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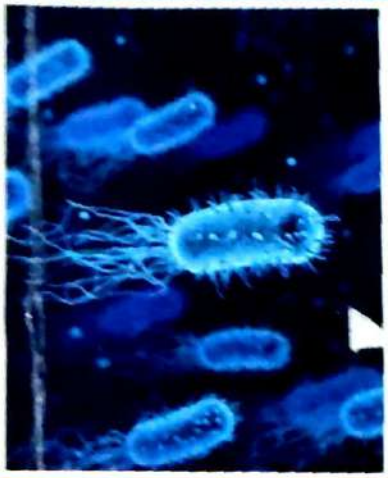
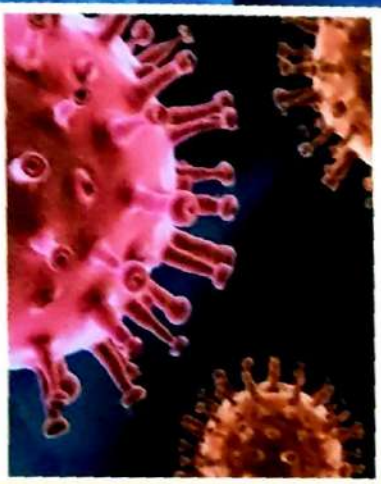
Anwar Microbiology

Review Book of Microbiology

By
Muhammad SanaUllah

Zubair Book Depot

ANWAR

MICROBIOLOGY

(Review Book of Microbiology)

For MBBS, BDS, DPT, MLT, & Pharma D, STUDENTS

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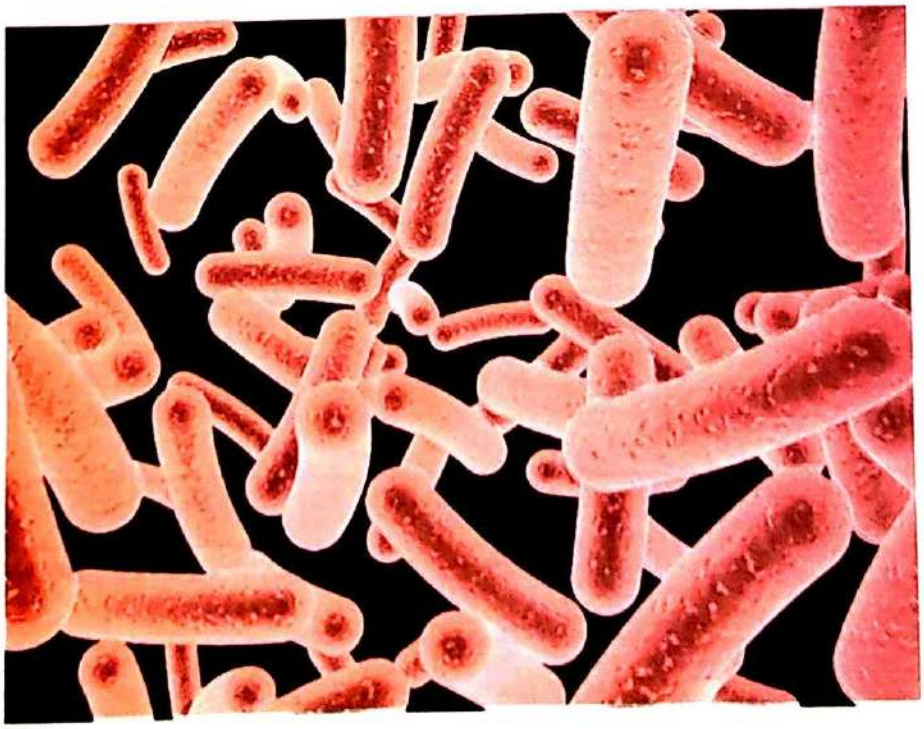


1 Bacteria Compared with other Microorganisms

IMPORTANT FEATURES OF MICROBES

Structure	Cells have a nucleus or nucleoid which contains DNA
Method of replication	Replicate either by binary fission or by mitosis
Nature of the nucleic acid	Cells contain both DNA and RNA

Characteristics of Prokaryotic and Eukaryotic Cells

Characteristic	*Prokaryotic Cells*	*Eukaryotic Cells*
DNA within a nuclear membrane	No	Yes
Mitotic division	No	Yes
DNA associated with histones	No	Yes
Chromosome number	One	More than one
Membrane-bound organelles	No	Yes
Size of ribosome	70S	80S
Cell membrane	Sterol absent (except *Mycoplasma*)	Contain sterol

Characteristics of Bacteria & Viruses

Property	*Viruses*	*Bacteria*
Type of nucleic acid	DNA or RNA but not both	DNA and RNA
Cell wall	No	Yes
Ribosomes	Absent	70S
Motility	None	Some
Multiplication by binary fission or mitosis	No	Yes

UNIVERSITY QUESTIONS

1. Give differences between viruses and bacteria. **[Annual 2007]**

2 Structure of Bacterial Cells

CELL WALL

The cell wall is the outermost component common to all bacteria (except *Mycoplasma*).

	Gram +ve	Gram -ve
Peptidoglycan	Thick & Multilayered	Thin & Single layered
Outer membrane	Present	Absent
Periplasmic space	Absent	Present
Lipoprotein content	Low	High
Teichoic acid	Present	Absent
Lipopolysaccharides	Absent	Present

Periplasmic space: space between outer membrane layer & cytoplasmic membrane. Beta lactamase present here

Teichoic acid: Fibers located in the outer layer of gram-positive cell wall & protrude outside peptidoglycan. Induce septic shock

Medically Important Bacteria That Cannot Be Seen in the Gram Stain

Organism	Reason	Alternative Microscopic Approach
Mycobacteria	Too much lipid in cell wall	Acid-fast stain
Treponema pallidum	Too thin to see	Dark-field microscopy or fluorescent antibody
Mycoplasma	No cell wall	None
Legionella pneumophila	Poor uptake of red counterstain	Prolong time of counterstain
Chlamydiae	Intracellular; very small	Inclusion bodies in cytoplasm
Rickettsiae	Intracellular; very small	Giemsa or other tissue stains

CYTOPLASMIC MEMBRANE

Lies inside the peptidoglycan layer of the cell wall composed of a phospholipid bilayer. Do not contain sterols; only *Mycoplasma* have sterols in their membrane.

Important functions:

1. Active transport of molecules into the cell.
2. Energy generation by oxidative phosphorylation.
3. Synthesis of precursors of the cell wall.
4. Secretion of enzymes and toxins.

CYTOPLASM

Ribosomes	Site of protein synthesis; 70S in size, with 50S and 30S subunits.
Granules	Serve as storage areas for nutrients. It appears as a "**metachromatic**" granule, which is a characteristic feature of *Corynebacterium diphtheriae*.
Nucleoid	Area of the cytoplasm in which DNA is located.
Plasmids	Extrachromosomal, double-stranded, circular DNA molecules that are capable of replicating independently of the bacterial chromosome.
Transposons	Transposons are pieces of DNA that move readily from one site to another either within or between the DNAs of bacteria, plasmids, and bacteriophages. Because of their unusual ability to move, they are nicknamed "jumping genes."

Plasmids

Types:

Transmissible plasmids	Can be transferred from cell to cell by conjugation. Responsible for synthesis of the sex pilus and for the enzymes required for transfer.
Non-transmissible plasmids	Do not contain the transfer genes

Functions:

1. Antibiotic resistance
2. Exotoxins
3. Pili (fimbriae)
4. Resistance to heavy metals
5. Resistance to ultraviolet light

STRUCTURES OUTSIDE THE CELL WALL

Capsule	Gelatinous layer covering the entire bacterium. It is composed of polysaccharide, except in the anthrax bacillus. **Medical importance:** (1) Limits the ability of phagocytes to engulf the bacteria. (2) Specific identification of an organism. (3) Used as the antigens in certain vaccines. (4) Adherence of bacteria to human tissues.
Flagella	Flagella are long, whip-like appendages that move the bacteria toward nutrients and other attractants, a process called **chemotaxis.** **Medical importance:** 1. Causes of urinary tract infections.

