiotics are virtually useless in treating viral infections. To date, relatively few antiviral chemotherapeutic agents are available and used to treat just a few limited viruses.

Most of the antiviral agents work by inhibiting viral DNA synthesis. These drugs chemically resemble normal DNA nucleosides, molecules containing deoxyribose and either adenine, guanine, cytosine, or thymine. Viral enzymes then add phosphate groups to these nucleoside analogs to form DNA nucleotide analogs. The DNA nucleotide analogs are then inserted into the growing viral DNA strand in place of a normal nucleotide. Once inserted, however, new nucleotides can't attach and DNA synthesis is stopped. They are selectively toxic because viral polymerases are more prone to incorporate nucleotide analogs into their nucleic acid than are host cell polymerases.

Antivirals used for viruses other than HIV		
Antiviral	**Brand Name**	**Use**
Amantadine	Symmetrel	used prophylactically against influenza A) in high-risk individuals. It prevents influenza A viruses from the uncoating step necessary for viral replication.
Rimantidine	Flumadine	used for treatment and prophylaxis of influenza A. It prevents influenza A viruses from the uncoating step necessary for viral replication.
zanamivir:	Relenza	used to limit the duration of influenza A and B infections. It is an inhibitor of the influenza virus surface enzyme called neuraminidase that is needed for release of newly formed influenza viruses from the infected cell.
Oseltamivir	Tamiflu	used limit the duration of influenza infections. It is an inhibitor of the influenza virus surface enzyme called neuraminidase that is needed for release of newly formed influenza viruses from the infected cell.
Acyclovir	Zovirax	used against herpes simplex viruses (HSV) to treat genital herpes, mucocutaneous herpes in the immunosuppressed, HSV encephalitis, neonatal herpes, and to reduce the rate of recurrences of genital herpes. It is also used against varicella zoster viruses (VZV)) to treat shingles. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.
Trifluridine	Viroptic	used to treat eye infection (keratitis and conjunctivitis) caused by HSV. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.

Antivirals used for viruses other than HIV		
Antiviral	**Brand Name**	**Use**
Famciclovir	Famvir	used to treat HSV and VZV infections. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.
Valacyclovir	Valtrex	used to treat HSV and VZV infections. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.
Penciclovir	Denavir	used in treating HSV infections. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.
Gancyclovir	Cytovene; Vitrasert	used in treating severe cytomegalovirus (CMV) infections such as retinitis. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.
valganciclovir	Valcyte	used in treating severe CMV infections such as retinitis). It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.
Foscarnet	Foscavir	used in treating severe CMV infections such as retinitis. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.
Cidofovir	Vistide	used in treating CMV retinitis. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.

Antivirals used for viruses other than HIV		
Antiviral	**Brand Name**	**Use**
Fomivirsen	Vitravene	used in treating CMV retinitis. Fomivirsen inhibits cytomegalovirus (CMV) replication through an antisense RNA (microRNA or miRNA mechanism. The nucleotide sequence of fomivirsen is complementary to a sequence in mRNA transcripts (Figure 10.10.110.10.1) that encodes several proteins responsible for regulation of viral gene expression that are essential for production of infectious CMV. Binding of fomivirsen to the target mRNA results in inhibition of protein synthesis, subsequently inhibiting virus replication.
Ribavirin	Copegus; Rebetol; Virazole	used in treating severe acute respiratory syndrome (SARS). In combination with other drugs it is used to treat hepatitis C virus (HCV). It chemically resembles a normal RNA nucleoside. Once inserted into the growing RNA chain it inhibits further viral RNA replication.
Telaprevir	Incivek	for the treatment of chronic hepatitis C (hepatitis C virus or HCV genotype 1). It is a protease inhibitor that binds to the active site of an HCV-encoded protease and prevent it from cleaving the long polyprotein from polycistronic HCV genes into proteins essential to the structure and function of HCV.
boceprevir	Victrelis	for the treatment of chronic hepatitis C (hepatitis C virus or HCV genotype 1) infection. It is used in combination with peginterferon alfa and ribavirin. Boceprevir is a protease inhibitor that binds to the active site of an HCV-encoded protease and prevent it from cleaving the long polyprotein from polycistronic HCV genes into proteins essential to the structure and function of HCV.
Simeprevir	Olysio	use for the treatment of chronic hepatitis C (hepatitis C

Antivirals used for viruses other than HIV		
Antiviral	**Brand Name**	**Use**
		virus or HCV genotype 1) infection. Used in combination with peginterferon alfa and ribavirin. Simeprevir is a protease inhibitor that binds to the active site of an HCV-encoded protease and prevent it from cleaving the long polyprotein from polycistronic HCV genes into proteins essential to the structure and function of HCV.
Sofosbuvir	Sovaldi	Use for the treatment of chronic hepatitis C infection. Used in combination with ribavirin for hepatitis C virus or HCV genotypes 2 and 4; used in combination with peginterferon alfa and ribavirin for HCV genotypes 1 and 4. The second indication is the first approval of an interferon-free regimen for the treatment of chronic HCV infection. Sofosbuvir is a nucleotide polymerase inhibitor that binds to the active site of an HCV-encoded RNA polymerase preventing the synthesis of the viral RNA genome.
Lamivudine	Epivir-HBV	used in treating chronic hepatitis B. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.
adefovir dipivoxil	Hepsera	used in treating hepatitis B.

Antisense RNA. When an antisense RNA (microRNA or miRNA) that is complementary to a mRNA coding for a particular protein or enzyme binds to the mRNA by complementary base pairing, that mRNA cannot be translated and the protein or enzyme is not made.

Current anti-HIV drugs include the following (classified by their action):

HIV nucleoside-analog reverse transcriptase inhibitors

To replicate, HIV uses the enzyme reverse transcriptase to make a DNA copy of its RNA genome. A complementary copy of this DNA is then made to produce a double-stranded DNA intermediate which is able to insert into host cell chromosomes to form a provirus. Most reverse transcriptase inhibitors are nucleoside analogs. A nucleoside is part of the building block of DNA, consisting of a nitrogenous base bound to the sugar deoxyribose but no phosphate group. A nucleoside analog chemically resembles a normal nucleoside.

Zidovudine: A comparison of zidovudine (AZT, ZDV) and the deoxyribonucleotide containing the base thymine.

Once phosphate groups are added by either viral or host cell enzymes, the drugs now chemically resemble normal DNA nucleotides, the building block molecules for DNA synthesis. The nucleotide analog binds to the active site of the reverse transcriptase which, in turn, inserts it into the growing DNA strand in place of a normal nucleotide. Once inserted, however, new DNA nucleotides are unable to attach to the drug and DNA synthesis is stopped. This results in an incomplete provirus. For example, zidovudine (AZT, ZDV, Retrovir), as shown in resembles the deoxyribonucleotide containing the base thymine. Once zidovudine is inserted into the growing DNA strand being transcribed from the viral RNA by reverse transcriptase, no further nucleotides can be attached.

Zidovudine, (left) Step-1: In order for a DNA strand to elongate, the phosphate group of a free deoxyribonucleotide bonds to the hydroxyl (OH) on the 3' carbon of the deoxyribose of the last deoxyribonucleotide in the strand. (middle) **Step-2:** To see how zidovudine interferes with this process. (right) **Step-3:** Zidovudine (ZDV, AZT) has an azide (N3) group instead of a hydroxyl (OH) group on its pentose sugar. Once the phosphate group of the zidovudine bonds to OH of the last deoxyribonucleotide in the strand, no further free deoxyribonucleotides can attach. (The phosphate groups of free deoxyribonucleotides can only bond to OH groups, they are unable to bond to N3groups.) This results in an incomplete provirus.

Examples of nucleoside reverse transcriptase inhibitors include:

a) zidovudine (AZT; ZDV; Retrovir)

b) didanosine (ddI; dideoxyinosine; Videx)

c) stavudine (d4T; Zerit)

d) lamivudine (3TC; Epivir)

e) abacavir (ABC; Ziagen)

f) emtricitabine (FTC; Emtriva, Coviracil)

Nucleotide Reverse Transcriptase Inhibitors (NtRTIs)

A NtRTI inhibitor is a a nucleotide analog. A nucleotide is the building block of DNA, consisting of a nitrogenous base bound to the sugar deoxyribose, and a phosphate group. A nucleotide analog chemically resembles a normal nucleotide. The nucleotide analog binds to the active site of the reverse transcriptase which, in turn, inserts it into the growing DNA strand in place of a normal nucleotide. Once inserted, however, new DNA nucleotides are unable to attach to the drug and DNA synthesis is stopped. This results in an incomplete provirus. An example of nucleoside reverse transcriptase inhibitor is tenofovir (TDF;Viread).

3. HIV Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

These drugs do not resemble regular DNA building blocks. They bind to an allosteric site that regulates reverse transcriptase activity rather than to the enzyme's active site itself as do the above nucleoside analogues. This also prevents HIV provirus formation.

a) nevirapine (NVP; Viramune)
b) delavirdine (DLV;Rescriptor)
c) efavirenz (EFV; Sustiva)
d) rilpivirine (Edurant)
e) etravirine (ETR, TMC125; Intelence)

In order for maturation of HIV to occur, a HIV enzyme termed a protease has to cleave a long HIV-encoded gag-pol polyprotein to produce reverse transcriptase and integrase (coded by the HIV pol gene) and gag polyprotein (coded by the HIV gag gene). The HIV protease then cleaves the gag polyprotein into capsid protein p17, matrix protein p24, and nucleocapsid protein p7, as well as proteins p6, p2, and p1 whose functions are not yet fully understood .Proteases also cleave the env-polyprotein (coded by the HIV env gene) into the envelope glycoproteins gp120 and gp41 . Protease inhibitors are drugs that bind to the active site of this HIV-encoded protease and prevent it from cleaving the long gag-pol polyprotein and the gag polyprotein into essential proteins essential to the structure of HIV and to RNA packaging within its nucleocapsid. As a result, viral maturation does not occur and noninfectious viral particles are produced.

Protease inhibitors include:

a) saquinavir (SQV; Inverase)
b) ritonavir (RTV; Norvir)
c) idinavir (IDV; Crixivan)
d) nelfinavir (NFV; Viracept)
e) amprenavir (APV; Agenerase)

f) atazanavir (ATV; Reyataz)

g) fosamprenavir (FPV; Lexiva)

h) ritonavir (RTV; Norvir)

i) darunavir (DRV; TMC114; Prezista)

j) tipranavir (TPV; Aptivus)

Entry Inhibitors (EIs)

EIs are agents interfering with the entry of HIV-1 into cells. During the adsorption and penetration stages of the life cycle of HIV, a portion or domain of the HIV surface glycoprotein gp120 binds to a CD4 molecule on the host cell. This induces a change in shape that brings the chemokine receptor binding domains of the gp120 into proximity with the host cell chemokine receptor. This brings about another conformational change that exposes a previously buried portion of the transmembrane glycoprotein gp41 that enables the viral envelope to fuse with the host cell membrane. EIs interfere with various stages of this process.

a. Agents that block the binding of gp120 to host chemokine receptor 5 (CCR5).

After the gp120 on the envelope of HIV binds to a CD4 molecule on the host cell, it must then also bind to a co-receptor - a chemokine receptor. CCR5-tropic strains of HIV bind to the chemokine receptor CCR5. (An estimated 50%-60% of people having previously received HIV medication have circulating CCR5-tropic HIV.)

Maraviroc (MVC; Selzentry; Celsentri) is a chemokine receptor binding blocker that binds to CCR5 and blocks gp120 from binding to the co-receptor thus blocking adsorption of HIV to the host cell.

b. Agents that block the fusion of the viral envelope with the cytoplasmic membrane of the host cell.

Enfuvirtide (ENF; T-20; Fuzeon) binds a gp41 subunit of the viral envelope glycoprotein and prevents the conformational changes required for the fusion of the viral envelope with the cellular cytoplasmic membrane.

5. Integrase Inhibitors

Integrase inhibitors disable HIV integrase, the enzyme that inserts the HIV double-stranded DNA intermediate into host cell DNA. It prevents production of a provirus.

raltegravir (Isentress)

6. Fixed-dose combinations

Tablets containing two or more anti-HIV medications:

1. abacivir + lamivudine (Epzicom)

2. abacivir + lamivudine + zidovudine (Trizivir)
3. efavirenz + emtricitabine + tenofovir DF (Atripla)
4. emtricitabine + tenofovir DF (Truvada)
5. lamivudine + zidovudine (Combivir)

Certain antiviral cytokines called type-1 interferons have been produced by recombinant DNA technology and several are used to treat certain severe viral infections. These include:

1. Recombinant interferon alfa-2a (Roferon-A): a cytokine used to treat Kaposi's sarcoma, chronic myelogenous leukemia, and hairy cell leukemia.
2. Peginterferon alfa-2a (Pegasys) : used to treat hepatitis C (HCV).
3. Recombinant interferon-alpha 2b (Intron A): a cytokine produced by recombinant DNA technology and used to treat Hepatitis B; malignant melanoma, Kaposi's sarcoma, follicular lymphoma, hairy cell leukemia, warts, and Hepatitis C.
4. Peginterferon alfa-2b (PEG-Intron; PEG-Intron Redipen): used to treat hepatitis C (HCV).
5. Recombinant Interferon alfa-2b plus the antiviral drug ribavirin (Rebetron): used to treat hepatitis C (HCV).
6. Recombinant interferon-alpha n3 (Alferon N): used to treat warts.
7. Recombinant iInterferon alfacon-1 (Infergen) : used to treat hepatitis C (HCV).

Most of the current antiviral agents don't kill and eliminate the viruses, but rather inhibit their replication and decrease the severity of the disease. As with other microbes, resistant virus strains can emerge with treatment.

Since there are no antiviral drugs for the vast majority of viral infections and most drugs that are available are only partially effective against limited types of viruses, to control viruses, we must rely on the body's immune responses. As will be seen in detail in Units 5 and 6, the immune responses include innate immunity as well as adaptive immunity (antibody production and cell-mediated immunity). Adaptive immunity can be either naturally acquired or, in some cases, artificially acquired.

UNIT -IV

MEDICAL PARASITOLOGY

INTRODUCTION

Medical parasitology traditionally has included the study of three major groups of animals. They are parasitic protozoa, parasitic helminths (worms) and those arthropods that directly cause disease or act as vectors of various pathogens. A parasite is a pathogen that simultaneously injures and derives sustenance from its host. In this part pathogenic protozoan diseases are described.

AMOEBIOSIS

Introduction

Amoebiosis is one of the intestinal disorder, which affect 10% of all the world population. Annual deaths are estimated between 40,000 and 100,000. Amebiosis is caused by Entamoeba histolytica.

Systemic Position

- Phylum - Protozoa
- Sub phylum - Sarcomastigophora
- Superclass - Sarcodina
- Class - Rhizopoda
- Sub class - Lobosia
- Order - Amoebida
- Genus - Entamoeba
- Species - histolytica.

Amoebas are unicellular organisms common in the environment, many are parasites of vertebrates and invertebrates. Relatively few species inhabit the human intestine and only Entamoeba histolytica is identified as a human intestinal pathogen.

Causative agent

Distribution - Worldwide distribution. Commonly available in Tropical, Subtropical, Temporate regions. Infection rate is higher in rural and densely populated areas.

Habitat – Endoparasite, Present in man and other mammals, Alive in mucous layer of colon

Habit - Feeds dissolved tissues, bacteria and RBCs, Causes fatal and serious disease. Infected individual discharge mucous and blood in their stool

Structure

E. histolytica has a relatively simple life cycle that alternates between Trophozoite and Cyst stages.

Trophozoite

The Trophozoite is the actively metabolizing, mobile stageand the cyst is dormant and environmentally resistant. Trophozoites vary remarkably in size-from 10 to 60 µm or more in diameter. When they are alive they may be actively motile (Unidirectional motility). Amoebas are anaerobic organisms and do not have Mitochondria. The finely granular endoplasm contains the nucleus and food vacuoles, which in turn may contain bacteria or red blood cells. The parasite is sheathed by a clear outer ectoplasm. Nuclear morphology is best seen in permanent stained preparations. The nucleus has a distinctive central karyosome and a rim of finely beaded chromatin lining the nuclear membrane. Finger like Pseudopodia is available.

Cyst

The cyst is a spherical structure, 10-20 µm in diameter, with a thin transparent wall.Fully mature cysts contain four nuclei with the characteristic amebic morphology. Rod-like structures (chromatoidal bars) are present variably, but are more common in immature cysts. Inclusions in the form of glycogen masses also may be present.

Clinical Manifestations

Fever, Amoebic dysentery -fulminant ulceration, Non dysentery gastroenteritis, Amoeboma formation, Amoebic colitis, Hepatomegaly, Amoebic abscess, Visceral amoebiosis

Multiplication, Life Cycle and Pathogenesis of E. histolytica.