	2. Identified in the clinical laboratory by the use of specific antibodies against flagellar proteins.
Pili (fimbriae)	Hair-like filaments that extend from the cell surface. They are found mainly on gram-negative organisms. **Medical importance:** 1. They mediate the attachment of bacteria to specific receptors on the human cell surface. 2. A specialized kind of pilus, the sex pilus, forms the attachment between the male (donor) & the female (recipient) bacteria during conjugation.
Glycocalyx	Polysaccharide coating secreted by many bacteria. It covers surfaces like a film and allows the bacteria to **adhere firmly** to various structures (e.g., skin, heart valves, prosthetic joints, and catheters). **Medical importance:** 1. Glycocalyx-producing strains of *P. aeruginosa* that cause respiratory tract infections in cystic fibrosis patients. 2. Glycocalyx-producing strains of *Staphylococcus epidermidis* and viridans streptococci that cause endocarditis.

BACTERIAL SPORES

Spores are medically important because they are **highly heat resistant** and are not killed by many disinfectants.

Important features:

1. Highly resistant to heating.
2. Highly resistant to many chemicals.
3. They can survive for many years, especially in the soil.
4. They exhibit no measurable metabolic activity.
5. Spores form when nutrients are insufficient but then germinate to form bacteria when nutrients become available.
6. Spores are produced by members of only two genera of bacteria of medical importance, *Bacillus* and *Clostridium*, both of which are gram-positive rods.

UNIVERSITY QUESTIONS

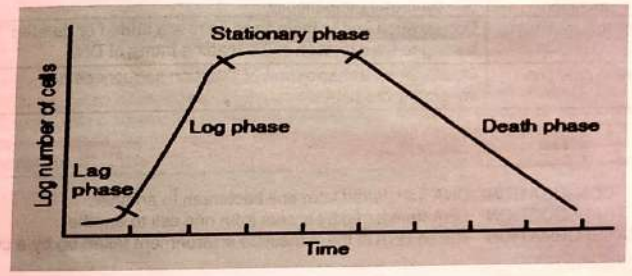
1.a) What are **plasmids**? Give its different **types**.
b) Enumerate the **function** and **structures** of medical importance whose genes are carried by plasmids. **[Annual 2009]**
2.a) Compare the **cell walls** of gram positive and gram-negative bacteria.
b) Enumerate any 3 bacteria of medical importance that can't be seen in Gram Stain. Give reason and alternate microscopic approach. **[Supple 2009]**

3 GROWTH

GROWTH CYCLE

The growth cycle of bacteria has four phases;

LAG phase	Metabolic activity occurs but cells do not divide.
LOG phase	Cell division occurs.
Stationary phase	Number of new cells produced equals to number of cells that die.
Death phase	Decline in number of bacteria.



AEROBIC & ANAEROBIC GROWTH

Obligate aerobes	Require oxygen to grow	M. *tuberculosis*
Facultative anaerobes	Use oxygen, in its presence; can use fermentation pathway in the absence of O_2	E. *coli*
Obligate anaerobes	Cannot grow in presence of oxygen	C. *tetani*

UNIVERSITY QUESTIONS

1. What are the different **phases** of bacterial growth curve? Give the different **cellular events** that take place in each phase. **(3) [Annual 2015]**
2. Explain diagrammatically the four **phases** of bacterial growth curve. **(2.5) [Supple 2016 held in 2017]**

4 GENETICS

MUTATIONS

Change in the base sequence of DNA that usually results in insertion of a different amino acid into a protein and the appearance of an altered phenotype.

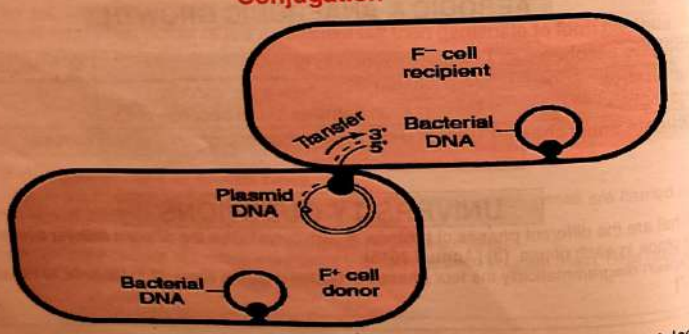
Types:

Base substitution a. Missense mutation b. Nonsense mutation	Occurs when one base pair is inserted in place of another. a. Results in a codon that causes a different amino acid to be inserted. b. Generates a termination codon that stops protein synthesis permanently.
Frameshift mutation	Occurs when one or more base pairs are added or deleted leading to the alteration in the reading frame of DNA.
Insertion mutation	Occurs when **transposons** or insertion sequences are integrated into DNA

Transfer of DNA between Bacterial Cells

CONJUGATION	DNA transferred from one bacterium to another.
TRANSDUCTION	DNA transferred by a virus from one cell to another.
TRANSFORMATION	Naked DNA in the immediate environment taken up by a cell.

Conjugation



(Reference: Levinson Microbiology)

Programmed rearrangements

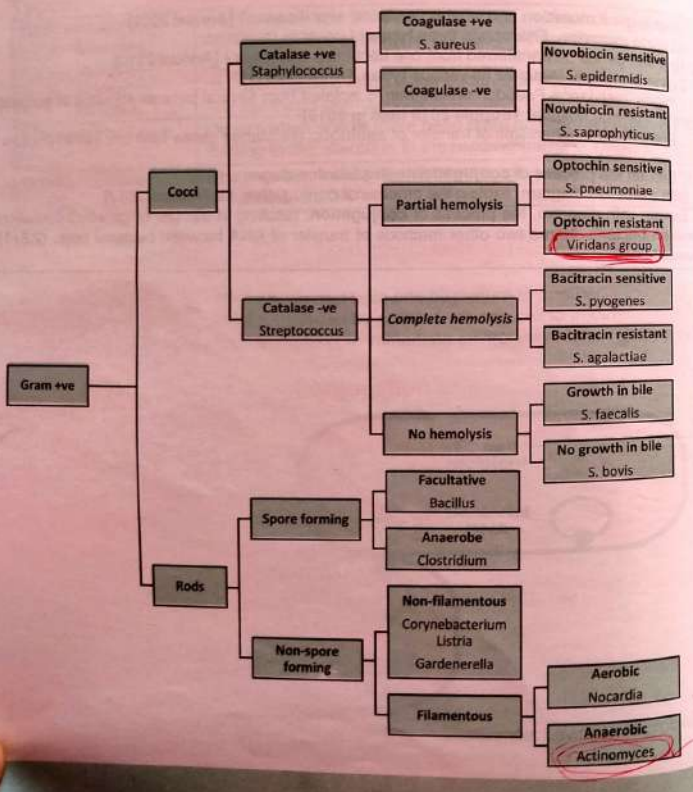
Movement of genes from inactive (storage) sites into active sites, where they are expressed as new proteins.

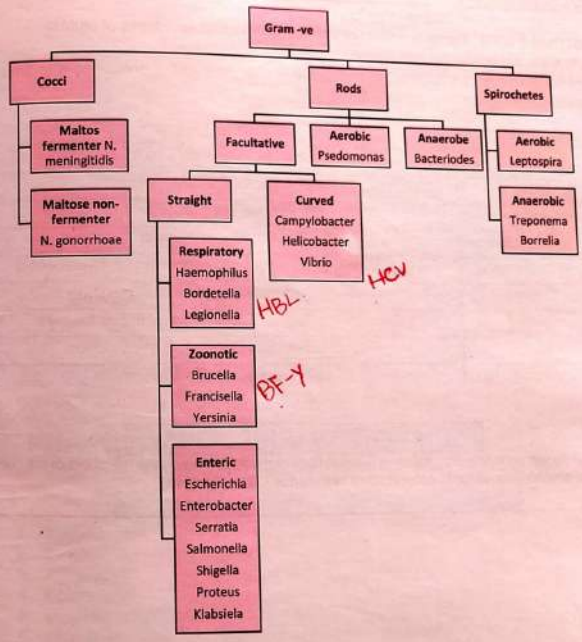
Importance: Medically, this is important because bacteria can acquire new proteins (antigens) on their surface and evade the immune system

UNIVERSITY QUESTIONS

1. What is gene **mutation** and what is its clinical **significance**? **[Annual 2009]**
2. a) Define **mutation**. Enumerate three **types** of possible mutations.
   b) How can genes be transferred from one bacterium to another? **[Annual 2010]**
3. Define **mutation**. Describe its various **types**. **[Annual 2011]**
4. A highly resistance Pseudomonas strain is isolated from several patients admitted in surgical ward of a private hospital. **[Supple 2015 held in 2016]**
   a) Enlist three mechanisms of transfer of **antibiotic resistance** genes from one cell to another. **(1.5)**
   b) Explain the process of **conjugation** with a labelled diagram. **(3.5)**
5. With a labelled diagram, explain the process of **conjugation**. **(3) [Annual 2017]**
6. Explain with diagram, the process of **conjugation,** resulting in transfer of genetic information between bacteria. Name two other methods of transfer of DNA between bacterial cells. **(2.5+1) [Annual 2019]**

5 Classification of Medically Important Bacteria





6 The Human Microbiome

Normal Flora: Bacteria and fungi that are **permanent residents** of certain body sites, especially the skin, oropharynx, colon, and vagina.

Colonization resistance: The ability of members of the normal flora to limit the growth of pathogens.

Location	Organisms
Skin	S. *epidermidis*, Candida *albicans*
Nose	S. *aureus*, S. *epidermidis*
Mouth	Viridans streptococci
Dental plaque	Streptococcus *mutans*
Gingival crevices	Bacteroides, Fusobacterium, Actinomyces, streptococci
Throat	Viridans streptococci
Colon	Bacteroides *fragilis*, E. *coli*, Enterococcus *faecalis*
Vagina	Lactobacillus, E. *coli*, group B streptococci
Urethra	E. *coli*, streptococci, S. *epidermidis*

UNIVERSITY QUESTIONS

1. What do you understand by the term **normal flora** of the body? Name the members of the norma flora of **oropharynx**. What is **colonization resistance**? **[Supple 2013]**

7 PATHOGENESIS

PRINCIPLES OF PATHOGENESIS

Pathogenicity	Ability of organism to cause disease (i.e., to harm the Host)
Pathogen	Microbes capable of causing disease.
Opportunistic pathogens	Microbes that are capable of causing disease only in immunocompromised people.
Virulence	Measure of a microbe's ability to cause disease (i.e., a highly virulent microbe requires fewer organisms to cause disease than a less virulent one).
ID_{50}	Number of organisms required to cause disease in 50% of the population. A low ID_{50} indicates a highly virulent organism.

TYPES OF BACTERIAL INFECTIONS

Bacteria cause disease by two major mechanisms:

1. Toxin production
2. Invasion and inflammation

Epidemiologic terms

Endemic	Infections occur constantly at a low level in a specific population.
Epidemics	Infections occur at a much higher rate than usual.
Pandemics	Infections spread rapidly over large areas of the globe.

STAGES OF BACTERIAL PATHOGENESIS

1. Transmission
2. Evasion
3. Adherence
4. Colonization
5. Disease symptoms
6. Host responses
7. Progression or resolution of the disease

DETERMINANTS OF BACTERIAL PATHOGENESIS

1. Transmission
2. Adherence to cell surfaces
3. Invasion, Inflammation & Intracellular survival
4. Toxin Production
5. Immunopathogenesis

1. Transmission

Vertical transmission	Transmission from mother to offspring
Horizontal transmission	Person to person transmission that is not from mother to offspring.

Vertical Transmission of Some Important Pathogens

Transplacental	Treponema *pallidum* Listeria *monocytogenes*	Congenital syphilis Neonatal sepsis
Within birth canal	E. *coli* N. *gonorrhea* S. *agalactiae*	Neonatal sepsis Neonatal conjunctivitis Neonatal sepsis
Breast milk	S. *aureus*	Oral or skin infections

Portals of entry

Respiratory Tract	S. *pneumoniae* H. *influenzae* M. *tuberculosis*	Pneumonia Meningitis Tuberculosis
GIT	Salmonella Shigella Vibrio	Typhoid fever Dysentery Cholera
Genital tract	N. *gonorrhea* Treponema *pallidum* Chlamydia *trachomatis*	Gonorrhea Syphilis Urethritis
Skin	Clostridium *tetani*	Tetanus

2. Adherence to cell surface

Many bacteria have specialized structures that allow them to adhere to the surface of human cells, thereby enhancing their ability to cause disease.

Pili	N. *gonorrhea*; E. *coli*
Capsule	S. *aureus*
Glycocalyx	Staphylococcus *epidermidis*

3. Invasion, inflammation and intracellular survival

Enzymes secreted by invasive bacteria

Collagenase	S. pyogenes
Hyaluronidase	S. pyogenes
Coagulase	S. aureus
Immunoglobulin proteases	S. pneumoniae

Types of Inflammation

Pyogenic	Neutrophils are the predominant cells
Granulomatous	Macrophages & T-lymphocytes predominate

Virulence factors

- Polysaccharide capsule
- M protein
- Protein A
- Protein Pilli

4.Toxin Production

There are two types of toxins.

Property	Exotoxin	Endotoxin
Source	Gram-Positive & Gram-Negative bacteria	Cell wall Gram-Negative bacteria
Secreted From cell	Yes	No
Chemistry	Chemistry Polypeptide Lipopolysaccharide	Lipopolysaccharide
Location of genes	Plasmid or Bacteriophage	Bacteriophage chromosome
Toxicity	High	Low
Clinical effects	Many	Fever, shock
Mode of action	Many	TNF & IL-1
Vaccines	Toxoid	No
Heat stability	Destroyed rapidly	Stable at 100°C
Typical diseases	Tetanus	Meningococcemia

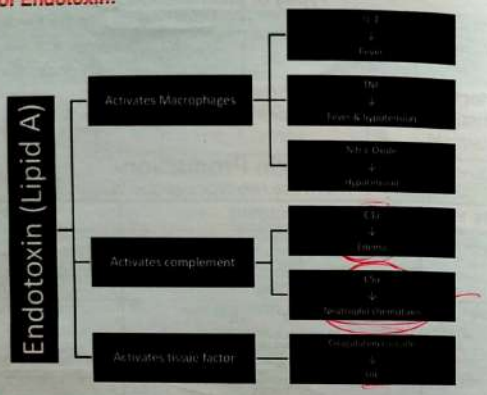
Important exotoxins

Diphtheria toxin	Corynebacterium *diphtheriae*	Inhibit protein synthesis by ADP- ribosylation of elongation factor 2.
Tetanus toxin	Clostridium *tetani*	Prevents release of inhibitory neurotransmitter glycine.
Botulinum toxin	Clostridium *botulinum*	blocks release of acetylcholine at the synapse producing flaccid paralysis.
Heat labile toxin (enterotoxin)	E. *coli*	Adenylate cyclase → ↑cAMP → cAMP phosphorylates ion transporter in the membrane → Export ions → Outpouring of fluid → Diarrhea

Effects of endotoxin:

- Fever
- Hypotension
- Inflammation
- Disseminated intravascular coagulation

Mode of action of Endotoxin:



STAGES OF AN INFECTIOUS DISEASE

1. Incubation period	Period between the acquisition of organism and the beginning of symptoms.
2. Prodrome period	Period during which non-specific symptoms such as fever, malaise and loss of appetite occur.
3. Specific-disease period	Period during which characteristic signs and symptoms of a disease occur.
4. Recovery period	Period during which the illness abates & the patient returns to the healthy state.