Encystment occurs apparently in response to desiccation as the amoeba is carried through the colon. After encystment, the nucleus divides twice to produce a quadrinucleate mature cyst. Encysted quadrinucleate cysts of E histolytica is ingested through fecal-oral transmission or food and water. Ingested cysts of E histolytica excyst in the small intestine. Trophozoites are carried to the colon, where they mature and reproduce. Excystment occurs after ingestion and is followed by rapid cell division to produce four amoebas which undergo a second division. Each cyst thus yields eight tiny amoebas (Trophozoites).

Quadrinucleated Amoeba adheres to the colonic mucosal cells. The amoeba adherence molecule has been identified as a lectin, which can bind to either of two common carbohydrate components of cell membrane, galactose and N-acetyl glactoseamine. Amoeba attacks and kills the host cell. This cytolytic event is a result of incorporation in the host cell membrane of an amoeba-produced, pore-forming protein, amoebapore. This protein forms ion channels in lipid cell membranes and results in cell death within minutes of cell contact with the amoeba.

The initial lesion is in colonic mucosa, most often in the cecum or sigmoid colon. The slow transit of the intestinal contents in these two locations seems an important factor in invasion of the mucosa, both because it affords the amoeba greater mucosal contact time and because it permits changes in the intestinal milieu that may facilitate invasion. The initial superficial ulcer may deepen into the submucosa and muscularis to become the characteristic flask-shaped, chronic amoebic ulcer. Spread may occur by direct extension, by undermining of the surrounding mucosa until it sloughs, or by penetration that can lead to perforation or fistulous communication to other organs or the skin. If the amoebas gain access to the vascular or lymphatic circulation, metastases may occur first to the liver and then by direct extension or further metastasis to other organs, including the brain. If amoebas pass down the colon they encyst under the stimulus of desiccationand then are evacuated with the stool.

Epidemiology

Fecal-oral transmission occurs when food preparation is not sanitary or when drinking water is contaminated. Contamination may come directly from infected food handlers or indirectly from faulty sewage disposal.

Lab Diagnosis

Sample – Stool, Aspirates from intestinal and other organs, Exudates, Biopsy materials, Mucous from rectal ulcer

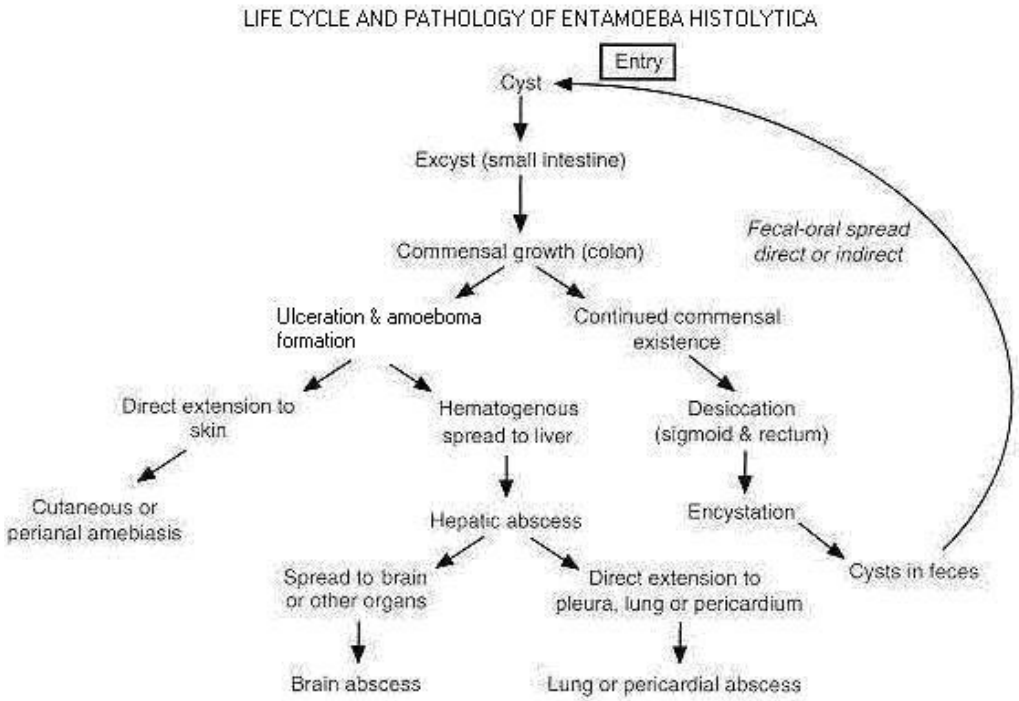
Microscopy - Saline and Iodine Wetmount & Concentration methods

The microscopic examination of direct smear has several purposes. They are To assess the worm burden of a patient. To provide quick diagnosis of a heavily infected specimen. To check organisms motility.

Serology - Serological methods include Gel diffusion, Immunoelectrophoresis, Countercurrent electrophoresis, Indirect Hemagglutination, Indirect Fluorescent Antibody, Skin tests, Enzyme-Linked Immuno Sorbent Assay (ELISA) and Latex Agglutination.

Control

Preventive measures are limited to environmental and personal hygiene. Treatment depends on drug therapy, Acute intestinal disease is best treated with Metronidazole at a dose of 750 mg three times a day orally for 10 day. In children the dose is 40 mg/kg/day divided into three doses and given orally for 10 days. Iodoquinol at an adult dose of 650 mg orally three times daily for 20 days or Diloxanide furoate at an adult dose of 500 mg orally three times daily for 10 days. Amoebic liver abscess is best treated with Metronidazole, Dihydroemetin, Chloroquine or Dehydroemetine.



MALARIA

Introduction

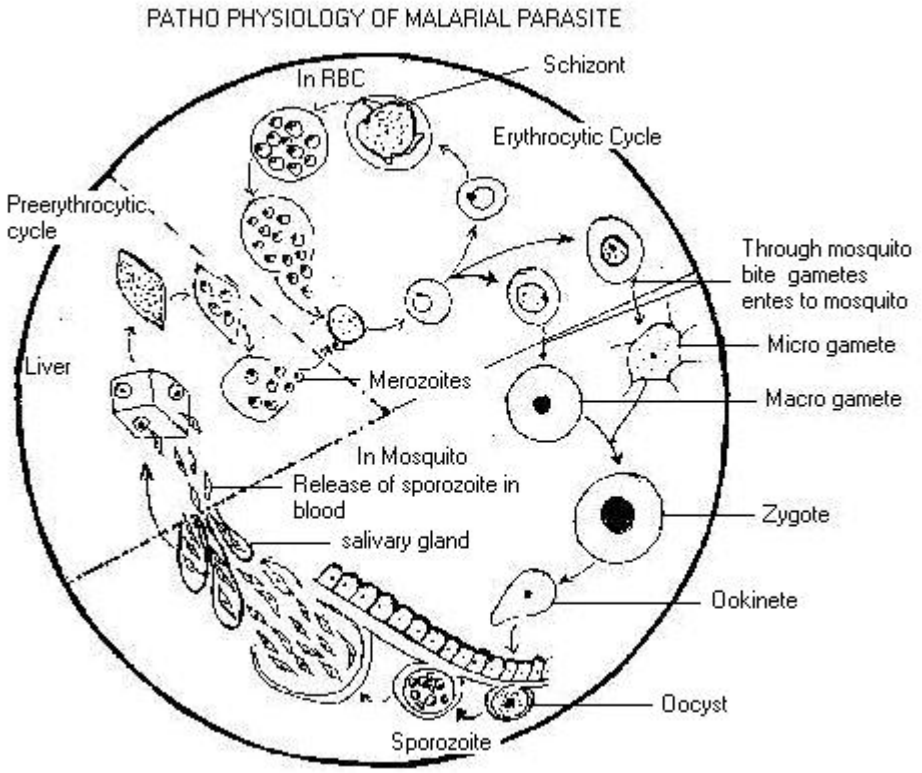
Malaria has been a major disease of humankind for thousands of years. Malaria is caused by protozoa of the genus Plasmodium.

Causative agent

Four species cause disease in humans: P falciparum, P vivax, P ovale and P malariae. Malaria is spread to humans by the bite of female mosquitoes of the genus Anopheles. P falciparum and P vivax account for the vast majority of cases. P falciparum causes the most severe disease.

Structure And Life Cycle

Plasmodia pass through a number of stages in the course



Plasmodia pass through a number of stages in the course of their two host life cycle. The stage infective for humans is the uninucleate, lancet shaped sporozoite (approximately 1 X 7 µm). Sporozoites are produced by sexual reproduction in the midgut of vector anophelies mosquitoes and migrate to the salivary gland. When an infected Anopheles mosquito bites a human, she may inject sporozoites along with saliva into small blood vessels. Sporozoites are thought to enter liver parenchymal cells within 30 minutes of inoculation. In the liver cell, the parasite develops into a spherical, multinucleate liverstage schizont which contains 2,000 to 40,000 uninucleate merozoites. This process of enormous amplification is called Exoerythrocytic Schizogony. This Exoerythrocytic or liver phase of the disease usually takes between 5 and 21 days, depending on the species of Plasmodium. However, in P vivax and P ovale infections, maturation of liverstage schizonts may be delayed for as long as 1 to 2 years. These liver-phase parasites are called hypnozoites.

The mature schizonts eventually rupture, releasing thousands of uninucleated merozoites into the bloodstream. Each merozoite can infect a Red Blood Cell. Within the red cell, the merozoite develops to form either an erythrocytic stage (blood-stage) schizont (by the process of erythrocytic schizogony) or a spherical or bananashaped, uninucleate gametocyte. The mature erythrocytic stage schizont contains 8 to 36 merozoites, each 5 to 10 µm long, which are released

into the blood when the schizont ruptures. These merozoites proceed to infect another generation of erythrocytes.

The gametocyte, which is the sexual stage of the Plasmodium, is infectious for mosquitoes that ingest it while feeding. Within the mosquito, gametocytes develop into female and male gametes (macrogametes and microgametes, respectively), which undergo fertilization and then develop over 2 to 3 weeks into sporozoites that can infect humans. The delay between infection of a mosquito and maturation of sporozoites means that female mosquitoes must live a minimum of 2 to 3 weeks to be able to transmit Malaria. This fact is important in Malaria control efforts.

Pathogenesis

Clinical illness is caused by the Erythrocytic stage of the parasite. No disease is associated with sporozoites, the developing liver stage of the parasite, the merozoites released from the liver, or gametocytes. The first symptoms and signs of Malaria are associated with the rupture of erythrocytes when erythrocytic stage schizonts mature. This release of parasite material presumably triggers a host immune response. The cytokines, reactive oxygen intermediatesand other cellular products released during the immune response play a prominent role in pathogenesisand are probably responsible for the fever, chills, sweats, weaknessand other systemic symptoms associated with malaria. In the case of Falciparum Malaria (the form that causes most deaths), infected erythrocytes adhere to the endothelium of capillaries and postcapillary venules, leading to obstruction of the microcirculation and local tissue anoxia. In the brain this causes cerebral malaria; in the kidneys it may cause acute tubular necrosis and renal failure; and in the intestines it can cause ischemia (deficiency of blood) and ulceration, leading to gastrointestinal bleeding and to bacteremia secondary to the entry of intestinal bacteria into the systemic circulation.

Symptoms

The most characteristic symptom of Malaria is fever. Other common symptoms include Chills, Headache, Myalgias, Nauseaand Vomiting. Diarrhoea, Abdominal painand Cough are occasionally seen.

The first is a 15-to-60 minute cold stage characterized by shivering and a feeling of cold.

Second comes the 2-to-6 hour hot stage, in which there is fever, sometimes reaching 41°C, flushed, dry skinand often headache, nauseaand vomiting.

Finally, there is the 2-to-4 hour sweating stage during which the fever drops rapidly and the patient sweats.

In all types of Malaria the periodic febrile response is caused by rupture of mature schizonts. In P vivax and P ovale malaria, a brood of schizonts matures every 48 hours, so the periodicity of fever is Tertian ("tertian malaria"), whereas in P malariae disease, fever occurs every 72 hours ("quartan malaria"). The fever in Falciparum Malaria may occur every 48 hours, but is usually irregular, showing no distinct periodicity.

Lab Diagnosis

Diagnosis of Malaria generally requires direct observation of Malaria parasites in Giemsa-stained thick and thin blood smears. Thick blood smears are more difficult to interpret than thin blood smears but they are much more sensitive, as more blood is examined. Thin blood smears, in which parasites are seen within erythrocytes, are used to determine the species of the infecting parasite.

New diagnostic methods include a rapid antigen-capture dip stick test and a technique for detecting parasites with a fluorescent stain. Both of these tests are fast, easy to perform and are highly sensitive and specific

Other diagnostic methods include assays to detect Malaria antibodies and antigensand Polymerase Chain Reaction/DNA and RNA probe techniques. These techniques are used primarily in epidemiologic studies and immunization trials and rarely in the diagnosis of individual patients.

Control

Drugs used for treatment are Primaquine, Chloroquine, Mefloquine, Quinine, Quinidine, Pyrimethamine-sulfadoxine, Doxycycline, Halofantrine, Artemisnin, Proguanin

Prevention of Malaria

Individuals should avoid contact with the mosquito by wearing protective clothing. Use an insect repellent containing N,N-diethyl mtoluamide (DEET). Sleeping under insecticide-impregnated bednets. For most of travelers, Mefloquine is the drug of choice and doxycycline is as acceptable alternative. Chloroquine plus Proguanil is another possible regimen for chloroquine-resistant areas, but this regimen is much less effective than Mefloquine or Doxycycline. Prophylaxis with Chloroquine or Mefloquine should begin 2 weeks before entering the Malarious area.

GIARDIA

Giardiasis is a major diarrheal disease found throughout the world. The flagellate protozoan Giardia intestinalis (previously known as G lamblia or G duodenalis), its causative agent, is the most commonly identified intestinal parasite in the United States and the most common protozoal intestinal parasite isolated worldwide. Infection is more common in children than in adults.

G intestinalis can cause asymptomatic colonization or acute or chronic diarrheal illness. The organism has been found in as many as 80% of raw water supplies from lakes, streams, and ponds and in as many as 15% of filtered water samples. It is a common cause of chronic diarrhea and growth retardation in children in developing countries.

Giardiasis usually represents a zoonosis with cross-infectivity between animals and humans. Giardia intestinalis has been isolated from the stools of beavers, dogs, cats, and primates. Beavers may be an important reservoir host for G intestinalis. Other Giardia species

include G muris in rodents; G agilis in amphibians; G psittaci and G ardeae in birds; and G microti in voles and muskrats.

Giardia species are endemic in areas of the world that have poor sanitation. In developing countries, the disease is an important cause of morbidity. Water-borne and food-borne outbreaks are common. Because ingestion of as few as 10 Giardia cysts may be sufficient to cause infection, giardiasis is common in daycare center attendees and institutionalized patients in developed countries. G intestinalis is a particularly significant pathogen for people with malnutrition, immunodeficiencies, or cystic fibrosis.

High-risk groups for giardiasis include travelers to highly endemic areas, immunocompromised individuals, and sexually active homosexual men. Cyst passage rates as high as 20% have been reported among certain groups of sexually active homosexual men; these individuals were frequently symptomatic.

The traditional basis of diagnosis is identification of Giardia intestinalis trophozoites or cysts in the stool of infected patients via a stool ova and parasite (O&P) examination. Stool antigen enzyme-linked immunosorbent assays also are available. (See Workup.) Standard treatment for giardiasis consists of antibiotic therapy. Metronidazole, tinidazole, and nitazoxanide are the drugs of choice. Metronidazole is the most commonly prescribed antibiotic for this condition; tinidazole is considered a first-line agent outside the United States.

Historical background

Giardia was originally observed by von Leeuwenhoek in 1681, in his own diarrheal stool, and was described by Vilem Dusan Lambl in 1859 and by Alfred Giard in 1895. The organism's previous name, honoring the contributions of Giard and Lambl, was bestowed in 1915.

Although G intestinalis was the first protozoan parasite described, its role as a pathogenic organism was not recognized until the 1970s, after community outbreaks and after the appearance of the disease in travelers returning from endemic regions. Prior to that time, the organism was thought to be a harmless commensal organism of the intestine.

Pathophysiology

Infection with Giardia intestinalis most often results from fecal-oral transmission or ingestion of contaminated water. Contaminated food is a less common etiology. Person-to-person spread is common, with 30% of family members with infected children themselves becoming infected.

Most infections are asymptomatic. The rate of symptomatic infection in the natural setting varies from 5% to 70%. Giardia is found in healthy people in endemic areas, and asymptomatic carriage with excretion of high numbers of cysts in stools is common.

Predisposing factors to symptomatic infection include hypochlorhydria, various immune system deficiencies, blood group A, and malnutrition. The incubation period averages 1-2 weeks, with a mean of 9 days. The average duration of symptoms in all ages ranges from 3 to 10 weeks.

Giardia life cycle

Giardia has one of the simplest life cycles of all human parasites. The life cycle is composed of two stages: (1) the trophozoite (see the first image below), which exists freely in the human small intestine; and (2) the cyst (see the second image below), which is passed into the environment. No intermediate hosts are required.

Giardiasis. Giardia lamblia trophozoites in culture.

View Media Gallery

Giardiasis. Giardia lamblia, cyst form.

View Media Gallery

Upon ingestion of the cyst, contained in contaminated water or food, excystation occurs in the stomach and the duodenum in the presence of acid and pancreatic enzymes. The trophozoites pass into the small bowel where they multiply rapidly, with a doubling time of 9-12 hours. As trophozoites pass into the large bowel, encystation occurs in the presence of neutral pH and secondary bile salts. Cysts are passed into the environment, and the cycle is repeated.

The trophozoite form of G lamblia is teardrop-shaped and measures 9-21 micrometers long by 5-15 micrometers wide. The trophozoite has a convex dorsal surface and a flat ventral surface that contains the ventral disk, a rigid cytoskeleton composed of microtubules and microribbons. The trophozoite also contains four pairs of flagella, directed posteriorly, that aid the parasite in moving. Two symmetric nuclei with prominent karyosomes produce the characteristic facelike image that appears on stained preparations.

The ventral disk, which is often referred to as the sucking or adhesive disk, provides the parasite with powerful adhesion, catching, and holding abilities. In the murine model of giardiasis, the ventral disk adhesion imprints are marked but less impressive than in the human small intestine. However, this direct injury is an unlikely cause of the more extensive reduction in microvillus surface area, the reduction in disaccharidase activities, and the more pronounced abnormalities of villous architecture that are seen in giardiasis.

The cyst form of the protozoan is smooth-walled and oval in shape, measuring 8-12 micrometers long by 7-10 micrometers wide. As the cyst matures, nuclear division occurs and readies the cyst to release two trophozoites upon excystation. Once the host is infected, trophozoites may appear in the duodenum within minutes. [26] Excystation occurs within 5 minutes of exposure of the cysts to an environment with a pH between 1.3 and 2.7.

After infection, the trophozoites attach to the enterocytes via the ventral adhesive disk. This may occur through the presence of lectin on the surface of the trophozoite or through other mechanical means. Encystation is a continuous process during infection.

As the trophozoites encounter neutral pH and/or secondary bile salts, encystation-specific secretory vesicles (ESVs) appear. After 15 hours, cyst wall proteins are visible. Within 24 hours

after the appearance of ESVs, the trophozoite is covered with these cyst wall proteins, the form of the cyst has emerged, and new antigenic differences are present.

Mechanism of injury

The mechanisms by which Giardia causes diarrhea and intestinal malabsorption are probably multifactorial and not yet fully elucidated. Postulated mechanisms include damage to the endothelial brush border, enterotoxins, immunologic reactions, and altered gut motility and fluid hypersecretion via increased adenylate cyclase activity.

Adhesion of trophozoites to the epithelium has been demonstrated to cause increased epithelial permeability. Giardia- induced loss of intestinal brush border surface area, villus flattening, inhibition of disaccharidase activities, and eventual overgrowth of enteric bacterial flora appear to be involved in the pathophysiology of giardiasis but have yet to be causatively linked to the disease's clinical manifestations.

Marked or moderate partial villous atrophy in the duodenum and jejunum can be observed in histologic sections from asymptomatic individuals who are infected. In addition to disrupting the mucosal epithelium, effects in the intestinal lumen may contribute to malabsorption and the production of diarrhea. Nevertheless, diarrhea can still occur in individuals in the absence of obvious light microscopic changes in small intestinal structure.

Varying degrees of malabsorption of sugars (eg, xylose, disaccharides), fats, and fat-soluble vitamins (eg, vitamins A and E) may contribute to substantial weight loss. The histopathologic response to giardiasis varies and does not strongly correlate with clinical symptoms.

G intestinalis may release cytopathic substances that damage the intestinal epithelium. Giardia species contain thiol-dependent and thiol-independent proteinases, which may find substrates in the microvillus membrane. A 2018 report suggests that three main cysteine proteases (CP14019, CP16160 and CP16779) secreted by G intestinalis disrupt intestinal epithelial cell junctional complexes and degrade chemokines. In addition, the surface mannose-binding lectin of G intestinalis may contribute to epithelial damage. Whatever the mechanism by which G intestinalis damages villous epithelial cells, the result consistently appears to be an increase in crypt length and crypt cell proliferation.

Enterocytic injury is mediated by activated host T lymphocytes. Pathophysiological activation of lymphocytes is secondary to Giardia-induced disruption of epithelial tight junctions, which, in turn, increases intestinal permeability. Loss of epithelial barrier function is a result of Giardia-induced enterocyte apoptosis.

Epithelial barrier dysfunction in cases with chronic giardiasis is associated with increased rates of enterocyte apoptosis. Consistent with these observations, microarray analyses of the effects of G intestinalis on human CaCo2 cells found that the parasite–host interactions lead to a pronounced up-regulation of genes implicated in the apoptotic cascade and the formation of reactive oxygen species.

Giardia can also prevent the formation of nitric oxide, a compound known to inhibit giardial growth, by consuming local arginine, which effectively removes the substrate needed by enterocytes to produce nitric oxide. This mechanism may contribute to Giardia-induced enterocyte apoptosis, because arginine starvation in these cells is known to result in programmed cell death.

G intestinalis is genetically heterogeneous with eight genetically distinct genotypes or assemblages, designated A-H; assemblages A and B can infect humans. Genotypes vary within group A and B, which could explain why the role of animals in the epidemiology of human infection remains poorly understood. Some strains appear more biologically suitable than other strains. This feature is potentially important in giardiasis pathogenesis. Genotypically diverse isolates of Giardia species may vary in their ability to produce morphologic changes in the small intestine epithelium and to impair fluid, electrolyte, and solute transport.

PROGNOSIS

The prognosis for patients with giardiasis is often good. Most patients are asymptomatic, and most infections are self-limited. Giardiasis is not associated with mortality except in rare cases of extreme dehydration, primarily in infants or malnourished children.

Several antibiotic agents are available with acceptable efficacy rates to shorten the disease course, although drug resistance has been observed in clinical experience. Untreated, symptomatic giardiasis can last for weeks.

When the parasite persists in the stool, reinfection is possible.

Weight loss, disaccharidase deficiency, malabsorption, and growth retardation are possible complications. G intestinalis has been implicated as the chief cause of growth retardation in infected children, even after other diarrhea-causing agents are controlled.

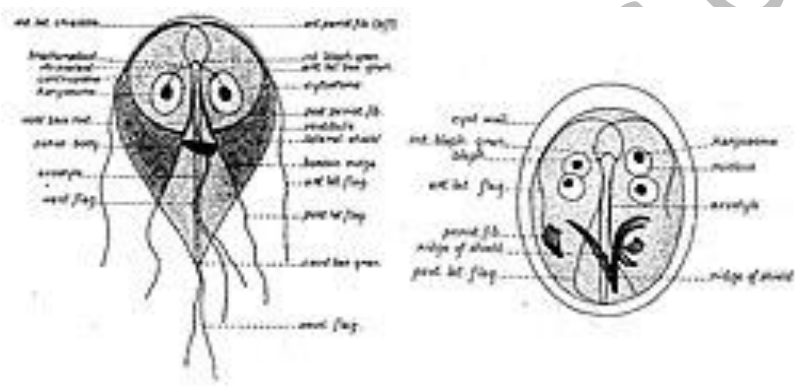
Some patients may experience persistent symptoms (eg, chronic diarrhea/steatorrhea, malabsorption) despite apparently effective antibiotic treatment, although these usually subside over weeks to months. However, Hanevik et al found symptoms consistent with irritable bowel syndrome (IBS) and/or functional dyspepsia in 76 of 82 patients at least 6 months after eradication of Giardia infection. Patients reported bloating, diarrhea, and abdominal pain, which were exacerbated by specific foods or by physical or mental stress. Another study by Hanevik and colleagues associated giardiasis with the presence of IBS and chronic fatigue even 6 years after infection.

Similarly a longitudinal cohort study (2006-2010) of data from the MarketScan commercial database that evaluated the relationship between a diagnosis of giardiasis and that of IBS found that, even accounting for confounding factors, patients with giardiasis had a greater 1-year incidence of IBS than those without giardiasis. The study included a matched cohort of 3935 patients with giardiasis and 19,663 without giardiasis.

Complications

Complications of giardiasis may include the following:

- Development of chronic illness with weight loss
- Malabsorption syndrome in adults
- Failure to thrive in children
- Disaccharidase deficiency
- Zinc deficiency in schoolchildren
- Growth retardation
- Persistent gastrointestinal symptoms



Treatment

Children and adults who have giardia infection without symptoms most often don't need treatment unless they're likely to spread the parasites. Many people who do have symptoms often get better on their own in a few weeks.

When symptoms are bad or the infection doesn't go away, the following medicines can treat giardia infection:

- **Metronidazole:** Metronidazole is often prescribed to be taken three times a day for five days. Side effects may include upset stomach and a taste of metal in the mouth. Don't drink alcohol while taking this medicine.
- **Tinidazole:** Tinidazole works as well as metronidazole and has many of the same side effects. But you can take it in a single dose.
- **Nitazoxanide:** Because it comes in a liquid form, nitazoxanide may be easier for children to swallow. Side effects may include upset stomach, gas, yellow eyes and brightly colored yellow urine.

LEISHMANIA DONOVANI

Leishmania donovani is a species of intracellular parasites belonging to the genus Leishmania, a group of haemoflagellate kinetoplastids that cause the disease leishmaniasis. It is a human blood parasite responsible for visceral leishmaniasis or kala-azar, the most severe form of leishmaniasis. It infects the mononuclear phagocyte system including spleen, liver and bone marrow. Infection is transmitted by species of sandfly belonging to the genus Phlebotomus in Old World and Lutzomyia in New World. The species complex it represents is prevalent throughout tropical and temperate regions including Africa (mostly in Sudan), China, India, Nepal, southern Europe, Russia and South America. The species complex is responsible for thousands of deaths every year and has spread to 88 countries, with 350 million people at constant risk of infection and 0.5 million new cases in a year.

L. donovani was independently discovered by two British medical officers William Boog Leishman in Netley, England, and Charles Donovan in Madras, India, in 1903. However, the correct taxonomy was provided by Ronald Ross. The parasite requires two different hosts for a complete life cycle, humans as the definitive host and sandflies as the intermediate host. In some parts of the world other mammals, especially canines, act as reservoir hosts. In human cell they exist as small, spherical and unflagellated amastigote form; while they are elongated with flagellum as promastigote form in sandflies. Unlike other parasitic protists they are unable to directly penetrate the host cell, and are dependent upon phagocytosis. The whole genome sequence of L. donovani obtained from southeastern Nepal was published in 2011.

L. donovani sensu stricto is in a species complex with the closely related L. infantum, which causes the same disease. The former is commonly found in East Africa and the Indian subcontinent, while the latter is found in Europe, North Africa, and Latin America. The split is done in 2007, and references to L. donovani often still refer to the entire complex (sensu lato). As of 2022, the parasite causes 50,000 to 90,000 infections worldwide.

Discovery

One of the earliest known epidemics of L. donovani infection (kala-azar as it was called in Hindi) was known in India just after the Indian Rebellion of 1857. The first medical record was however only in 1870 by British medical officers from Assam. In 1900, an English soldier stationed at Dum Dum, West Bengal, died at the Army Medical School in Netley, England. The autopsy was performed by William Boog Leishman. He processed the tissue sample of the enlarged spleen using a staining technique (now known as Leishman's stain) which he had just developed, and discovered the protozoan parasites using microscopy. But he mistakenly considered the parasites to be degenerate trypanosomes, already known protozoan parasites in Africa and South America.[10] In 1903, Leishman published his discovery as "On the possibility of the occurrence of trypanosomiasis in India" in the British Medical Journal, which appeared on 11 May.

Another British medical officer Charles Donovan, who was serving in the Indian Medical Service, had found the parasites in April of that year at the Government General Hospital in Madras. After

reading Leishman paper, Donovan confirmed on 17 June that the parasites (by then known as "Leishman bodies") were definitely the causative agents of kala-azar. He wrote a commentary of his discovery in relation to that of Leishman in the same journal using the same title as that of Leishman's that appeared on 11 July 1903.

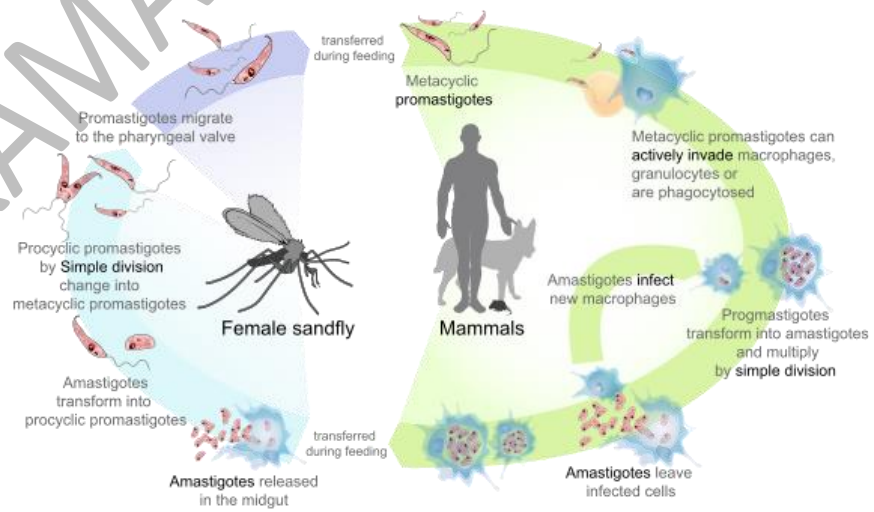
Soon a controversy arose as to whom such a monumental discovery should be credited. Donovan sent some of his slides to Ronald Ross, who was at the time in Liverpool, and to Alphonse Laveran at the Pasteur Institute in Paris. Laveran and his colleague Félix Mesnil identified the protozoan (and yet wrongly) to be members of Piroplasmida, and gave the scientific name Piroplasma donovanii. It was Ross who resolved the conflict of priority in the discovery and correctly identified the species as member of the novel genus Leishmania. He gave the popular name "Leishman-Donovan bodies", and subsequently the valid binomial Leishmania donovani, thereby equally crediting the two rivals.

Structure

Leishmania donovani is a unicellular eukaryote having a well-defined nucleus and other cell organelles including a kinetoplast and a flagellum. This species has n=36 chromosomes. Depending on its host it exists in two structural variants, as follows:

1. Amastigote form found in the mononuclear phagocyte and circulatory systems of humans. It is an intracellular and non-motile form, being devoid of external flagellum. The short flagellum is embedded in the anterior end without projecting out. It is oval in shape, and measures 3–6 µm in length and 1–3 µm in breadth. The kinetoplast and basal body lie towards the anterior end.
2. Promastigote is formed in the alimentary tract of the sandfly. It is an extracellular and motile form. It is considerably larger and more highly elongated, measuring 15–30 µm in length and 5 µm in width. It is spindle-shaped, tapering at both ends. A long flagellum (about the body length) is projected externally at the anterior end. The nucleus lies at the centre, and in front of which are kinetoplast and basal body.

Infection and life cycle



Life cycle of Leishmania donovani

Leishmania donovani is a digenetic parasite passing its life cycle in two different hosts.

Definitive host

In humans the metacyclic promastigotes are injected by sandfly through the skin during its blood meal. When sandfly bites using its proboscis it ejects the parasites that are stored inside the hollow tube. Some promastigotes may enter the blood stream directly where some are destroyed by macrophagic cytolysis. But many are also taken up through phagocytosis by mononuclear phagocytes in liver, spleen and bone marrow. Inside the cells they undergo spontaneous transformation into oval-shaped amastigotes.

Granulocytes selectively kill the promastigotes by oxidative mechanism, while amastigotes are resistant. Then the surviving amastigotes undergo cell division using simple binary fission. Multiplication continues until the host cell can no longer hold and ruptures. In a fully congested cell there can be as many as 50 to 200 amastigotes, which are released into tissue cavities. Each individual amastigote is then capable of invading fresh cells. As a result, the entire tissue is progressively infected and destroyed. A number of free amastigotes then enters the blood stream where many are phagocytosed by macrophages. These free and phagocytosed amastigotes in peripheral blood are then sucked up by blood-feeding sandfly.