KOCH'S POSTULATES

1. The organism must be isolated from every patient with the disease.
2. The organism must be isolated free from all other organisms and grown in pure culture in vitro.
3. The pure organism must cause the disease in a healthy, susceptible animal.
4. The organism must be recovered from the inoculated animal.

Exceptions:

1. Pathogens isolated from patients who are not manifesting symptoms (i.e., asymptomatic carriers) such as Salmonella *typhi*.
2. Pathogens that cannot be cultivated on existing media, such as Treponema *pallidum* or prions.
3. Exposure to pathogens, e.g., M. *tuberculosis*, that do not always result in disease in all hosts.

UNIVERSITY QUESTIONS

1. a) Define pathogenicity and virulence. **[Supple 2010]**
   b) Name four components an organism must have to be capable to cause an infectious disease.
2. Enumerate four major differences between exotoxins and endotoxins. **(2) [Annual 2016]**
3. Enlist six major differences between exotoxins and endotoxins. **(3) [Supple 2018 held in 2019]**
4. Enlist the generalized sequence of steps involved in bacterial pathogenesis. **(1.5) [Annual 2019]**
5. Enlist the generalized sequence of different stages of bacterial pathogenesis. **(2) [Annual 2020]**

8 LAB DIAGNOSIS

Methods of Laboratory Diagnosis

(1) Microscopic Examination
(2) Culture-Based Methods
- Blood Cultures
- Throat Cultures
- Sputum Cultures
- CSF Cultures
- Stool Cultures
- Urine Cultures
- Genital Tract Cultures
- Abscess Cultures

(3) Serological Methods
- Slide Agglutination Test
- Tube Agglutination Test
- Fluorescent Antibody Test
- Latex Agglutination Test
- Cold Agglutinin Test
- Enzyme-Linked Immunosorbent Assay

(4) Molecular Diagnostic Methods
- Genomic Tests
- Proteomic Tests

Commonly Used Bacteriologic Agars and Their Function

NAME OF AGAR	BACTERIA ISOLATED ON THE AGAR	PROPERTIES OF THE AGAR
BLOOD	Various bacteria	Detect hemolysis
BORDET-GENGOU	Bordetella *pertussis*	Increased concentration of blood allows growth
CHARCOAL-YEAST EXTRACT	Legionella *pneumophila*	Increased concentration of iron and cysteine allows growth
CHOCOLATE	Neisseria *meningitidis* and Neisseria *gonorrhoeae*	Heating the blood inactivates inhibitors of growth
CHOCOLATE AGAR PLUS X AND V FACTORS	Haemophilus *Influenzae*	X and V factors are required for growth
EGG YOLK	Clostridium *perfringens*	Lecithinase produced by the organism degrades egg yolk to produce insoluble precipitate
EOSIN-METHYLENE BLUE	Various enteric gram-negative rods	Differentiates between lactose fermenters and non-fermenters
LÖWENSTEIN-JENSEN	Mycobacterium *tuberculosis*	Contains lipids required for growth
MACCONKEY	Various enteric gram-negative rods	Differentiates between lactose fermenters and non-fermenters
TELLURITE	Corynebacterium *diphtheriae*	Causes tellurite to become tellurium, which has black color
THAYER-MARTIN	N. *gonorrhoeae*	Chocolate agar with antibiotics to inhibit growth of normal flora
TRIPLE SUGAR IRON (TSI)	Various enteric gram-negative rods	Distinguishes H_2S producers from nonproducers

9 ANTIBACTERIAL DRUGS: Mechanism of Action

Bacteriostatic Drug that inhibits bacterial growth but does not kill them.
Bactericidal Drug that kills bacteria.

Mechanism of Action of Important Antibacterial Drugs

Mechanism of Action	Drugs
Inhibition of cell wall synthesis a) Inhibition of cross-linking of peptidoglycan b) Inhibition of other steps in peptidoglycan synthesis	Penicillin; cephalosporins Cycloserine; bacitracin
Inhibition of protein synthesis a) Action on 50S ribosomal subunit b) Action on 30S ribosomal subunit	Chloramphenicol; erythromycin Tetracyclines; aminoglycosides
Inhibition of nucleic acid synthesis a) Inhibition of nucleotide synthesis b) Inhibition of DNA synthesis c) Inhibition of mRNA synthesis	Sulfonamides; trimethoprim Quinolones Rifampin
Alteration of cell membrane function a) Disrupts membrane	Polymyxin; daptomycin
Other mechanisms of action a) Inhibits mycolic acid synthesis b) Acts as electron sink & damages DNA c) Inhibits arabinogalactan synthesis d) Inhibit fatty acid synthesis	Isoniazid Metronidazole Ethambutol Pyrazinamide

UNIVERSITY QUESTIONS

1. Define the terms **bacteriostatic** and **bactericidal. (2) [Annual 2015]**
2. **a)** Name four **mechanisms of action** of antimicrobial drugs.
   b) Briefly discuss the mechanism acting on bacterial cell wall. **[Supple 2015]**

10 ANTIBACTERIAL DRUGS: Resistance

Mechanisms of Drug Resistance

Mechanism	Important Example	Drugs
Inactivate drug	Cleavage by β-lactamase	Penicillin; cephalosporins
Modify drug target in bacteria	1. Mutation in penicillin-binding proteins 2. Mutation in protein in 30S ribosomal subunit 3. Replace alanine with lactate in peptidoglycan 4. Mutation in DNA gyrase 5. Mutation in RNA polymerase 6. Mutation in catalase-peroxidase	Penicillin Aminoglycosides Vancomycin Quinolones Rifampin Isoniazid
Reduce permeability of drug	Mutation in porin proteins	Aminoglycosides
Export of drug from bacteria	Multidrug-resistance pump	Tetracyclines

Genetic Basis of Resistance

1. Chromosome-Mediated Resistance
2. Plasmid-Mediated Resistance
3. Transposon-Mediated Resistance

Non-Genetic Basis of Resistance

1. Bacteria can be walled off within an abscess cavity that the drug **cannot penetrate** effectively.
2. Bacteria can be in a **resting state** (i.e., not growing).
3. Under certain circumstances, organisms would be survived as **protoplasts.**
4. The presence of **foreign bodies** makes successful antibiotic treatment more difficult.
5. Several **artifacts** can make it appear that the organisms are resistant.

UNIVERSITY QUESTIONS

1. MRSA (Methicillin-Resistant Staphylococcus Aureus) is isolated from the wound culture of a patient admitted in surgical ward. Enlist the four major **mechanisms** that mediate bacterial resistance to drug with one example of each. **(2) [Supple 2018 held in 2019]**
2. Give the four **reasons** for the failure of drugs to inhibit the growth of bacteria. **(2)**

[Annual 2017]

11 STERILIZATION & DISINFECTION

Sterilization: killing or removal of microorganisms, including bacterial spores, which are highly resistant.

Disinfection: killing of many but not all microorganism.

Antiseptics → chemicals used to kill microorganisms on the surface of skin & mucous membrane.

Methods of Sterilization

Chemical Agents: act primarily by one of the three mechanisms:

- Disruption of cell membranes
- Modification of Proteins
- Modification of DNA

Disruption of cell membranes

Alcohol	• 70 % ethanol → antiseptic to clean the skin prior to venipuncture. • 70 % ethanol → disinfect the stethoscope.
Detergents	• Surface active agents • Benzalkonium chloride → disinfectant for floors and other surfaces.
Phenols	• Phenol → disinfectant in the operating room. • Chlorhexidine → used as a hand disinfectant prior to surgery and in the cleansing of wounds.

Modification of Proteins

Chlorine	Purify the water supply and to treat **swimming pools**.
Iodine	Tincture of Iodine → prepare the skin prior to blood culture. Iodophors → prepare the skin prior to surgery.
Heavy Metals	Thimerosal (mercury) → skin antiseptics. Silver nitrate drops → prevent gonococcal neonatal conjunctivitis. Silver sulfadiazine → prevent infection of burn wounds.
Hydrogen peroxide	Disinfect contact lenses.
Formaldehyde & Glutaraldehyde	Sterilize respiratory therapy equipment, endoscopes, and hemodialysis equipment.
Ethylene oxide	Sterilization of heat sensitive materials such as surgical instruments & plastics.
Acids & Alkalis	Weak acids, such are frequently used as food preservatives because they are bacteriostatic.