Intermediate host

L. donovani undergo further development only in the digestive tract of the female sandfly. Hence only females are responsible for transmitting the infection. Once the amastigotes are ingested, they enter the midgut of the sandfly. Then they undergo structural modification into flagellated promastigotes, becoming larger and considerably elongated. They get attached to the gut epithelial lining where they multiply rapidly by binary fission. (They are also capable of sexual reproduction by genetic hybridisation in the sandfly gut.) They then migrate back towards the anterior part of the digestive system such as pharynx and buccal cavity. This process is known as anterior station development, which is unique in Leishmania. A heavy infection of pharynx can be observed within 6 to 9 days after initial blood meal. The promastigotes become infective only by this time, and the event is called the metacyclic stage. The metacyclic promastigotes then enter the hollow proboscis where they accumulate and completely block the food passage. Immediately upon biting a human, the parasites are released, which invariably results in infection.

The stages of development in sandfly can be described as follows:

1. Soon after entering the gut, the amastigotes get coated with peritrophic matrix, which is composed of chitin and protein complex. This protects the parasites from the digestive enzymes of the host.

2. The amastigotes travel as far as the abdominal midgut and first transform into a weakly motile "procyclic promastigotes" on the gut wall within 1–3 days.

3. The young promastigotes secrete a neuropeptide that stop peristalsis of the gut. The surface lipophosphoglycan (LPG) of the promastigote serves as an attachment to the gut epithelium. These factors prevent the expulsion of promastigotes during excretion of the insect.

4. During 4–7 days the peritrophic matrix is degraded by the activity of chitinases. This release the more actively motile "nectomonad promastigotes" which migrate anteriorly until they reach the opening of the thoracic gut.

5. Another transformation takes place by which they turn into "leptomonad promastigotes". These are fully motile and capable of binary fission. Multiplication and migration towards thoracic midgut cause congestion of the pharynx and buccal cavity. Here they secrete promastigote secretory gel (PSG), which is composed of soluble acid phosphatase and phosphoglycoprotein.

6. After 6–9 days the promastigotes become metacyclic. Some are also transformed into non-replicating promastigotes, which also become metacyclic. The sandfly is able to regurgitate and eject the parasites from its proboscis with the help of PSG when it bites.

Reservoir host

Dogs are known to be susceptible to L. donovani infection. Especially in the New World, infection is a zoonotic disease, involving different canine species, including domestic dog and the two fox species, Lycalopex vetulus and Cerdocyon thous. In the Mediterranean region domestic dogs and the three fox species Vulpes vulpes, V. corsac and V. zerda are common reservoir hosts. In Africa and Brazil, some marsupials and rodents are also reported to harbour L. donovani.

Epidemiology

It is estimated that visceral leishmaniasis (VL) affects more than 100 million people worldwide, with 1.5 to 2 million new cases and more than 70,000 deaths each year. As of the 2022 report, there were 50,000 to 90,000 infections worldwide in 2020, and the World Health Organization estimates that only between 25 and 45% of the cases were reported. Although L. donovani is only the second-most prevalent Leishmania causing VL, it is the most dangerous form and directly fatal to humans. Over 90% of reported cases are from India, Bangladesh, Nepal, Sudan and Brazil. In India, it is prevalent in the eastern region including Bihar, West Bengal, eastern Uttar Pradesh, Assam and foothills of Sikkim. It is responsible for tens of thousands of mortality among Africans in eastern and southern parts of Sudan. During the epidemic of 1984–1994 death toll was as high as 70% in the Sudanese population. Moreover, due to emergence of drug resistance the prevalence is not subsiding, and in fact has spread to central Europe. For example, during the late 1990s hundreds of cases were reported in Switzerland.

Pathogenicity

L. donovani is the causative agent of visceral leishmaniasis, traditionally known as kala-azar ("black fever", particularly in India), because of its characteristic symptoms. The disease is

highly lethal if not treated properly. The incubation period generally ranges from 3 to 6 months, and in some cases may be over a year. In Indian leishmaniasis, incubation can be as short as 10 days. The target cells are those of mononuclear phagocyte system. The two main tissues of infection are spleen and liver.

Clinical symptoms include pyrexia (recurring high fever which may be continuous or remittent), enlargement of spleen and liver, and heavy skin pigmentation which darkens the physical appearance (the reason for naming "black fever"). To a lesser extent, mucosa of the small intestine and lymph nodes are also invaded by the parasite. Morphological symptoms are noticeable particularly on facial and abdominal regions. Skin becomes coarse and hard. In African infections, warty eruptions are common. In a fully developed stage, the patient shows emaciation and anaemia. Where medical facilities are poor, mortality can be as high as 75–95% within 2 years of epidemics. The disease is often accompanied by complications with dysentery, tuberculosis, septicaemia and even HIV infection.

The conventional treatment method is an intravenous injection with antimony compounds, such as pentostam. Unfortunately, these chemotherapeutics are so poisonous that about 15% of the patients die from the treatments. To compound the situation, drug resistance has evolved in the parasites against the traditional antimonials. According to rough estimates, about 40% of patients in India are already resistant to this therapy

Another antimicrobial drug amphotericin B is also commonly used. Liposomal amphotericin B (L-AmB) has been a drug of choice in India, but is practically useless in Africa because of low effectiveness in the African strain of the parasite. Further, amphotericin B has severe adverse effects. Its acute effects includes nausea, vomiting, rigors, fever, hypertension or hypotension, and hypoxia, and its chronic effect is nephrotoxicity.

In 1999, an anticancer drug miltefosine was demonstrated to be highly effective (95% cure rate) among Indian patients. This was the first time an oral drug is effective for visceral leishmaniasis. Clinical trials showed that the new drug is relatively harmless. The most adverse effects were only vomiting and diarrhoea in 20–28% patients, which were rather mild. The drug has been officially approved in India. The recommended dosage is 100 mg per day over a period of four weeks.

Because of resistance development and harsh side effects, new therapeutics are needed to treat leishmaniasis. The gp65 metalloprotease have been proposed as a new drug target, due to its important role during an infection.

UNIT V

Helminth Parasites

The word 'helminth' is a general term meaning 'worm', but there are many different types of worms. Prefixes are therefore used to designate types: platy-helminths for flat-worms and nemat-helminths for round-worms. All helminths are multicellular eukaryotic invertebrates with tube-like or flattened bodies exhibiting bilateral symmetry. They are triploblastic (with endo-, meso- and ecto-dermal tissues) but the flatworms are acoelomate (do not have body cavities) while the roundworms are pseudocoelomate (with body cavities not enclosed by mesoderm). In contrast, segmented annelids (such as earthworms) are coelomate (with body cavities enclosed by mesoderm).

Many helminths are free-living organisms in aquatic and terrestrial environments whereas others occur as parasites in most animals and some plants. Parasitic helminths are an almost universal feature of vertebrate animals; most species have worms in them somewhere.

Biodiversity

Three major assemblages of parasitic helminths are recognized: the Nemathelminthes (nematodes) and the Platyhelminthes (flatworms), the latter being subdivided into the Cestoda (tapeworms) and the Trematoda (flukes):

Nematodes (roundworms) have long thin unsegmented tube-like bodies with anterior mouths and longitudinal digestive tracts. They have a fluid-filled internal body cavity (pseudocoelum) which acts as a hydrostatic skeleton providing rigidity (so-called 'tubes under pressure'). Worms use longitudinal muscles to produce a sideways thrashing motion. Adult worms form separate sexes with well-developed reproductive systems.

Cestodes (tapeworms) have long flat ribbon-like bodies with a single anterior holdfast organ (scolex) and numerous segments. They do not have a gut and all nutrients are taken up through the tegument. They do not have a body cavity (acoelomate) and are flattened to facilitate perfusion to all tissues. Segments exhibit slow body flexion produced by longitudinal and transverse muscles. All tapeworms are hermaphroditic and each segment contains both male and female organs.

Trematodes (flukes) have small flat leaf-like bodies with oral and ventral suckers and a blind sac-like gut. They do not have a body cavity (acoelomate) and are dorsoventrally flattened with bilateral symmetry. They exhibit elaborate gliding or creeping motion over substrates using compact 3-D arrays of muscles. Most species are hermaphroditic (individuals with male and female reproductive systems) although some blood flukes form separate male and female adults.

Unlike other pathogens (viruses, bacteria, protozoa and fungi), helminths do not proliferate within their hosts. Worms grow, moult, mature and then produce offspring which are voided from the host to infect new hosts. Worm burdens in individual hosts (and often the severity of infection) are therefore dependent on intake (number of infective stages taken up). Worms develop slowly

compared to other infectious pathogens so any resultant diseases are slow in onset and chronic in nature. Although most helminth infections are well tolerated by their hosts and are often asymptomatic, subclinical infections have been associated with significant loss of condition in infected hosts. Other helminths cause serious clinical diseases characterized by high morbidity and mortality. Clinical signs of infection vary considerably depending on the site and duration of infection. Larval and adult nematodes lodge, migrate or encyst within tissues resulting in obstruction, inflammation, oedema, anaemia, lesions and granuloma formation. Infections by adult cestodes are generally benign as they are not invasive, but the larval stages penetrate and encyst within tissues leading to inflammation, space-occupying lesions and organ malfunction. Adult flukes usually cause obstruction, inflammation and fibrosis in tubular organs, but the eggs of blood flukes can lodge in tissues causing extensive granulomatous reactions and hypertension.

Life-cycles

Helminths form three main life-cycle stages: eggs, larvae and adults. Adult worms infect definitive hosts (those in which sexual development occurs) whereas larval stages may be free-living or parasitize invertebrate vectors, intermediate or paratenic hosts. Nematodes produce eggs that embryonate in utero or outside the host. The emergent larvae undergo 4 metamorphoses (moults) before they mature as adult male or female worms. Cestode eggs released from gravid segments embryonate to produce 6-hooked embryos (hexacanth oncospheres) which are ingested by intermediate hosts. The oncospheres penetrate host tissues and become metacestodes (encysted larvae). When eaten by definitive hosts, they excyst and form adult tapeworms. Trematodes have more complex life-cycles where 'larval' stages undergo asexual amplification in snail intermediate hosts. Eggs hatch to release free-swimming miracidia which actively infect snails and multiply in sac-like sporocysts to produce numerous rediae. These stages mature to cercariae which are released from the snails and either actively infect new definitive hosts or form encysted metacercariae on aquatic vegetation which is eaten by definitive hosts.

Collection, transport and examination of specimen Laboratory techniques in parasitology

Faecal specimens should be collected in clean, wide-mouthed containers most often in a wax cardboard container with a tight lid. The specimen should not be contaminated with water or urine which may destruct the motile organisms. A minimum of three samples should be submitted. For accuracy especially in the case of amoebiasis, at least six samples should be provided to the laboratory. Collection of specimens on alternate days will give good results. Liquid specimens should be examined within 30 min. of passage or it should be preserved in polyvinyl alcohol (PVA). Of the PVA, SAF later gives excellent results. Stained smear gives an accurate examination of intestinal protozoa. In some laboratories copper sulphate is the component that is tried most frequently but does not yield expected results. Many laboratories try to avoid mercury compounds, but substitute compounds may not yield good results on preservation for protozoan morphology especially in a permanent stained smear.

PVA FIXATIVE

This is the most excellent and highly recommended for preserving protozoan cysts and trophozoites (Brooke and Goldman, 1949). PVA is a combination of modified Schaudins fixative and a water-soluble resin. The ratio of fixative to faecal matter should be 3 : 1, three parts of preservative to one part of faecal matter. This fixative keeps for several years. It can be either prepared in the laboratory or can be purchased. Protozoan, helminth eggs, and larvae could be used for helminth eggs which will prevent further development. The ratio of fixative to faecal matter for formalin fixative is the same as for PVA (3 : 1). This fixative is used for protozoan, helminth eggs and larvae. It is especially good for helminth eggs as this will prevent further development.

MIF SOLUTION

Merthiolate–iodine–formalin solution of Sapero and Lawless (1953) is good for helminth eggs, larval stages and also for some protozoans. However there are certain disadvantages with this fixative, since the iodine part in the fixative is not soluble.

SAF SOLUTION

This fixative is a combination of formalin and sodium acetate. It is a liquid fixative. The difficulty with this fixative is that the material may not adhere to the slide. So Mayers albumin is recommended which serves as an adhesive. The fixative furnishes best results in recombination with haematoxylin stain rather than trichrome stain. Consistency of the stool may indicate the type of parasite or stage of the life cycle. Trophozoites are rarely found in liquid stools, cyst stages are found in formed or semi-formed specimens and rarely in liquid stools. Ascaris lumbricoides, Enterobius vermicularis, Tapeworm proglottids are rarely seen in the surface of the stool whereas Hymenolepis nana, Trichuris trichiura and hook worms are found. Blood in stool may indicate certain facts. High bleeding in stool indicates bleeding in lower bowel. Amoebic infection is certain in a mucous and bloody stool.

DIRECT SMEAR

Mix 2 mg of faecal material with a drop of physiological saline. This is in the form of uniform suspension under 22 × 22 mm coverslip. These smears are useful in detecting mobile trophozoite stages of Protozoa, helminth eggs and larvae. Helminth eggs, larval and protozoan cysts are also seen in the wet forms. A drop of iodine is placed at the edge of the coverslip. A weak iodine solution is recommended, or Lugols Dobell O'Conors and Autonis are also suggested. Gram's iodine often used for microorganisms is not used. Protozoan cysts stained with iodine reveal golden yellow cytoplasm, brown glycogen and pale nuclei. Chromatoidal bodies are not clear. Buffered methylene blue is very effective at low pH (Nair, 1953). It shows nuclear details. It stains cytoplasm in a pale blue shade and nuclei are dark blue.

When faecal matter is directly mounted on a slide, it does not give a clear picture of the parasitic organisms present in the gastrointestinal tract. To overcome this, concentration procedures are adopted and a good number of procedures are available.

Concentration procedures will enable us to identify cysts of Giardia lamblia, Entamoeba histolytica, Entamoeba coli and Iodamoeba beutchlii, but trophozoites could rarely be seen in concentration procedures. So to overcome this, a number of procedures are available such as floatation or sedimentation procedures.

Floatation procedure permits the Stool Examination ˆ' separation of protozoan cysts and some helminth eggs, with the help of a liquid with high specific gravity. Parasites come to the surface and debris will remain at the bottom. This technique yields a clearer picture than the sedimentation procedure. However some dense eggs such as Ascaris egg (unfertilized), some helminth eggs, and some protozoa do not concentrate well with floatation method. Sedimentation procedure may give good result of protozoan eggs and larvae, but the sediment contains more faecal debrisIn this method some helminth eggs and parasitic cysts float to the surface of a liquid with high specific gravity like zinc sulphate.

ZINC SULPHATE FLOATATION PROCEDURE

1. Faecal suspension with half teaspoonful of faecal matter in 10–50 cc tap water.

2. Filter this suspension into a tube through two layers of gauze. Fill the tube with tap water up to 3 mm below the top and then centrifuge for one min. at 500 xg.

3. Decant, fill the tube with water and resuspend and centrifuge for 1 min. at 500 xmg.

4. Decant the water, add 2–3 ml of zinc sulphate solution resuspend the sediment, and fill the tube with zinc sulphate solution.

5. Centrifuge for two min. at 500 xg.

6. Without taking out the tubes from the centrifuge touch the surface of the suspension with a loop and then add the material on the loop onto a slide. Parasites should be detected.

In this procedure, eggs, larvae and cysts are fixed in formalin. Morphological features are well-preserved.

FORMALIN ETHER SEDIMENTAION TECHNIQUE

1. Mix ½ teaspoonful of faeces with 70 ml of 10 per cent formalin, allow it to stand for 30 min. to fix properly.

2. Filter this suspension through two layers of gauze into a centrifuge tube.

3. To this add physiological saline up to ½ inch of top and then centrifuge for one min. at 500 xg.

4. Decant, resuspend the sediment in saline and centrifuge for one min. at 500 xg.

5. Decant and resuspend the sediment in 10 per cent formalin.

6. Add 3 ml of ether, stopper and shake vigorously.

7. Centrifuge for 3 min. at 500 xg. There are four layers in the tube. One layer is sediment at the bottom containing parasites, a layer of faecal matter on the top and a layer of the ether above this. Decant all fluid and prepare a wet mount.

8. Examine the slide.

PERMANENT STAINED SMEARS

With permanent stained smears, intestinal protozoa can be easily detected and identified. Smaller protozoan organisms are often seen in the stained smear. Permanent stained smear is recommended for stool sample for routine examination. A good number of staining techniques are in vogue. The most commonly used method is iron–haemotoxylin method. Of late, trichrome stain is preferred in many laboratories.

PREPARATION OF FRESH SMEAR

As soon as the specimen arrives, take an applicator stick or brush to smear a small amount of stool on a clean slide and immerse it in Schaudins fixative and fix for 30 min. On the other hand if it is a liquid stool mix 3 or 4 drops of PVA with one or two drops of faecal matter on a slide and smear and allow it to dry at 35°C for several hours.

BLOOD FILM EXAMINATION

A large number of parasites are found in blood such as malarial parasites, microfilaria and trypanosomes. To study these two types of blood films (a) thin blood film (b) thick blood film have to be prepared. (a) Thin film can be made by a finger prick. A small drop is spread on a clean non-greasy slide and with a spreader uniform smear is made. It is allowed to dry and later stained. (b) Thick film is made with a big drop of blood which is put in the centre of the slide and spread with the help of a needle or slide at least ½ an inch area and dried.

Blood films must be prepared within one hour after the blood is drawn. Otherwise organism morphology may not be clear. Blood films should be stained as soon as possible and delay of more than three days may result in failure to demonstrate staining characteristics of individual species. The most common stains are of two types, the Wright's stain and Giemsa stain. In Wright's stain the fixative is combined with the staining solution. As a result of this both fixation and staining occur at the same time. Thick films are lysed while processing. Giemsa stain is the other one. Here the fixative and stain are separate. So the thick film is fixed with absolute methanol (acetone free) before staining thick films as it will be lysed during the staining process. When slides are removed from either staining, they are air-dried in a vertical position. Then they can be examined under oil immersion by placing oil directly on the uncovered blood film. If permanent slides are required for record, permanent mount should be made.

Blood cells have both acidophilic and basophilic structures. The nuclei are basophilic and take a blue shade the basophilic granules also take a blue shade. Haemoglobin takes a red shade. Various stains such as Wright's, Leishman's, Geimsa and Jenner stain are in vogue. In most laboratories methylene blue is used as a basic stain and toluidine blue in some. Most laboratories use eosin as the acid stain though azure I and II are also used. Buffer solutions commonly used are Solution 1 Sodium hydroxide 8 g Distilled water 1000 ml Solution 2 Potassium dihydrogenphosphate 27.2 g Distilled water 1000 cc Take 23.7 cc of solution 1 and 50 cc of solution 2 and mix it. 20 cc of the mixture is added to 100 cc of distilled water (pH 6.8). Thick blood smear examination enable you to test larger quantity of blood but it has its own disadvantages in the sense that morphology is distorted resulting in non-identification. In this smear blood cells concentrate in the centre of the film. Initially the slide should be examined under low magnification (10 × objective) to detect microfilariae, Babesia spp., Trypanosoma spp and malarial parasites are best detected under oil immersion (100 × objective). Presence of brown pigment granules may indicate the presence of malarial parasites. RBC will concentrate at the peripheri of the thick film and this gives a clue for malarial diagnosis. Microfilaria occur in small numbers in thin films. They are commonly found at the edge of the film or at the feathered end of the film because they are carriers of these sites while spreading whereas RBC are drawn out into one single distinct layer of cells. These can be examined for malarial parasite, etc., using stains like Wright's stain. Stains used

1. Wright's Stain

Wright's stain powder (BDA) 200 mg Methylene blue (acetone free) 100 ml Allow it to stand for a few days.

1. Flood the slide with stain 1–2 min.

2. Dilute it with equal amount of buffer.

3. Allow it to stand for 5 min.

4. Flood off with tap water.

2. Leishman's Stain

Leishman's powder 150 mg Methyl alcohol 133 ml If it does not dissolve, powder the stain and dissolve and store it.

1. Flood the slide with stain for 20 min.

2. Dilute it with double amount of buffer.

3. Excess stain is flooded with distilled water.

4. Air-dry the slide.

3. Giemsa stain

Giemsa powder 300 mg Glycerine 25.0 ml Methyl alcohol 25.0 ml This is the stock solution. Just before use dilute 1 ml of stain with 9 ml of buffered distilled water.

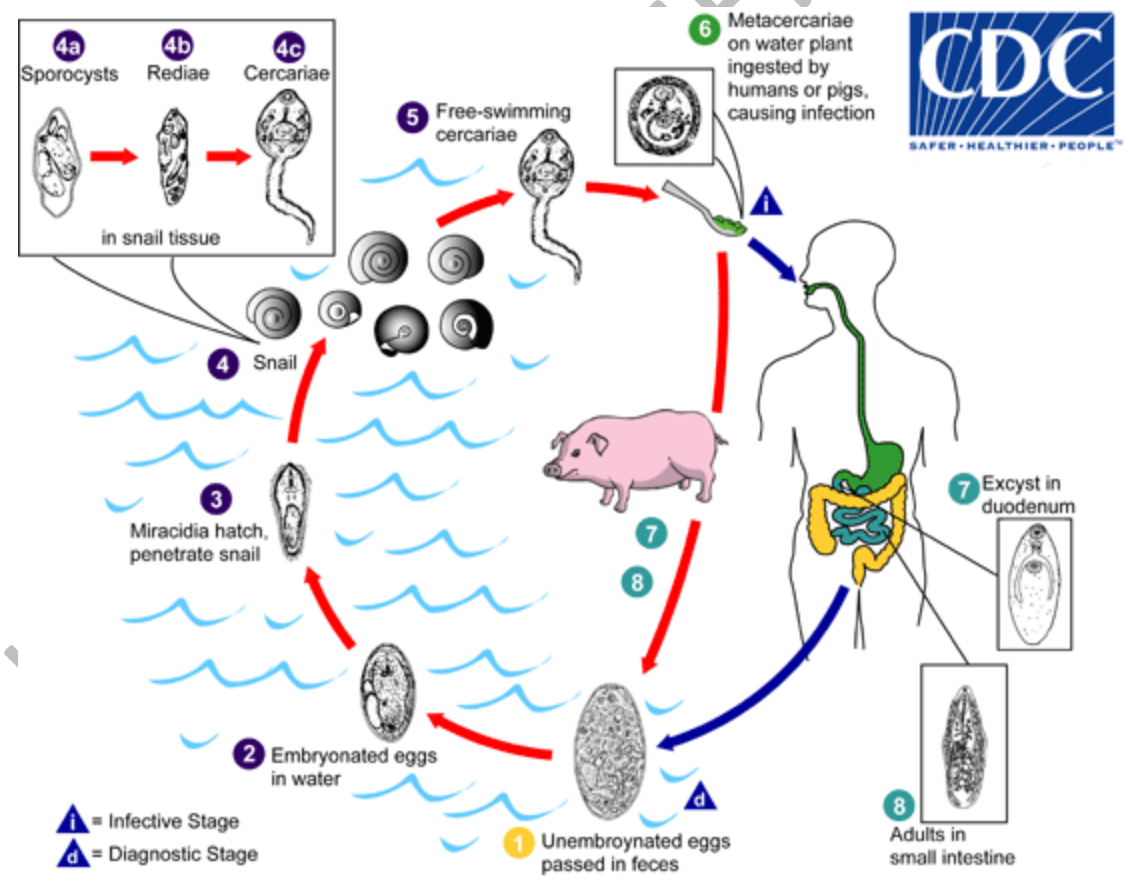
1. Fix the blood film with methyl alcohol for 5 min.

2. Air-dry.

3. Flood the slide with stain for 15 min.

FASCIOLA BUSKI

This is the giant intestinal fluke. Its infection is mostly confined to Asian countries like China, India, Bangladesh, Thailand, Malaysia, Myanmar, and Sumatra. It is a parasite found in pig and dog, and inhabits the small intestine. This is the largest trematode of man measuring 3 cm × 12 cm and is 3 cm thick. It is oval, elongated and flesh-coloured Body is covered by transverse rows of spines with numerous spines near the ventral sucker. Acetabulum is close to the ventral sucker and it is prolonged into a cavity dorsally and backwards. Intestinal caecae are without lateral branches. Genital pore is medially placed anterior to the ventral sucker. Both testis and ovary are branched.



Egg measures on an average 140 × 80 m and is operculated. Presence of eggs in the stool confirms the infection. After 3–7 weeks, the egg hatches into a miracidium in the snail host Segmentina hemisphaerula. Miracidium later develops into sporocyst within 3 days, which later develops into redia, daughter redia and finally cercaria. The whole cycle is completed in 2 months. Cercaria is oval in shape, lophocercous, with a well-developed digestive tract, a muscular bladder and collecting lobules. These cercariae as usual encyst on water plants and transform into metacercariae. Man and pig become infected through the consumption of viable metacercariae attached to the water plants like water hyacinth, water caltrops, water chestnut, water bamboo, lotus roots and wild rice shoots. F. buski encysts in the duodenum and gets attached to the duodenal and jejunal walls. The larvae mature in 3 months and start producing as many as 25,000 eggs per day. If these eggs reach water sources, the cycle repeats.

Epidemiology

In Thailand, certain places where there is heavy rainfall and flooding resulting in faecal contamination of water are sources of infection. In addition to this, there is large-scale usage of pig and human faeces as manure in certain areas and these regions are prone to infection. In China, there are reports of sources of infection through contaminated drinking water. Children over 5 years of age are more prone to infection since they eat and enjoy water plants while in play.

Pathogenesis

When the parasites occur in small numbers, they do not cause any harm, but when they occur in large numbers, they cause intestinal obstruction, followed by intestinal ulceration which will indirectly interfere with digestion. Ulcers sometimes bleed due to capillary damage.

Symptoms

Diarrhoea, flatulence, loss of appetite, vomiting, mild colic pain and ultimately fever and eosinophilia. Since eggs are laid in large numbers, faecal infection is quite easy.

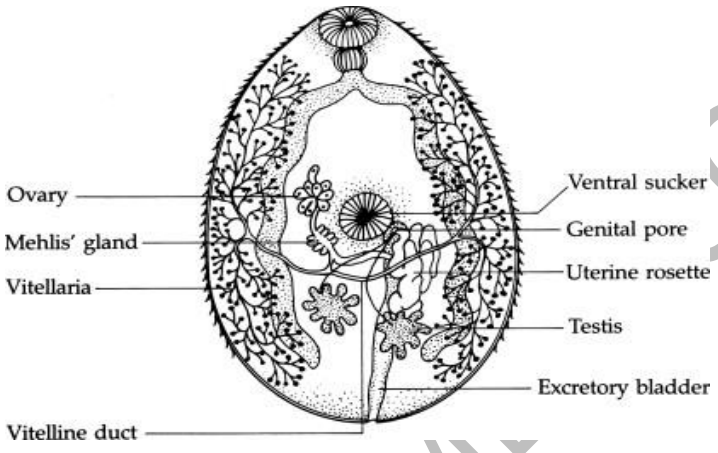
PARAGONIMUS

This parasite measures 8–20 × 6–9 m and is somewhat oval in shape (Figure 5.31). The oral sucker is subterminal. Ventral sucker is larger than the oral sucker, almost and somewhere in the central part of the body. Body is transluscent and reddish brown in colour. It has a short oesophagus, pharynx and a bifurcated intestine. Genital pore is situated near the ventral sucker. Testis is tubular, ovary is branched on either side of the median line posterior to the ventral sucker. Saclike uterus lies opposite to the ovary. Well-developed vitellaria extend the whole length of the body. There is a shell gland, Laurer's canal. There is no cirrus pouch and cirrus organ.

Life cycle

The eggs are in the form of pockets in the lungs and find their way into the water by sputum or faeces. Within 15 days to seven weeks, a ciliated miracidium emerges out. It has a ciliated

covering arranged in four rows at the anterior end. The excretory pore is in the form of a rosette. It makes entry into the snail host. In about two months it develops into sporocyst and later rediae. Each redia contains cercariae which are microcercous and which meaure 200 × 70–80 m and are ellipsoidal in shape. Tail is in the form of a knob. It has an anterior stylet and the whole body is covered with spines. The second intermediate hosts are crabs or crayfish (fresh water). The cercariae bore into these hosts. In crabs, the metacercariae encyst in the liver, muscles and the gills. When humans consume such infected crabs, the metacercariae enter the stomach where the cyst wall is digested. From the abdominal cavity, they penetrate the diaphragm, lungs and finally the bronchiole.



Symptoms of paragonimiasis include:

- Fever.
- Chest pain.
- Fatigue.
- Cough. This might be a dry cough at first and progress to producing rusty-colored or blood-tinged mucus.
- Unintended weight loss.
- Diarrhea.
- Abdominal pain.
- Bumps under the skin of your abdomen (belly). These may hurt to touch and they might gradually move upward toward your chest.

Diagnosis

- Sputum (mucus coughed up from your lungs).
- Poop (stool).

- Fluid from around your lungs.
- Skin or other tissue samples.

Treatment

Healthcare providers use antiparasitic medications (praziquantel or triclabendazole) to treat paragonimiasis. If you have cerebral paragonimiasis, they may also treat you with corticosteroids to reduce inflammation in your brain and spinal cord. Rarely, you might need surgery to remove cysts.

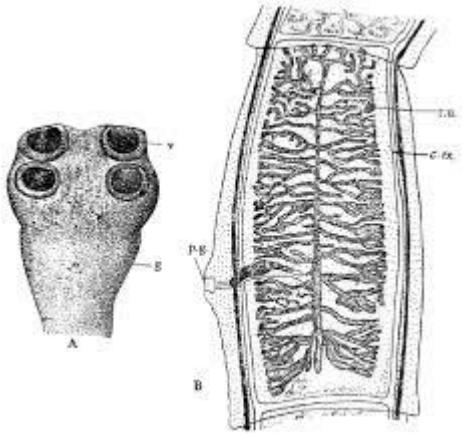
Prevention

Preparations to avoid include:

- Raw or undercooked crab.
- Pickled crab.
- Salted crab.
- “Drunken” crab (dipped or marinated in wine).
- Crab juice.

Taenia saginata

Taenia saginata is a whitish, semi-transparent worm reaching a length of 4–10 m and contains as many as 2,000 segments. The scolex is pear-shaped and without a rostellum or rostellar hooks. Adult worms live with their heads firmly attached to the mucous membrane of the small intestine. The scolex has four hemispherical suckers which are frequently pigmented. The suckers are the sole organs of attachment. Neck is slender and long, not more than one half as broad as the head. This is followed by a chain of segments—immature, mature (Figure 5.35b) and gravid proglottids—which is three times longer. As many as testes (300 – 400) and in the absence of accessory ovarian lobe. There are as many as 97,000 eggs in each gravid segment (Figure 5.35c). The uterus has 15–20 main lateral branches which is a diagnostic character. When the gravid proglottid detaches itself from the strobila, the ova are expelled. The eggs are globular, 30–40 × 20–30 µ m, and cannot be distinguished from T. solium. These eggs have a double-shelled embryophore with an oncosphere inside. The egg has an outer shell, a chorionic membrane and two oncospharal membranes.



Gravid proglottids pass out in stool to the exterior and get settled on grass. There they disintegrate. When the intermediate host ox happens to eat the grass the eggs reach the duodenum, where the oncospheres are set free. They migrate to the small intestine where they penetrate the wall and from there they get into circulation and are carried on to the pterygoid muscle, diaphragm and the tongue. In the muscle in 60–75 days they metamorphose into the bladderworm (cysticercus).

CYSTICERCUS

This cysticercus measures 7.5–9 × 5.5 m. The scolex is unarmed, ovoid in shape and milky white. The cysticercus is devoid of hooks on the scolices and this is a distinguishing character in which they differ from other cysticerci. They live for eight months in the ox and their further development depends upon their entry into humans who are the definitive host and who get infected by eating flesh with cysticercus. The bladder is digested and the liberated scolex gets a firm hold on the wall of the intestine.

It has a small invaginated scolex). It has hairlike processes, a peripherous collaginous fibrous layer, two muscle layers, calcareous corpuscles, peripheral cells, flame cells, a duct system embedded in a loose fibrous net and a central band of muscles. The different cestode larvae could be distinguished in human tissues by variations in their structures.

EPIDEMIOLOGY

Humans are infected by eating raw beef containing cysticercus larvae. Cattle get infected by grazing on ground polluted by human faeces containing the eggs.

PATHOGENESIS

Because of the size of the worm, they produce acute intestinal stoppage. Sometimes proglottids get lodged in the lumen of the appendix and cause appendicitis. When the incubation period is over, diarrhoea starts and there is loss of weight.

NEMATODES

The group Nematoda is a widespread and successful group consisting of a number of small worms, which occupy almost every habitat. i.e., terrestrial, marine and fresh water. Added to this they gain fame as the best known parasites of every class of vertebrates and invertebrates including insects as well as plants. In vertebrate hosts they infect almost all tissues and organs. Some of them are much larger in size than their free-living counterparts. The larval or the juvenile stages are spent in the intermediate host and the adult stage in definitive hosts. In some instances, all the stages are passed in the same host as in the case of Trichinella spiralis.