Modification of DNA

Crystal violet	• Used as a skin antiseptic. • Its action is based on binding of the positively charged dye molecule to the negatively charged phosphate groups of the nucleic acids.

Malachite green	• It is a **component of Lowenstein-Jensen's medium**, which is used to grow M. tuberculosis. • The dye inhibits the growth of unwanted organisms in the sputum during the 6-week incubation period.

Physical Agents:

Heat

Moist heat	• **Autoclaving** is the most frequently used method of sterilization. • It consists of exposure to steam at **121°C** under a pressure of **15 lb./in²** for **15-20 minutes.**
Dry heat	• Requires temperatures in the range of **180°C for 2 hours.** • This process is used primarily for glassware and is used less frequently than autoclaving.
Pasteurization	• Used primarily for milk. • Consists of heating the milk to **62°C for 30 minutes** followed by rapid cooling. • Flash" pasteurization at **72°C for 15 seconds** is often used.

Radiation

UV Light	• The. most significant lesion caused by UV irradiation is formation of **thymine dimers.**
X-ray	• X-ray have higher energy and penetrating power than UV radiation and kill mainly by the production of free radicals • These highly active radicals can break covalent bonds in DNA thereby killing the organism.

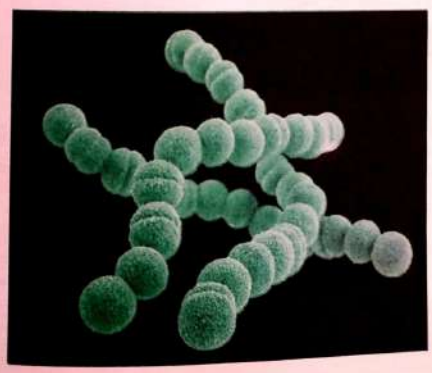
Filtration

- Solutions are filtered to make them pyrogen-free prior to autoclaving.
- The most commonly used filter is composed of **nitrocellulose**.
- This will retain all bacteria and spores.

UNIVERSITY QUESTIONS

1. Give any two mechanisms by which **chemical agents** kill microorganisms. Give two **examples** of chemical agents from each category. **[Supple 2013]**
2. A laboratory technician is asked to sterilize instruments used in Operation Theatre to prevent sepsis and Hepatitis B and C infection in patients undergoing delivery. Define **sterilization** and enlist its **physical methods** with **examples. (2.5) [Supple 2016 held in 2017]**
3. Enumerate the different **physical methods** of sterilization with one **example** each. **(1.5) [Annual 2018]**

Clinical Bacteriology



12 GRAM – POSITIVE COCCI

- Grape-like
- Catalase +ve

- In chains
- Catalase -ve

STAPHYLOCOCCUS

Species:

- S. Aureus (Nose)
- S. Epidermidis (Skin)
- S. Saprophyticus (UTI)

Disease:

S. Aureus:

- Abscesses
- Cellulitis
- Impetigo
- Folliculitis
- Septicemia
- Bacterial Conjunctivitis
- Endocarditis
- Septic Arthritis
- Osteomyelitis
- Food Poisoning
- Scalded Skin Syndrome
- Hospital acquired pneumonia
- Surgical Wound Infections
- Toxic Shock Syndrome
- Kawasaki Syndrome

S. Epidermidis:

- Endocarditis
- CNS shut infection
- Prosthetic Joint Infection

S. Saprophyticus: Urinary tract Infections

Important Properties:

S. Aureus:

- Coagulase production
- Staphyloxanthin pigment
- Golden Green Colonies
- β-hemolytic
- Ferment Mannitol

Cell Wall Components:

Protein A	Binds **Fc portion** of IgG & prevent activation of compliment
Teichoic Acid	Mediate adherence to mucosal cells
Polysaccharide capsule	Virulence factor
Surface Receptors	Permits **phage typing**

Peptidoglycan	Endotoxin-like properties

Pathogenesis: Produces several toxins & enzymes:

Enterotoxin	• Acts as a **supra-antigen** within GIT to stimulate release of IL-1 & IL-2. • Causes **Food Poisoning**.
Toxic Shock Syndrome Toxin	• Stimulate release of large amounts of IL-1, IL-2 & TNF. • Causes **Toxic Shock Syndrome.**
Exofoliatin	• Acts as a protease that cleaves desmoglein in desmosomes, leading to the separation of epidermis at granular cell layer. • Causes **Scalded Skin Syndrome.**
Leucocidins	Kill leucocytes & cause necrosis of tissues. **α– toxin**: necrosis of skin & hemolysis **P-V leucocidin**: pore forming toxin
Enzymes	Includes Coagulase, Fibrinolysin, Hyaluronidase, Proteases, Nucleases, Lipases.

Clinical Findings:

Pyogenic Diseases:
- Endocarditis
- Septicemia
- Pneumonia
- Conjunctivitis
- Metastatic abscess
- Septic Arthritis
- Skin and Tissue Infections
- Osteomyelitis

Toxin Mediated Disease:
- Food Poisoning
- Toxic Shock Syndrome
- Scalded Skin Syndrome

Kawasaki Disease: Cardiac Involvement
- Myocarditis
- Arrhythmias
- Aneurysm of Coronary arteries

Laboratory Diagnosis:

S. Aureus:
- Gram stained smear ⟹ Gram +ve cocci
- Coagulase Test ⟹ +ve
- Catalase Test ⟹ +ve
- Colonies ⟹ Golden or yellow on blood agar
- Mannitol salt agar ⟹ Ferments Mannitol
- Hemolysis ⟹ β-hemolytic

S. epidermidis	*S. saprophyticus*
Novobiocin sensitive	Novobiocin resistant

STREPTOCOCCUS

Species	Lancefield Group	Typical Hemolysis	Diagnostic features
S. pyogenes	A	β	Bacitracin-sensitive
S. agalactiae	B	β	Bacitracin-resistant; Hippurate hydrolyzed
E. faecalis	D	α or β or none	Growth in 6.5% NaCl
S. bovis	D	α or none	No growth in 6.5% NaCl
S. pneumoniae	Not Applicable	α	Bile-soluble; optochin-sensitive
Viridans group	Not Applicable	α	Not bile-soluble; optochin-resistant

Disease:

S. pyogenes	**Pyogenic**: Impetigo, Cellulitis, Pharyngitis, Sepsis **Toxigenic**: Scarlet fever, Toxic Shock **Immune-mediated** (non-suppurative): Rheumatic fever, Acute glomerulonephritis
S. agalactiae	Neonatal sepsis, Meningitis
E. faecalis	Hospital-acquired UTI, Endocarditis
S. bovis	Endocarditis (uncommon cause)
S. pneumoniae	Pneumonia, Otitis Media, Meningitis
Viridans group	Endocarditis (most common cause)

Important Properties:

Types of Hemolysis

α–hemolysis	Incomplete lysis of RBCs
β–hemolysis	Complete lysis of RBCs
γ–hemolysis	No hemolysis

Antigens of β–hemolytic streptococci

C carbohydrate	Distinguish S. pyogenes from S. agalactiae
M protein	Virulence factor of S. pyogenes

Normal Flora:

E. faecalis	Colon
S. bovis	Colon
Viridans group	Oropharynx

S. pyogenes

Pathogenesis: Causes disease by three mechanisms;

1. Pyogenic Inflammation:
   - Hyaluronidase
   - Streptokinase
   - DNase
   - IgG degrading enzyme

2. Exotoxin production:

Erythrogenic toxin	Rash of **scarlet fever**
Streptolysin O	β–hemolysis; antigenic; O_2 labile
Streptolysin S	β–hemolysis; not antigenic; O_2 stable
Exotoxin A	Streptococcal Toxic Shock Syndrome
Exotoxin B	Necrotizing fasciitis