The following are some of the important characters of parasitic nematodes. Cuticle is non-cellular which is shed periodically. Provided with musculature. Fully developed digestive system is present. Reproductive system is well-developed. Eggs and the larval stages are well suited to

survive in the external environment or in the intermediate host. More than a dozen species of nematodes are human parasites and more than a dozen species are zoonotic human parasites.

More than 1 billion people are hosts of Ascaris lumbricoides (roundworm), more than 800 million people have hookworm (Ancylostoma duodenale) infection and several hundreds of millions of people suffer from pinworm (Enterobius vermicularis and Wuchereria bancrofti) infection.

ASCARIS LUMBRICOIDES

It is the largest human intestinal parasite. Adult female is larger than the male with an elongated cylindrical body, with a blunt anterior en × 3–8 mm and the male 15–30 cm × 2–4 mm. The worm is pale, brown or whitish in colour with prominent lateral lines longitudinally running the entire length of the body. The mouth is situated at the anterior end and is guarded by 3 lips with fine denticulated ridges. Of the three lips, one is dorso-median and the other two are ventro-lateral. Buccal cavity is triangular. The posterior end of male is curved. Reproductive system consists of testes and vas deferens, ejaculatory duct which is coiled towards its posterior end and opens into the cloaca. Associated with male reproductive system is a pair of spicules situated in a pocket. Gubernaculum is absent. The male tail is conical with two rows of tactile papillae mostly pre-anal and few post-anal. In the female, the vagina is directed backward, paired genital tubes are present, each having uterus, receptaculum seminis, oviduct and an ovary. At a time these worms can lay as many as 27 million eggs and per day as many as 200,000 eggs are laid. d and tapering posterior end. It measures 20–35 cm.

Egg measures 50–70 × 40–50 m with an elliptical shape or ovoidal broad (Figure 5.44 f) with a transparent shell having a rough albuminous coat and with lipoidal vitelline membrane inside (not found in unfertilized eggs) and a thick transparent middle layer. These eggs when they pass in the faeces do not have a differentiated embryo, and no segmentation. These eggs are resistant to desiccation, low temperatures, putrefaction of the medium and strong chemicals, and they lie dormant. With advent of Parasitology favourable conditions, the eggs are stimulated. Within 2–4 months, the coiled up embryo inside the egg is seen moving. The embryo will come out only when the egg is swallowed. Once it is swallowed, it passes down to the duodenum, and is softened by the digestive juices. It releases the larva which becomes activated and is called the rhabditiform larva. It penetrates the mucous membrane, enters the blood via the heart and the lungs and then to the alveolus capillaries. Here they burrow in the wall of the alveolus, enter the respiratory tree and finally move to the trachea. Most of the larvae are again swallowed for the second time from the trachea and they reach the small intestine. This second invasion brings about severe allergy and low blood pressure. The whole process takes place in 10–14 days during which period the larva moults twice. The larva measures about 1.75–2.37 mm. Then the larva migrates to the intestine by the fifth day. The larva moults again between the 25th and 29th day. Incubation period in humans is about 60–70 days. Humans gets infection by ingesting fully embryonated eggs from the soil, or food and consuming drinks, contaminated with viable eggs.

Epidemiology

Human infection occurs by ingestion of fully embryonated eggs present in polluted soil, food and drink. All age groups may be heavily infected. In areas where human faeces are used as fertilizers, infections occur through vegetables contaminated with eggs. Ascariasis is known as household infection propagated within the house.

Pathogenesis

Complications due to A. lumbricoides arise out of the need for extra-intestinal migration. The migration of the larva through the lungs may precipitate in pneumonia. In most nematode infections eosinophilia is common. Adults in the intestine cause abdominal discomfort, colic pains, vomiting and diarrhoea. In heavy infections, worms become tangled masses blocking the host intestine. In severe infections worms up to 1500 have been recovered from the host. Irritation of mucous membrane and appendicitis occur. Release of toxic substances from the worm cause convulsions, delirium, etc. Ascaris-infected school children have stunted growth.

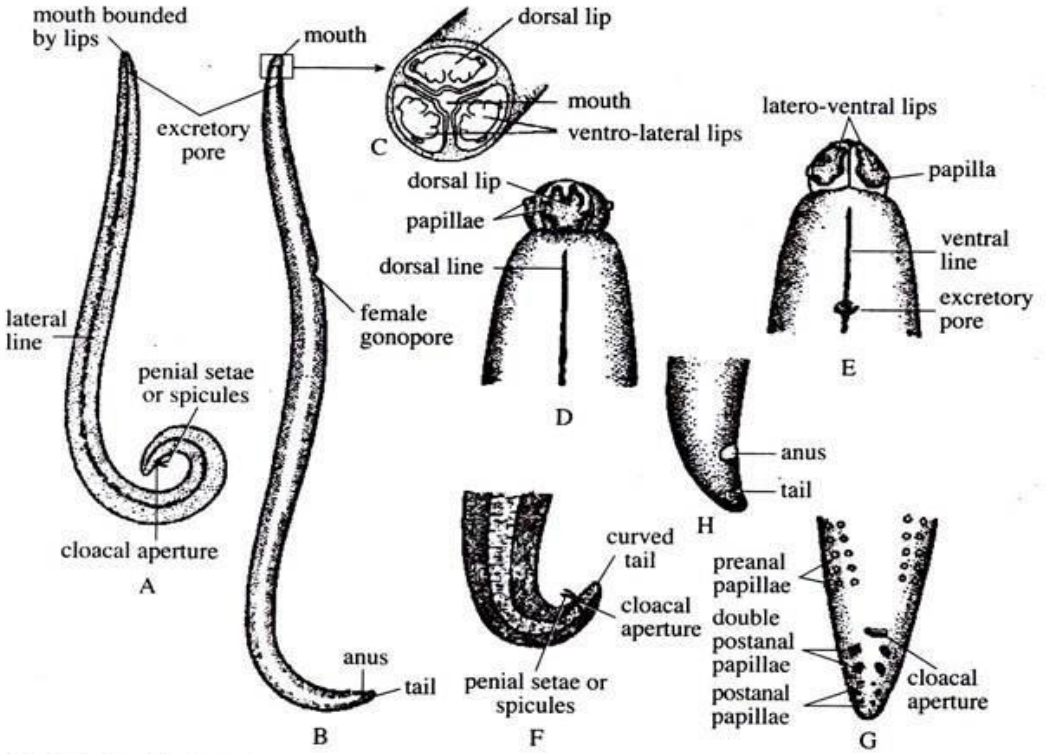


Fig. 1.64 : *Ascaris lumbricoides*. A. Adult male in lateral view. B. Adult female in lateral view, C. Enlarged view of head end, D. Anterior end in dorsal view, E. Anterior end in ventral view, F. Posterior end of male in side view, G. Posterior end of male in ventral view showing papillae, H. Posterior end of female in side view.

ENTEROBIUS

The adult worms live in the caecum, appendix, the adjacent regions of the ascending colon and the ileum. They live with their heads attached to the mucosa of the intestinal wall. The parasite is small, white and spindle-shaped. A true buccal capsule is absent but the worm has 3 lips and a

dorso-ventral bladder transversely striated. Male is rarely seen. It measures 2.5 mm in length and 0.1 to 0.2 in greatest diameter. Posterior end is strongly curved spirally. The curved end bears 6 sensory papillae and a single spicule. A gubernaculum is lacking. Copulatory bursa is reduced and termed as “caudal aloe” supported by a pair of anterior pedunculate papillae.

Life cycle

No intermediate host is involved in the life cycle for subsequent development and there is no multiplication of worms inside the body. The shell of egg gets weakened by the digestive juices and the larva breaks out of the shell and starts invading the glandular crypts and penetrates into the glands and stroma where it coils up. The lifespan of E. vermicularis is 37 to 93 days. The female worm comes down the intestine and lies in the faeces. The fertilized worm migrates to the anal region to lay eggs in the perianal skin and perineum. The crawling of the gravid females produces an itching sensation. After a few hours the embryo develops rapidly and grows to a length of 140–150 m. The eggs enter the mouth due to nails soiled with faecal matter under finger nails, reaches the digestive system where it hatches. Liberated larvae after 2 months reach the large intestine where they mature. The whole cycle is completed in 2–4 weeks.

Epidemiology

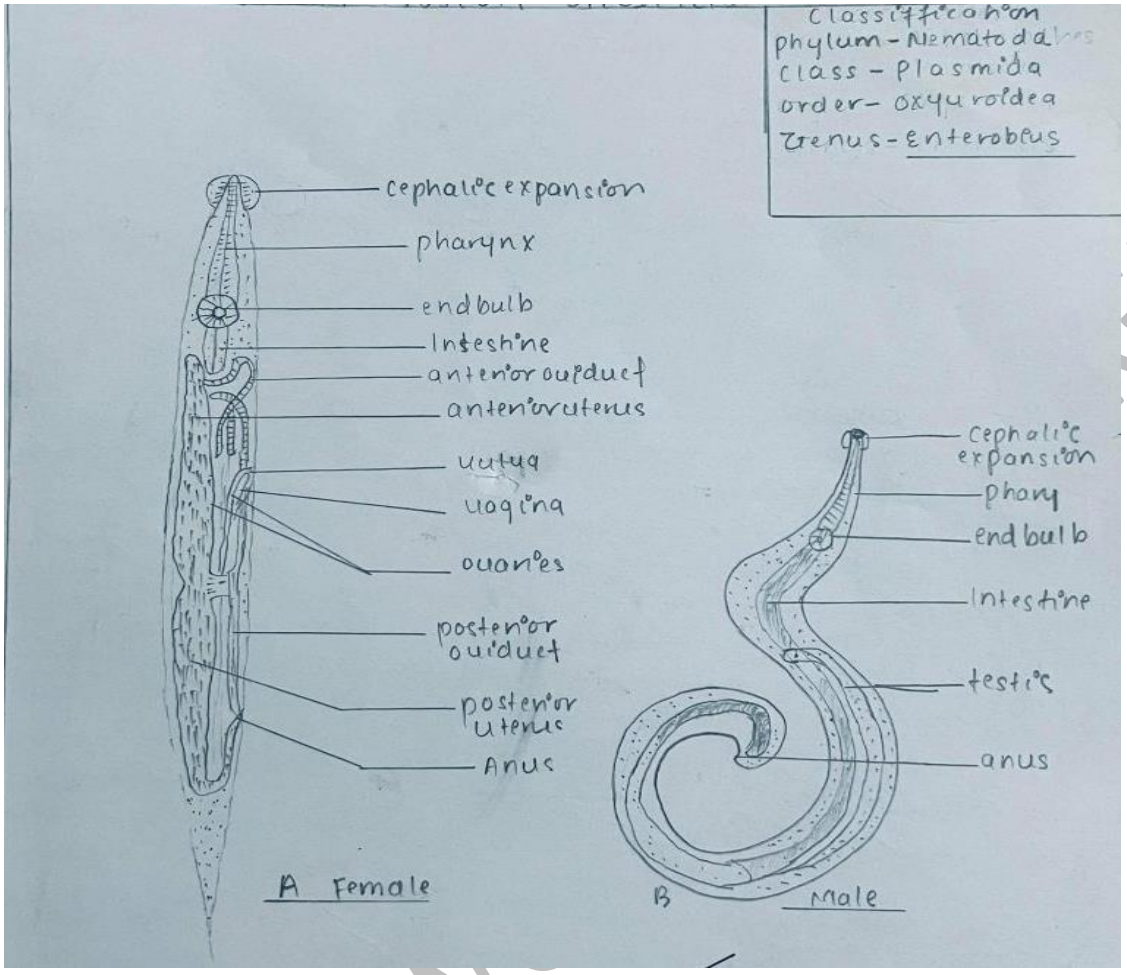
It is worldwide in distribution and children are more prone to this disease. It is common in groups or institutions like school and asylums. Though it is a human infection, chimpanzees and gibbons could also be infected.

Pathology

Due to its habitation in upper part of colon, caecum and lower ileum, minute ulcerations are seen. Sometimes haemorrhage could be noticed.

Symptoms

Theyare more obvious when gravid females migrate out by the anus on to perianal skin to deposit eggs where they cause pruritus.



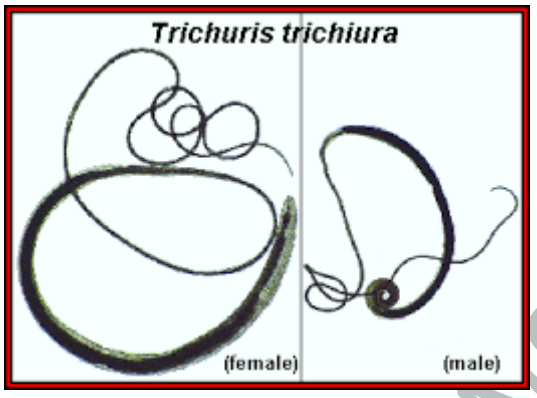
Trichuris trichuria

This parasite lives attached to the wall of the caecum. Humans are the typical hosts, although there are reports of the parasites infecting pigs and some monkeys. Worms are creamy with a fleshy posterior end. The oesophagus is delicate with anterior muscle with stylet and posterior muscles dilating at its more distal end. Male grows to a length of 30–40 mm, and has an anterior attenuated portion with cellular oesophagus which is half as long again as thicker posterior portion. The caudal portion is curved with a single spicule armed with spines. The female grows to a length of 30–34 mm with an anterior attenuated portion, twice as long as the posterior portion. Uterus occupies the posterior portion fully packed with eggs. Ovary is saccular and runs forward from the posterior end. Females preponderate over males in a proportion of over 400 to 1.

Eggs measure 50 × 22 m, and are brown in colour and have a peculiar barrel shape. It has a thick shell with a plug at both ends. Eggs contain an unsegmented embryo. They are discharged and passed in the stool

Epidemiology

It is common in places where rainfall and, humidity are high, and which are shady and have poor sanitation and in contaminated soil. Mostly it is transmitted by soil contaminated with eggs. In Malaysia, children are more prone to this infection. This infection is associated with Ascaris. Pathology When they occur in large numbers they spread to the colon and then to rectum. Haemorrhage, stools with lot of mucus and dysentery are some of the symptoms.



Dracunculus medinensis

The male measures 40 mm in length 1.2–2.9 × 0.4 mm in breadth. The posterior end is coiled with 10 pairs of caudal papillae of which 4 are pre-anal, and six post-anal. The copulatory spicules are subequal. After copulation, the male dies. Figure 5.55 shows the various parts of male and female of this organism. The embryo measures 500–750 × 17 m and is flattened. Body shows transverse striation. Tail is long, slender with a rounded head. The alimentary canal consists of a bulblike oesophagus and rudimentary anus.