3. Immunologic

Laboratory Diagnosis:

- Gram stained smear ⟹ Gram +ve cocci
- Catalase Test ⟹ -ve
- Blood agar ⟹ β-hemolytic
- Bacitracin sensitive
- Serological test
  - In suspected Rheumatic fever patient ⟹ Elevated ASO titer
  - In suspected Acute Glomerulonephritis patient ⟹ Anti DNase B elevated

S. *agalactiae*

Laboratory Diagnosis:

- Gram stained smear ⟹ Gram +ve cocci
- Catalase Test ⟹ -ve
- Blood agar ⟹ β-hemolytic
- Bacitracin resistant
- Hydrolyze Hippurate
- **CAMP test** ⟹ +ve

Neonates Sepsis + Meningitis

Viridans Group

Members:

- S. *mutans*
- S. *mitis*
- S. *milleri*
- S. *salivarus*

Pathogenesis:

- Bacteremia from **dental procedures** spreads organism to damaged heart valves.
- **Glycocalyx** composed of polysaccharide enhances adhesion to heart valves.

S. *pneumoniae*

Important Properties:

- Lancet Shaped diplococci
- C-substance ⟹ Teichoic acid in the cell wall → Induces Septic Shock.
- Reacts with C-reactive protein (β-globulin)

Pathogenesis:

Capsular polysaccharide	Most important virulence factor
Lipoteichoic acid	Induces inflammation
Pneumolysin	Causes α–hemolysis
IgA protease	Enhances ability to colonize the mucosa of URT by cleaving IgA.

Pre-disposing factors:

At risk of developing disease.

- Alcohol intoxication
- Drug intoxication
- Chronic disease
- Splenectomy
- Abnormal circulatory dynamics
- Abnormality of respiratory tract

Laboratory Diagnosis:

- Gram stained smear ⟹ Gram +ve cocci
- Catalase Test ⟹ -ve
- Blood agar ⟹ α-hemolytic
- Optochin sensitive
- Lysed by bile
- Quelling reaction ⟹ Capsule swells with type specific anti-serum
- Latex agglutination test ⟹ Capsular polysaccharide in spinal fluid
- Urinary antigen ⟹ C-substance in case of bacteremia

Vaccines:

- Prevnar-13 (given to immunocompromised & under 5 years child)
- Pneumovax-23 (given to healthy persons or 50 years old)
- Booster dose (given to people older than 65 years old; or between 2-65 years of age who are asplenic)

UNIVERSITY QUESTIONS

1. A female infant was delivered by a midwife at home. Within a day she developed meningitis and dies the next day. **[Supple 2010]** Pg 423 (Q 21).

a) Name two common **organisms** which are likely to cause this disease. **(2)**

b) Name three **risk factors** which increase the chances of a new born acquiring this infection. **(1.5)**

c) Name three **laboratory tests** that are helpful in identifying the causative organism. **(1.5)**

2. A 14-year-old girl develops a rapidly spreading painful, erythematous rash on her leg. The rash was warm and tender and her temperature was 38° C. Gram positive cocci were seen in the aspirate from the lesion. Culture of the aspirate on the blood agar grew colonies surrounded by β-hemolysis. Growth of the organism is inhibited by bacitracin. **[Annual 2010]** Pg 421 (Q 17)

a) What is your most likely **diagnosis**? **(1)**

b) Enumerate the **mechanism** and prominent clinical features of two **immunologic diseases** caused by this organism. **(4)**

3. A 10-year-old boy is brought to pediatrician with complaints of weakness, fever, malaise and passing of low quantity of brownish urine. The urine microscopic examination was positive for RBC casts. Mother gives history of child having a severe attack of sore throat 2-3 weeks back. **[Annual 2012]** Pg 430 (Q 32)

a) If throat swab of the child was cultured at the time of active throat infection, which **organism** would have been isolated? **(1)**

b) Give an account of the **toxins** and **enzymes** produced by these bacteria. **(4)**

4. A patient with suspected brain abscess is admitted to the neurosurgery ward. The abscess was drained and the pus was sent for culture and sensitivity. The isolate on the blood agar is beta-hemolytic, gram-positive coccus with positive coagulase and catalase test. **[Supple 2013]**

a) What is the likely **organism**? Name any four other typical **diseases** produced by this organism. **(3)**

b) Enumerate any two **cell wall components** or antigens of this organism. Give their **importance** in pathogenesis. **(2)**

5. A young, previously fit man presents with one-week history of flu-like illness which has worsened over the past 24 hours. His temperature is 39.5° C with heart rate of 100 and increased respiratory rate. His chest X-ray shows diffuse mottled shadowing in both lung fields. A diagnosis of Community Acquired Pneumonia is made.

a) What **specimens** will be sent for culture to the microbiology lab? What **organisms** are the most likely to cause community acquired pneumonia in a young patient? **(1+2)**

b) Cultures were positive for Staphylococcus Aureus. What **laboratory characteristics** will help to identify this organism? **(2) [Annual 2017]**

6. A 50-year-old female develops a pyogenic infection along the suture line after abdominal surgery. Pus is sent to microbiologist laboratory. A preliminary report of a beta-hemolytic, catalase-positive, gram-positive coccus is given. **[Annual 2018]**

a) What is most likely **diagnosis**? Briefly discuss three clinically important **exotoxins** produced by this organism. **(0.5+3)**

b) Enumerate three types of **diseases** produced by streptococcus pyogenes with one example for each. **(2)**

7. Pneumococcal pneumonia is diagnosed in a 24 years old drug addict brough to the clinic with severe pleuritic pain, fever & cough with rusty color sputum.

a) Discuss its **pathogenesis** with special reference to the role of different **virulence factors** produced by this pathogen. **(3.5)**

b) Enlist three **toxic mediated diseases** produced by Staphylococcus aureus with their associated disease. **(1.5) [Annual 2019]**

8. *Streptococcus pyogenes*is suspected in an 8-year-old girl presenting with repeated attacks of pharyngitis in the previous three months. **[Annual 2020]**

a) Discuss the **laboratory procedures** employed for the confirmation of the pathogen. **(2.5)**

b) Give a short account of the five important **toxins and hemolysins** produced by it. **(2.5)**

13 GRAM – NEGATIVE COCCI

NEISSERIA

Important Properties:

- Diplococci
- Bean shaped
- Cultured on chocolate agar
- Endotoxin consist of **lipooligosachharide**
- Oxidase positive
- Growth inhibited by toxic trace metals & fatty acids found in culture media

N. gonorrhoeae	*N. meningitidis*
Do not ferment Maltose	Ferment Maltose

N. *meningitidis*

Disease:

- Meningitis
- Meningococcemia

Important Properties:

- Polysaccharide capsule
- Capsule is immunogen in vaccine

Mode of Transmission: Airborne droplets

Pathogenesis:

Capsular polysaccharide	Resist phagocytosis by PMN leukocytes.
Endotoxin	Cause fever, shock & other pathophysiological changes
Factor H binding protein	Inhibitor of C3b
IgA protease	Enhances attachment to the membranes of URT by cleaving IgA.

Clinical Findings:

Meningitis:

- Fever
- Stiff neck
- Headache
- PMNs in spinal fluid

Meningococcemia (Waterhouse-Friderichsen syndrome):

- High fever
- DIC
- Widespread Purpura
- Thrombocytopenia
- Adrenal insufficiency

Laboratory Diagnosis:

- Gram stained smear ⇒ Gram -ve kidney shaped diplococci
- Oxidase Test ⇒ +ve
- Maltose fermentation ⇒ +ve
- Colonies ⇒ Grey colored on chocolate agar
- Latex agglutination test
- Immunofluorescence

N. gonorrhoeae

Disease:
- Gonorrhea
- Neonatal conjunctivitis
- Pelvic inflammatory disease

Important Properties:
- No polysaccharide capsule
- Three outer membrane proteins
- Pilus protein present
- Marked antigenic variation

Mode of Transmission:
- Sexual act
- Newborns acquire during birth

Pathogenesis:

Pilli	Mediate attachment to cell surface; antiphagocytic.
Endotoxin	Cause fever, shock & other pathophysiological changes.
Outer membrane proteins	Attachment of organisms to cells.
IgA protease	Enhances attachment by cleaving IgA.

At risk: Persons with deficiency of late acting complement components (C6-C9)

Clinical Findings:

In men	Urethritis, Epididymitis, Dysuria, Purulent discharge
In women	Cervicitis, Salpingitis, Purulent discharge, PID, Intermenstrual bleeding
In newborn	Purulent conjunctivitis (**ophthalmia neonatorum**)
Disseminated Gonococcal Infections	Arthritis, Pustules in skin, Tenosynovitis
Other infections	Pharyngitis, Bloody or purulent discharge

Laboratory Diagnosis:
- Gram stained smear ⇒ Gram -ve kidney shaped diplococci
- Oxidase Test ⇒ +ve
- Maltose fermentation ⇒ -ve
- Nucleic acid amplification tests
- Fluorescent antibody staining
- **In men,** finding of gram -ve diplococci within PMNs in urethral discharge.

UNIVERSITY QUESTIONS

1. A young fashion designer presents to the outdoor patient department with complaints of painfu urination, with discharge of yellowish creamy pus. The gram smear of this discharge was examine which revealed gram negative diplococci within the polymorphonuclear leukocytes.
   a) How would you proceed for further isolation and **identification** of this organism? **(2)**
   b) Name the other closely related human pathogen. Describe the role of **vaccination** for thi other organism. **(1+2) [Annual 2011]** Pg 425 Q23
2. A young female presented to infertility clinic for workup for her infertility status. Her detailed investigations revealed bilateral fallopian tubal blockade. She gave history of mucopurulent vagina discharge in the past and history of similar creamy yellowish urethral discharge in her husband. A common bacterial infection is suspected in the couple. **[Supple 2011]** Pg 428 Q28
   a) Name the **microorganism** responsible for producing this clinical scenario. **(1)**

   b) How would you proceed in the **lab** to isolate and identify the infecting agent in the urethral discharge of her husband? **(4)**
3. A young fashion designer presents to the urology ward with complaints of creamy yellowish urethral discharge and painful micturition. He is otherwise in good health. Gram-stained smear of discharge revealed pus cells with intracellular gram-negative cocci. **[Annual 2012]** Pg 430 Q33
   a) How would you proceed in the **lab** for identifying this bacterium? Describe systemically. **(3)**
   b) Name other intracellular gram-negative coccus and briefly describe its **antigenic structure**. **(2)**
4. A patient comes to hospital with complaints of fever, headache, neck rigidity and increased polymorphonuclear neutrophils in his CSF.
   a) What is probable **diagnosis**? Q48 Pg 436.
   b) What lab tests will be used to make a **lab** diagnosis? **[Annual 2014]**
5. A 25-year-old female was brought to hospital because of sudden onset of high-grade fever and headache. On examination there is stiffness of neck, irregularly shaped ecchymosis and purpuric flat lesions scattered over body. Her temperature is 40° C, BP is 40/20 mm Hg, pulse is 140/min. Culture smear of CSF showed gram negative diplococci. **[Supple 2015]** Q51, Pg 437
   a) What is most likely **diagnosis** and **bacteria** causing it?
   b) Briefly discuss the **pathogenesis** of this clinical condition.
6. A 5-year-old child develops high grade fever and headache for last 4-5 days. He is brought to Pediatrics emergency room. On examination, he has neck stiffness. A lumbar puncture is performed. Preliminary report of cerebrospinal fluid (CSF) analysis suggests the diagnosis of acute bacterial meningitis. **[Annual 2015]** Q55 Pg 439
   a) Name the possible **organism** responsible for disease in him and give its **portal of entry**. **(2)**
   b) How will you proceed in the **laboratory** to further confirm the diagnosis and establish the causative organism? **(3)**
7. A 20-year-old boy presents with purulent urethral exudate and dysuria for last two weeks. Gram smear of pus discharge shows a large number of Gram-negative intracellular diplococci suggestive of Neisseria Gonorrhea.
   a) Briefly discuss its **pathogenesis** and name another important sexually transmitted bacterium and the disease produced by it. **(2+1)**
   b) Enlist three **clinical complications** of this infection seen in women and one in infants. **(2) [Supple 2016 held in 2017]** Q64, Pg 444.
8. A 25-year-old patient was seen in emergency department of hospital with fever, headache and stiffness of neck. His Kernig's sign was positive. There were petechial rashes on his body. **[Supple 2017 held in 2018]** Q69 Pg 447
   a) What is the **diagnosis**? **(1)**
   b) If it is due to Gram-Negative diplococci, how would the diagnosis be confirmed in pathology **laboratory**? **(4)**
9. Discuss **lab investigations** for diagnosis of Neisseria Gonorrhoeae. **[Annual 2018]** Q28, Pg 428
10. A young man with sign & symptoms of meningitis is brought to the emergency. Gram smear of CSF shows a large number of Gram-negative diplococci within the neutrophils.
    a) What is the most likely clinical **diagnosis**? Give its **laboratory** diagnosis. **(1+2)**
    b) What is **Ophthalmic Neonatorum** & **Reiter's syndrome**? **(2) [Annual 2019]**

14 GRAM – POSITIVE RODS

- GRAM-POSITIVE RODS
  - SPORE-FORMING
    - BACILLUS
    - CLOSTRIDIUM
  - NON-SPORE-FORMING
    - CORYNEBACTERIUM
    - LISTERIA
    - GARDENERELLA

BACILLUS

Species:

- B. anthracis
- B. cereus

B. *anthracis*

Disease: Anthrax

Important Properties:

- Gram +ve rods
- No-motile
- Rods with square ends
- D-glutamate capsule

Habitat: Soil

Transmission:

Cutaneous anthrax	Spores in soil enter wound
Inhalation anthrax	Spore are inhaled into lungs
Gastrointestinal anthrax	Ingestion of contaminated meat

Pathogenesis:

Produces two exotoxins;

Edema factor	Adenylate cyclase → ↑cAMP → cAMP causes an outpouring of fluid from the cell into extracellular space → Edema
Lethal factor	Protease → Phosphokinase cleavage → no activation of Mitogen activated protein kinase (**MAPK**) signal transduction pathway → Inhibition of cell growth → Cell death

Subunits;

A subunit	Enzymatic activity
B subunit	Protective antigen (forms pores in human cell membrane)

Clinical Findings:

Cutaneous anthrax	Edema, Malignant pustule, Painless ulcer with a **black eschar**, Untreated cases progress to bacteremia
Inhalation anthrax	Dry cough, Substernal pressure, Hemorrhagic mediastinitis, Blood pleural effusions, Septic shock, Death
Gastrointestinal anthrax	Vomiting, Bloody diarrhea, Abdominal pain

Laboratory Diagnosis:

- Gram stained smear ⇒ Gram +ve rods in chains
- Blood agar ⇒ Non-hemolytic colonies (**comet's tail appearance**)
- PCR
- Fluorescent antibody test
- ELISA

B. *cereus*

Disease: Food Poisoning

Transmission: Spores on grains such as rice

Pathogenesis:

Produces two exotoxins;

1. Act like Cholera toxin → Adenylate cyclase → ↑cAMP → cAMP **phosphorylates** ion transporter in the membrane → Export ions → Outpouring of fluid → Diarrhea
2. Act like Staphylococcal enterotoxin → acts as a supra-antigen within GIT to stimulate release of IL-1 & IL-2.