Life cycle

Man is the definitive host. The adult worm discharges larvae when they are ingested by a crustacean (copepod), the intermediate host. When humans drink accidentally the contaminated water infected with Cyclops, they get the infection. This is the third-stage larvae present in the haemocoel. In the body of humans, the Cyclops is killed by the gastric juices and the worms are released and penetrate the duodenal wall and attack the sub-cutaneous tissues. In the tissues the larvae develop into male or female within a span of 4 months. After copulation, male dies. The fertilized female grows, migrates within the connective tissues and after six months they come to lie in the sites where it is likely to come in contact with water. The common site is the leg. Arms, shoulders, feet, genitalia are also affected. The female worm lies below the skin and secretes some kind of a toxin which irritates the skin and forms blister. This blister bursts and forms an ulcer with a small hole at its base through which the female protrudes. When the ulcer comes in contact with water, the female discharges a milky fluid loaded with a number of larvae. This process lasts for 3 weeks. The larvae swim about for a week and gain entry into the fresh water crustacean Cyclops. The larvae penetrate the gut wall of the crustacean, enter the body cavity

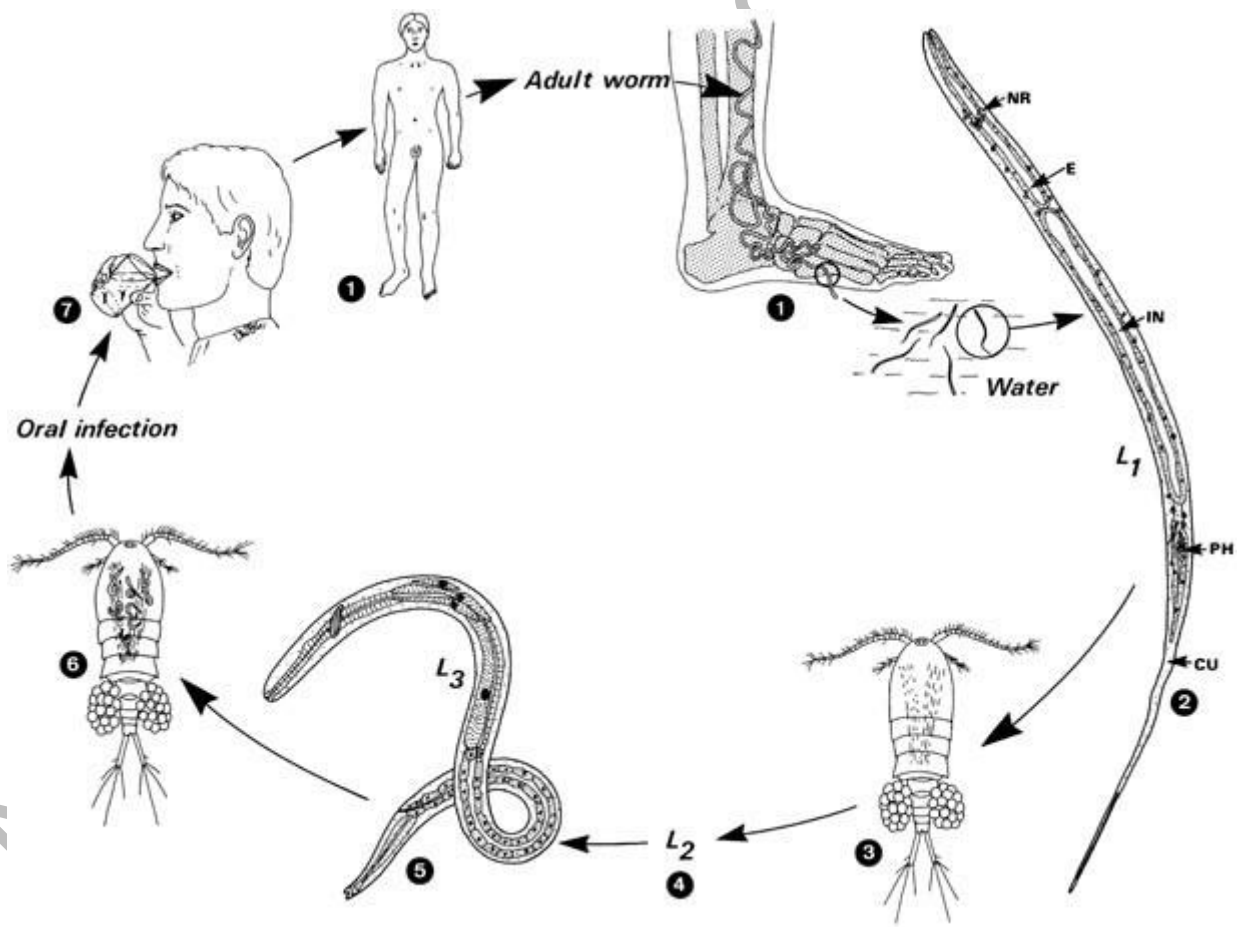
where 2 ecdyses take place in 2–4 weeks and enter into third-stage larva. The entire cycle takes about a year.

Epidemiology

Period of infection coincides with rainy season. Infection is common in children and adults up to 35 years. Later the incidence falls. Infections are common in areas of step wells. Jodhpur in Rajasthan is a highly endemic area.

Pathogenesis

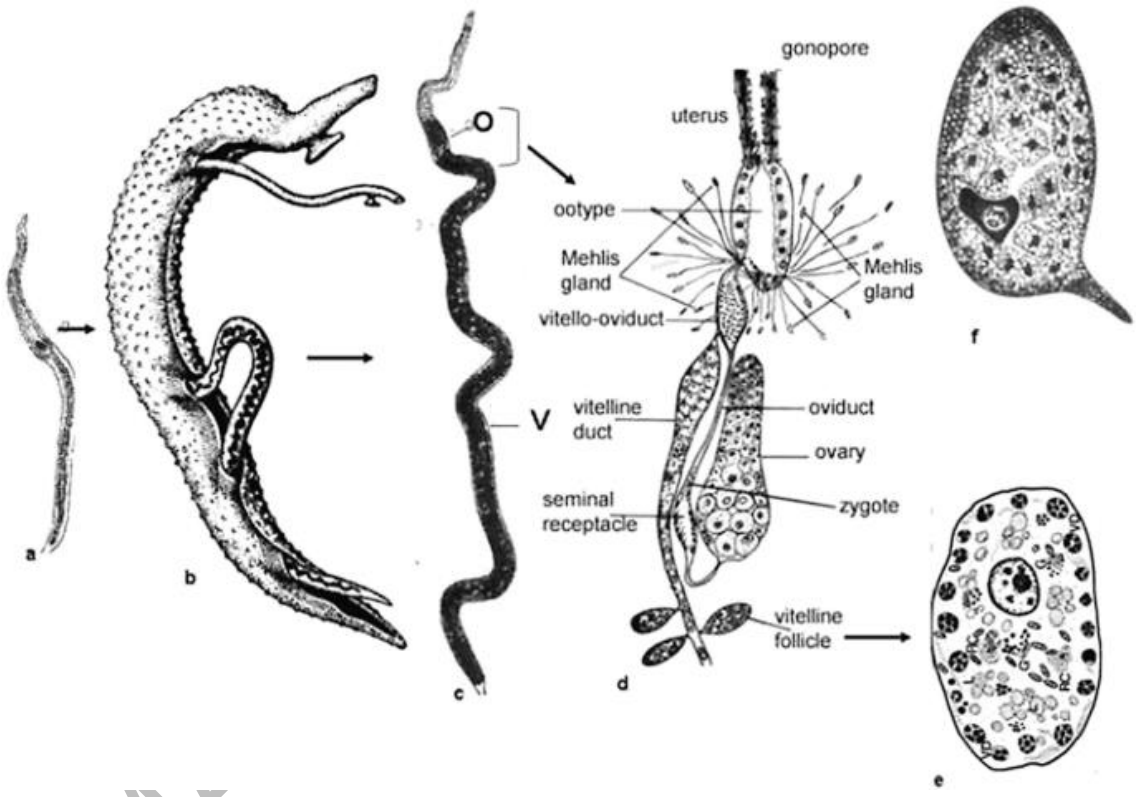
The guinea worm pierces the layers of the skin through the anterior end of the body and irritates the tissue to cause a blister. The blister breaks and exposes a shallow ulcer with a hole in the middle. When the ulcer comes into contact with water the worm's uterus projects out and a milky fluid containing larvae is issued. The larvae are released in puffs and ultimately the uterus dries up. is blister bursts and forms an ulcer with a small hole at its base through which the female.



Schistosoma mansoni

These worms are almost similar to S. haematobium. Male is 6.4 to 12 mm in length and females 7.2–17 mm. Body is covered over by tentacles which are prominent. In male, the testis is 6.9 m

with minute sensory papillae and with tufts of hairs. In female, the ovary is at the anterior end, the receptaculum seminis lies posterior to the ovary. Uterus is short and already contains eggs with characteristic lateral spines. The eggs break the venules, get into the mucosal layers of the intestine and escape into the lumen of the bowel and finally pass into faeces. The egg has a yellowish brown shell which is transparent with characteristic lateral spines. It measures 114–175 m in length and 45–68 m in breadth. The eggs are in mature stage when they are discharged. Miracidia escape from the egg and they possess penetration glands. A small primitive gut is present. Miracidia have the ability to locate the appropriate snail host which in this case is B. glabarata. In 4 weeks time the miracidia develops into one or two generations of sporocysts and finally cercaria. The cercaria resembles that of S.japonicum. It possesses 2 pairs of preacetabular and 3–4 pairs of post-acetabular penetration glands.



SCHICSTOSOMA

Wuchereria brancrofti

It is a threadlike worm, white in colour and inhabits lymph glands and vessels. Sexes live coiled together and it is a bit difficult to separate them. Cuticle is smooth. Male measures 4 mm × 0.1 mm, coiled tail with two spicules. Spicule is grooved on the ventral side. Distal portion is whiplike ending in a hood and there are 15 pairs of minute sensory caudal papillae. In the posterior wall of the cloaca, there is a saddle-shaped thickening forming a shield. In W. bancrofti there is a characteristic accessory process. There are 12 pairs of circumoral papillae of which eight pairs are pre-anal and 4 pairs are post-anal.

There are two pairs of large papillae which are sessile and at the tail a solitary pair of minute size. The female measures 6.5 cm × 0.2–2.8 m. The anterior end is tapering with a round swelling. There are some sessile papillae on the head. The oral aperture leads to oesophagus which is cylindrical in shape. The tubular intestine is 1/5th of the total diameter and posteriorly opens into the rectum. The caudal end is narrow ending abruptly in a rounded fashion. Vulva is behind the anterior extremity. Vagina is swollen and leads into the uterus which divides into 2 coiled tubes occupying a major portion of the body and wider than the intestine.

There is a pair of ovaries and oviducts. Eggs are enclosed in a chorionic membrane, which forms a protective sheath to microfilariae. The female liberates the eggs which migrate to the bloodstream via the lymphatics. The microfilariae measure 216 m. Within the egg the embryo lies curled up. The microfilaria which measure 280 × 7 m has no definite shape. Under high magnification, the microfilaria is enclosed in a sheath. Sheath is longer than the microfilaria. It takes a mauve shade with Giemsa stain. Within the sheath the microfilaria moves up and backwards and upwards. The nature of the sheath is the subject of controversy. The general opinion is that the sheath is nothing but an outstretched vitelline membrane but in certain other microfilaria, it is not the vitelline membrane but the sheath is developed during its sojourn in the blood. Somewhere in the middle third of the body, some kind of granular material is present which may be considered as the primitive gut. The whole body has transverse striations which could be considered as the muscular layer.

At one-seventh of the length from the head there is a break, which could be considered as the nerve ring and at the fifth of the length there is a triangular 'V' shaped path or 'V' spot which is supposed to be the excretory cell and excretory pore. Anus is at the posterior end, a short distance from the tail, or cloaca and is called the posterior 'V' spot. When stained, some cells get deeply stained and they form the G1 –G4 cells, the genital cells and the body appears to be fully packed with cells. A short fang shoots out at regular intervals from the uncovered cephalic end.

Nerve ring Excretory pore Excretory cell Genital cells Genital cells Anal pore f Microfilaria pass through the peripheral capillaries. They are more active at night than during day.

Life cycle

Microfilaria circulate in the bloodstream. In many Asian countries they exhibit nocturnal periodicity. They occur in large numbers in peripheral blood at night between 10 p.m. and 4 a.m. During daytime they are found in capillaries of the lungs, kidneys or in other great vessels. In certain countries like Malayan archipelago and Pacific Islands, they occur in peripheral circulation both at day and night with a peak period in the afternoon. The life cycle was first worked out by Mansoon in 1878 in China.

Life cycle in mosquito

The mosquito Culex pipiens quinquefasciatus, is the vector which transmits the organism from one human to another. Within an hour, after entering the mosquito, in the stomach the sheaths are cast off and they penetrate the stomach wall. While penetrating, there is a chance of the

microfilaria being damaged by the buccopharyngeal armature of the mosquito. The embryos collect at the anterior end of the stomach, later migrate to the midgut. Man is the definitive host. There is no reservoir host for W. bancrofti. The intermediate host is the female mosquito of the genus Culex. In India, the vector is Culex pipiens quinquefasciatus. Further development of microfilariae depends upon their entry into the male Culex mosquito.

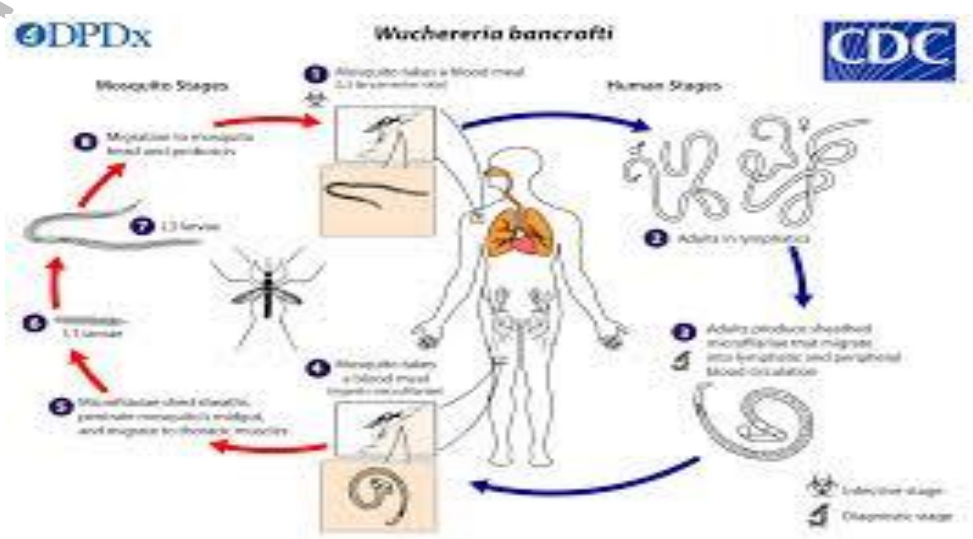
If this does not happen the microfilaria die. The lifespan is about 2–3 months when Culex feeds on blood of infected humans, the sheaths are cast off, then penetrate the stomach wall and finally reach the thoracic muscles where further development takes place. After 12 days, they metamorphose into the 1st stage larva. It has a sausage shape with a tail and measure 225–325 × 15–30 m. It moults twice a week grows considerably and develops into the second stage larva, which measures 225–325 × 15–30 m. It develops further in a week, becomes elongated and all external structures are developed. Now it is the third stage larva.

This is the filariform larva and measures 1500 –2000 × 5–28 m. This is infective, active, motile larva. They migrate to the proboscis of the mosquito. Now it is ready to infect the human host when mosquito takes a bite. Microfilaria does not develop further in the mosquito. From the time the microfilaria enters the mosquito, to the development of the infective stage in the proboscis, 10–20 days are required for the whole period.

This period varies with environmental factors like humidity, temperature and also vector species. When a mosquito with infective stages bites a person and takes a blood meal, it introduces microfilaria. The larva accumulates at the site of bite, penetrates the skin, enters the lymphatic vessels and is carried to lymph nodes where it develops into adult form either male or female. In about 6 months the larvae become sexually mature and mate. A female releases as many as 2,50,000 microfilaria per day. They pass through the thoracic duct and pulmonary capillaries to the peripheral circulation. From the time microfilaria enter the human host till the appearance of microfilaria in circulation, it takes 8–12 months. This is the incubation period.

Pathogenesis

The typical manifestation of filariasis is due to blockage of lymph nodes and vessels. The affected lymph nodes and vessels show hyperplasia. The wall of the vessels get thickened and the lumen narrows. As a result the lymph vessels dilate and finally result in oedema or filariasis.



ANKYLOSTOMA BRASILIENSE

It is found in dogs and cats. It is a rare parasite in the small intestine. It is part of mixed hookworm infection in humans in India, Malaysia and Thailand. Males measure 7.75–8.5 mm in length × 0.35 mm in diameter and females measure 9–10 m × 0.375 m (Figure 5.48). Unlike A. duodenale, A. braziliense has a buccal capsule within conspicuous median teeth and a pair of outer teeth. The bursa is also conspicuous, and is supported by short stubby rays. The eggs are almost similar to those of A. duodenale and are indistinguishable from the latter. The life cycle pattern is same as that of A. duodenale. Humans are not suitable hosts. The larva does not enter the bloodstream but wanders under the skin.

ANKYLOSTOMA DUODENALE

This is commonly called the hookworm. Both male and female are stout, cylindrical and anteriorly constricted. Females measure 1–1.3 cm × 0.6 m, cylindrical in shape, posteriorly expanded, creamy grey in colour and covered with a tough cuticle and provided with a pair of lateral cervical papillae below the circumesophageal ring (Figure 5.47). Vagina is at the posterior one-third of the body. Adult worms live in the duodenum, jejunum and ileum. The body is so curved that the anterior aspect is concave and the ventral aspect convex. Mouth is not at the tip but directed dorsally. Buccal capsule is prominent with two pairs of horselike teeth. Male worm is 8–11 mm in length and 0.4 m thick. The posterior end is expanded with copulatory bursa supported by rays. The ray pattern is species-specific. Cloaca is situated in the bursa. The rectum and genital canal open into the bursa. There is a pair of long retractile bristlelike copulatory spicules the tips of which project from the bursa. The female is longer than the male and measures 10–13 mm long and 0.6 mm thick. The posterior end is conical with sub-terminal anus. Vulva opens on the ventral side at the junction of the middle and posterior third of the body. The vagina leads into coiled tubes, the ovarian tubes.

During copulation the male attaches its copulatory bursa to the vulva. The copulatory pairs assume a Y-shaped figure. In both sexes, well-differentiated cephalic glands are present. They secrete an anti-coagulating ferment. The excretory pore is ventrally placed at the level of the oesophagus. The buccal capsule has a chitinous living with 2 pairs of sharp teeth on the ventral side. Eggs are ovoid and measure 60 × 60 µm .It has an acting hyaline shell membrane. When eggs are released, the egg contains an unsegmented ovum. When passed in the faeces, the egg contains a segmented ovum with 4 or 8 blastomeres and with a space between the ovum and the egg shell. A female can lay as many as 25,000 to 30,000 eggs a day and 55 million during its lifetime.

Eggs freshly passed in faeces are not infective for man. When eggs are deposited in soil under favourable conditions like shady environment, or moist and decaying vegetation, a rhabditiform larva emerges out.

It measures 250 m long and feeds on bacteria and organic matter in the soil, moults first time on 3rd day and second time on 5th day to become the infective filariform larva. It is 500 m long with

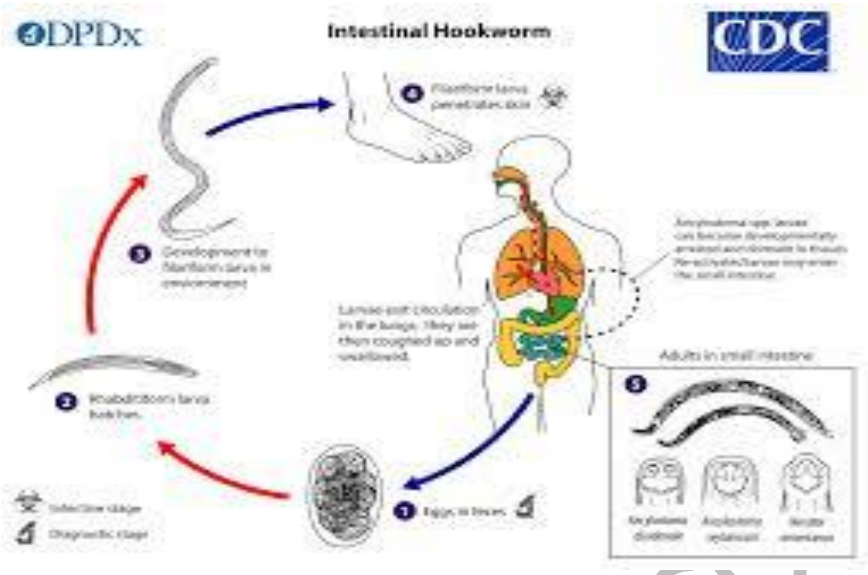
a pointed tail. This is the non-feeding stage. They live in the soil for about 5 weeks. They are attached to grass blades. When a person walks bare-footed in soil containing filariform larva, the latter penetrate the skin (sub-cutaneous tissue). The soft skin between the toes is the favourite site for penetration. For people working in farms, the larvae may penetrate the skin of the hands. The larvae enter the venules of the subcutaneous tissue, and is carried in the circulation to the right heart and to the lungs. In the lungs they break the capillaries and reach the alveoli, from where they migrate to the jejunum, where they moult and develop a temporary capsule with which they get attached to the mucosa. Here they feed and grow and undergo the fourth and final moult during which they develop a regular buccal capsule growing into adults. In about six weeks they become sexually mature and start laying eggs. Oral infection is rare. The larvae penetrate the buccal mucosa, reach the venous circulation and complete the migration via lungs. Alternatively the larvae may be swallowed and develop directly into adults in the small intestine without a tissue phase.

Epidemiology

Epidemiology of hookworms is linked to the following closely integrated factors: Defaecation habits—eggs deposited in areas of favourable conditions, appropriate environment for development of the eggs (moisture, warmth). Soil-shade and sandy. Opportunity for filariform larva to come into contact with human skin. In tropical and subtropical countries these conditions remain optimal throughout a greater part of the year. Prolonged rainfall or dry and cold seasons may be detrimental for the survival of larvae.

Pathogenesis

The hookworm larvae, itching and inflammation of the skin and development of pustular sores may occur. A. braziliense may fail to find these ways beneath the germinative layer and become unable to reach blood or lymph vessels. Thus they may enter airlessly under the skin-cutaneous larva migrans (creeping eruption). Next they cause pulmonary infection and even pneumonia symptoms. The larva may be encapsulated. Eosinophilia and leucocytoses are common. When the worms are migrating in the intestine, nausea, abdominal discomfort are common. The important effect's because of their habit of blood drinking anaemia is the frequent condition. In severe cases haemoglobin may be reduced to 30%. Appetite is capricious. In children, physical and mental retardation may appear. In pregnant mothers still births are common.



TRICHINELLA

Causal Agents

Trichinellosis (trichinosis) is caused by nematodes (roundworms) of the genus Trichinella. In addition to the classical agent T. spiralis (found worldwide in many carnivorous and omnivorous animals), several other species of Trichinella are now recognized, including T. pseudospiralis (mammals and birds worldwide), T. nativa (Arctic bears), T. nelsoni (African predators and scavengers), T. britovi (carnivores of Europe and western Asia), and T. papuae (wild and domestic pigs, Papua New Guinea and Thailand). Trichinella zimbabwensis is found in crocodiles in Africa but to date there are no known associations of this species with human disease.

Life Cycle

Depending on the classification used, there are several species of Trichinella: T. spiralis, T. pseudospiralis, T. nativa, T. murelli, T. nelsoni, T. britovi, T. papuae, and T. zimbabwensis, all but the last of which have been implicated in human disease. Adult worms and encysted larvae develop within a single vertebrate host, and an infected animal serves as a definitive host and potential intermediate host. A second host is required to perpetuate the life cycle of Trichinella.

The domestic cycle most often involved pigs and anthropophilic rodents, but other domestic animals such as horses can be involved. In the sylvatic cycle, the range of infected animals is great, but animals most often associated as sources of human infection are bear, moose and wild boar.

Trichinellosis is caused by the ingestion of undercooked meat containing encysted larvae (except for T. pseudospiralis and T. papuae, which do not encyst) of Trichinella species.

After exposure to gastric acid and pepsin, the larvae are released from the cysts and invade the small bowel mucosa where they develop into adult worms. Females are 2.2 mm in length; males 1.2 mm. The life span in the small bowel is about four weeks. After 1 week, the females release larvae that migrate to striated muscles where they encyst.

Diagnosis

It is usually made based on clinical symptoms, and is confirmed by serology or identification of encysted or non-encysted larvae in biopsy or autopsy specimens.

SYMPTOMS

Symptoms of trichinosis range from very mild to severe. Early symptoms, which start a few days after the worms enter your body, may include:

- Nausea and vomiting.
- Diarrhea.
- Pain in your abdomen.

Later symptoms may begin about two weeks after you eat the infected meat and may last as long as several weeks. They include:

- Muscle soreness.
- Fever.
- Headaches.
- Eye swelling.
- Chills.
- Extreme tiredness.
- Diarrhea or constipation.
- Cough.
- Rash or itchy skin.

In severe cases, trichinosis can cause:

- Difficulty with coordination and movement.
- Inflammation of your heart muscles.
- Difficulty breathing.

- Inflammation in your brain (encephalitis).

These symptoms can last from five to 45 days, but they usually begin to appear 10 to 14 days after consuming the infected meat. Abdominal symptoms can occur much sooner at one to two days after infection. Milder cases of trichinosis are often mistaken for the flu or other common illnesses. In extreme cases, trichinosis may result in death.

TREATMENT

Your healthcare provider might prescribe:

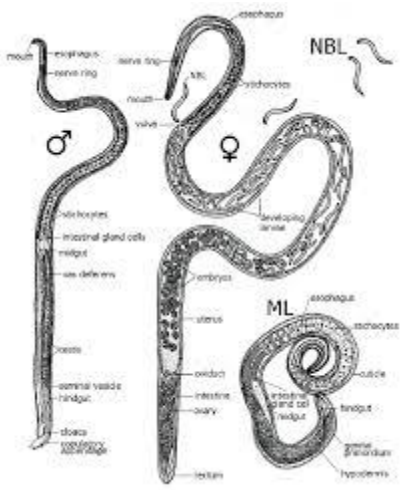
- Drugs to rid your body of parasites, including mebendazole and albendazole. If you take these within the first three days of being infected, they stop the infection from getting to the muscles and getting worse.
- Drugs to help with pain, such as nonsteroidal anti-inflammatory drugs (NSAIDS).
- Drugs to help with inflammation, such as steroids.

PREVENTION

Cooking

Be sure you cook any meat you eat, especially pork and wild game, thoroughly. Use a food thermometer to cook it to recommended temperatures, after washing your thermometer with soap and water.

- For pork, the recommended temperature is 160 degrees Fahrenheit.
- For game meat (including deer, elk, moose, bear, bison, rabbit and beaver), the recommended temperature is 165 degrees Fahrenheit.
- For game birds (including turkey, duck, goose, partridge or pheasant), the recommended temperature is 180 degrees Fahrenheit for whole birds.
- For game birds, it's recommended to cook parts (like breasts or thighs) at 165 degrees Fahrenheit. This includes birds cooked with stuffing inside the bird or dressed in the pan with the bird.



Freezing

Freeze pork, or any meat, that is less than 6 inches thick for 20 days at -15 degrees Centigrade, for 10 days at -23 Centigrade or six days at -30 degrees Centigrade. Freezing may not kill the worms in wild game meat because those particular parasites may be resistant to freezing.

Other processing

U.S. cases of trichinosis in previous years were caused by eating undercooked pork, but a successful education campaign reduced those numbers. However, many people still don't cook game meats correctly. Also, most people don't know that salting meat doesn't kill the cysts of trichinosis and neither does smoking meats.

CULTIVATION OF PARASITES

INTRODUCTION

Pathogenic microbes can be cultured to aid in identification and this is normally used as the gold standard for the majority of bacterial infections. For many other infections such as viral, rickettsial and amoebic, they represent a good reference method. In most of the parasitic infections, culture is not a routine identification technique. However, culture is useful for clinching the diagnosis in some protozoan parasitic infections, e.g. in case of Central Nervous System infections by free living amoebae; and also culture has immense role in research related to pathogenic parasites. Parasite cultivation is a tricky task, which requires expertise and knowledge of all kinds of microbiological cultures. The earliest attempt in this field was the Novy, Mc Neal and Nicole (NNN) medium for Trypanosoma and Leishmania, made by Novy and McNeal in 1904[1] and modified by Nicolle in 1908.[2] Success in establishing in vitro and in vivo cultures of parasites has allowed their morphology, physiology, behavior and metabolism to be studied dynamically; this understanding has helped tremendously in diagnosis, management, control and prevention of human parasitic diseases.

USES OF CULTIVATION OF PARASITES

Cultivation of parasites is invaluable for a number of reasons, which may be broadly divided as follows:

Patient care

Cultivation is an important adjunct to diagnosis of many clinically important parasites, for example, Entamoeba histolytica, Trichomonas vaginalis, Leishmania spp., free-living amoebae (FLA) and Trypanosoma spp. Live parasitic cultures are used for detection of drug-resistance and for screening of potential therapeutic agents.

Research

Parasite cultivation may be used to study the biochemistry, physiology, and metabolism of the parasites, determine their nutritional requirements, understand their ultra-structural

organization, elucidate their patho-physiology, life-cycle and host-parasite relationship, as well as assess functional antibodies and cell-mediated protective systems against the parasites. It is also of use for producing antigens used to prepare monoclonal and polyclonal antibodies against the organisms for use in immunological tests, for providing the inoculums used for experimental animals and for obtaining serological reagents. Identifying specific proteins that enhance the invasiveness of parasites may help in developing monoclonal antibodies to neutralize parasitic invasion. In vitro cultivation also provides a system to assess vaccine efficacy, since that can be done only by using intact parasites, obtained in large quantities and without the contaminating influences of host components.

Epidemiology

Cultivating parasites may help in differentiating clinical isolates. The techniques such as iso-enzyme electrophoresis, monoclonal antibody techniques, and/or DNA probe techniques can be easily applied on cultures; to explicate isolate and strain differences which are useful for epidemiological studies.

Teaching

For demonstrating the characteristics of pathogenic parasites to medical students to facilitate understanding the morphology and physiology; while keeping a stock of cultured parasites is of enormous worth.

DIFFICULTIES IN CULTIVATION OF PARASITES

Parasite cultivation techniques are complex procedures involving a number of issues, some of which are known while some are still undefined Most of the parasites have complex life-cycles with different morphological stages and may have both cold-blooded and warm-blooded animals as hosts within the life-cycle. These stages involve number of variables including parasitic form, host site, host temperature, host immune responses, parasite species and/or strain, and parasite-protective mechanisms. To simulate the host environment, especially in an in vitro culture system can be extremely demanding, assuming one can actually determine all the relevant variables.

Parasites are often fastidious and require medium components that may be toxic. Filter sterilization may be required in some cases. Human or animal sera, which are expensive and highly variable are usually required for successful culturing of parasites. In some cases, growth factors have been identified and substituted for serum. Much research has been devoted to the development of defined media, although, even with the elimination of serum, other components may not have been totally defined.

TERMINOLOGY

Three types of culture media may be used for cultivating parasites:

1. Xenic culture - It refers to culture of parasites grown in association with unknown microbiota, for example stool specimens cultured for E. histolytica in National Institute of Health medium. It is used for primary growth of parasites.
2. Monoxenic culture - If the parasites are grown with a single known bacterium, the culture is referred to as monoxenic, for example corneal biopsy specimens cultured with Escherichia coli as a means of recovering species of Acanthamoeba. It can be used for primary growth as well as a transitional phase in isolation.
3. Axenic culture - It is a pure culture without any bacterial associate or any other metabolizing cells. It is mainly used as isolation medium for the parasites, but can be used for primary growth also, for example TYI-S-33 medium in case of T. vaginalis.

GENERAL PRINCIPLES

Although the province of parasitic cultivation is very diverse, there are certain principles which are applicable at large to the subject:

1. Parasitic helminths are more difficult to cultivate than protozoa. The complexity of helminth body configuration and metabolism, and inability to meet essential environmental conditions account for failure to complete their life-cycles under artificial conditions.
2. Cell cultures are used for the obligate intracellular parasites, for example Plasmodium spp. and coccidia.
3. Various kinds of nutrients such as blood, serum, haem, egg, peptone, minerals and carbohydrates are used in the culture media.
4. Temperature required for optimum growth is usually 37°C though lower temperatures may be required in few cases, e.g. 25°C for Leishmania promastigotes.
5. Incubation condition is aerobic with some exceptions like microaerophilic conditions for amoebae and Giardia and 5% CO2 for Plasmodium spp.
6. Identification tools include parasite's characteristic morphology, direct fluorescent antibody assay, polymerase chain reaction, enzyme immunoassay, etc.
7. Positive controls need to be run in parallel to keep a check on the medium and the method used.

IN VITRO CULTIVATION OF DIFFERENT PARASITES

Luminal parasitic protists

Luminal protists are first grown in xenic cultures, gradually weaned, then isolated in axenic cultures. While T. vaginalis and Giardia intestinalis can be established directly into axenic cultures, E. histolytica and Blastocystis hominis have never been grown axenically without first

being established in xenic cultures. Dientamoeba fragilis and Balantidium coli have never been grown successfully in axenic culture to the best our knowledge. Some of the important media used for cultivating luminal parasitic protists are described.

Culture is a very sensitive (95%) procedure for the diagnosis of trichomoniasis. It is recommended when microscopy is negative. T. vaginalis grows best at 35°C-37°C under anaerobic conditions. Trussell and Johnson's medium and simplified trypticase serum medium with pH 5.5-6.0 are suitable for isolation of T. vaginalis. A commercial and proprietary product with a reasonable shelf life called InPouch TV (Biomed Diagnostics Inc. White City-OR, USA) gives comparable results and has the advantage of being self-contained.

Maintenance of cultures

Established cultures of all parasites are handled largely in the same way. Xenic cultures of E. histolytica, D. Fragilis, B. hominis and B. coli are routinely passaged at 48–72 h intervals. More inoculum should be used when incubating for longer duration than that for shorter incubation periods. Xenic cultures should be passaged using two or more inoculum sizes to ensure a successful subculture. Established axenic cultures of T. vaginalis, E. histolytica, G. intestinalis, and B. hominis are passaged at 72 and 96 h intervals. For doing subculture, cultures are chilled in an ice-water bath for 5 min (xenic and axenic cultures of E. histolytica) or 10 min (G. intestinalis) to release trophozoites attached to the glass culture tube. In T. vaginalis, B. coli, and B. hominis cultures, most organisms will be nonadherent and the tubes need not be chilled unless an accurate count is desired. Tubes are inverted several times to disperse the cells and a measured inoculum is passed aseptically to a culture tube containing fresh medium. The tubes are capped tightly and incubated at 37°C, either vertically (xenic cultures of T. vaginalis, and axenic cultures of B. hominis) or at 5° to the horizontal (established axenic cultures of E. histolytica and G. intestinalis). For axenic B. hominis cultures the medium must be pre-reduced for 48 h before inoculation in an anaerobic jar.