Clinical Findings:

Short incubation period	Vomiting, Nausea
Long incubation period	Watery non-bloody diarrhea

CLOSTRIDIUM

Species:

- C. tetani
- C. botulinum
- C. perfringens
- C. difficile

C. *tetani*

Disease: Tetanus

Habitat: Soil

Portal of entry: Wound site

Pathogenesis:

- Tetanus toxin is an exotoxin produced by the vegetative cells at the site of wound.
- Carried intra-axonal to CNS → Binds **ganglioside receptors** → Blocks release of inhibitory neurotransmitters (**Glycine** & **GABA**) at spinal synapses
- It is **protease** that cleaves the proteins involved in mediator release from the neurons.

Clinical Findings:

- **Spastic Paralysis**
- Lockjaw
- Exaggerated reflexes

- Strong muscle spasms
- Tetany
- Risus Sardonicus
- Opisthotonos
- Respiratory failure

Laboratory Diagnosis:

- No microbiological or serological diagnosis
- Produces terminal spore ⟹ Characteristic appearance of **tennis racket**

C. botulinum

Disease: Botulism

Habitat: Soil

Mode of Transmission: Ingestion of improperly preserved food

Pathogenesis:

- Botulinum toxin → absorbed from gut → carried via blood to peripheral nerve synapses → blocks release of acetylcholine.
- It is **protease** that cleaves the proteins involved in Acetylcholine release.

Clinical Findings:

- **Flaccid Paralysis**
- Diplopia
- Dysphagia
- Respiratory muscle failure

Clinical Forms;

Wound botulism	Spores contaminate a wound, germinate produce toxin at the site.
Infant botulism	Organisms grow in the gut & produce toxin there.

Laboratory Diagnosis:

- Not cultured
- **Mouse protection tests** ⟹ Demonstrable in uneaten food & patient's serum
- Enzyme-linked immunoassay
- PCR

C. perfringens

Disease:

- Gas Gangrene
- Food Poisoning

Gas Gangrene

Habitat: Soil

Transmission:

- War Wound
- Septic Abortion
- Automobile accidents

Pathogenesis:

- Organism grows in traumatized tissue.
- Produce various toxins
- Alpha toxin (**Lecithinase**) of RBCs (**hemolysis**) ⟹ damages cell membranes including those
- Degradative enzymes produce gas in tissues.

Clinical Findings:

- Pain
- Cellulitis
- Hemolysis
- Edema
- Jaundice
- Gangrene
- Hydrogen gas in affected tissues

Laboratory Diagnosis:

- Gram stained smear ⟹ Gram +ve rods
- Blood agar ⟹ Double zone of hemolysis
- Culture ⟹ Organic acid production, anaerobically
- Sugar fermentation reaction
- Egg yolk agar ⟹ Colonies produce a precipitate in egg yolk agar by action of Lecithinase.

Food Poisoning

Habitat: Soil

Transmission:

- Ingestion of contaminated food
- Heat resistant spores survive cooking & germinate

Pathogenesis:

- Enterotoxin acts like Staphylococcal enterotoxin → acts as a supra-antigen within GIT to stimulate release of IL-1 & IL-2.

Clinical Findings:

- Watery diarrhea with cramps
- Vomiting

C. difficile

Disease:

- Antibiotic associated pseudo membranous colitis
- Nosocomial cause of diarrhea

Habitat: Human Colon

Transmission: Fecal-Oral route

Pre-disposing drugs:

Antibiotics suppress normal flora;

- Clindamycin
- Ampicillin
- 3rd generation cephalosporins
- Fluoroquinolones
- Cancer Chemotherapy
- PPIs

Pathogenesis:

- Antibiotics suppress drug-sensitive members of the normal flora of the colon, allowing C. difficile to multiply & produce exotoxin A & B.
- Both exotoxins are **glycosyltransferases.**
- Exotoxins → **Depolymerization** of actin → Loss of cytoskeletal integrity → **Apoptosis** → Death of enterocytes
- Exotoxin B plays the leading role.

Clinical Findings:
- Diarrhea associated with pseudo membranes on colonic mucosa
- Neutrophils in stool
- Abdominal pain
- Fever
- Toxic megacolon

Laboratory Diagnosis:
- Stool specimen ⇒ Presence of exotoxins
- PCR
- ELISA
- Cytotoxicity test

CORYNEBACTERIUM *DIPHTHERIAE*

Disease: Diphtheria

Important Properties:
- Gram +ve rods
- Club shaped
- Non-Spore forming
- Beaded appearance
- Arranged in palisades or in V or L shaped
- Non-motile
- Metachromatic granules

Habitat: Upper Respiratory Tract

Transmission: Airborne droplets

Pathogenesis:
- Produces **exotoxin**.
- **Invasiveness** of organism.
- Diphtheria toxin → ADP-ribosylation of **Elongation Factor-2** → Inhibition of Protein synthesis.
- Pseudo membrane in throat caused by death of mucosal epithelial cells.

Functional Domains;

Active (A) domain	Enzymatic activity
Binding (B) domain	Binding of toxin to glycoprotein receptors.

Clinical Findings:
- Fever
- Sore Throat
- Cervical adenopathy
- Thick, gray, adherent pseudo-membrane over the tonsils & throat

Complications
- Airway obstruction
- Myocarditis
- Nerve weakness & paralysis

Laboratory Diagnosis:
- Gram stained smear ⇒ Gram +ve pleomorphic rods
- Tellurite culture ⇒ Gray black colonies
- Loeffler's medium ⇒ Reddish granules
- Antibody-based gel diffusion precipitin test
- PCR

LISTERIA *MONOCYTOGENES*

Disease:
- Meningitis
- Gastroenteritis
- Sepsis

Important Properties:
- Gram +ve rods
- Exhibits **tumbling movement**
- Arranged in V or L shaped formations
- Non-Spore forming

Habitat:
- ✓ Female genital tract
- ✓ GIT

Transmission: Ingestion of **unpasteurized** milk products

Pathogenesis:
- The pathogenesis of *Listeria* depends on the organism's ability to **invade** and **survive** within cells.
- Invasion of cells is mediated by **internalin** made by *Listeria* and **E-cadherin** on the surface of human cells.
- Upon entering the cell, the organism produces **listeriolysin,** which allows it to escape from the phagosome into the cytoplasm, thereby **escaping destruction** in the phagosome.
- *Listeria monocytogenes* can move from cell to cell by means of **actin rockets**

Clinical Findings:

During pregnancy;
- Abortion
- Premature delivery
- Sepsis
- Influenza-like illness in infected mother

Gastroenteritis;
- Fever
- Headache
- Myalgias
- Vomiting
- Watery diarrhea
- Abdominal cramps

Laboratory Diagnosis:
- Gram stained smear ⇒ Gram +ve rods
- Blood Agar ⇒ Gray colonies with narrow zone of β-hemolysis
- Sugar fermentation test

GARDENERELLA *VAGINALIS*

Disease: Bacterial vaginosis

Important Properties:

Gram-variable rods — Thin gram +ve cell wall

Clinical Findings:
- Malodorous, white or grey colored vaginal discharge with fishy odor
- Mild itching
- Morbidity & Mortality in new born

Laboratory Diagnosis:

- Microscopic examination ⟹ Clue cells (vaginal epithelial cells) covered with bacteria in vaginal discharge.
- **Whiff test** ⟹ +ve
- pH ⟹ greater than 4.5

UNIVERSITY QUESTIONS

1. A grandmother in remote village cow dung to the umbilical stump of a new born. Child develops strong muscular spams pronounced arching of back and dies of respiratory failure after a week. **[Annual 2007]** Q3 · Pg 415
   a) Name the most likely **etiological agent.**
   b) Name other **3 bacterial species** of the genus and **diseases** caused by them.
2. A 7-year-old girl is brought to pediatrics emergency with fever of 101.5 F, sore throat, malaise, and dyspnea. She has an incomplete vaccination history. Physical examination reveals cervical lymphadenopathy and whitish membrane covering most part of pharynx. **[Annual 2009]**
   a) What is the most likely **diagnosis** and **causative agent**? Q11, Pg 418
   b) How does this microorganism **cause** this presentation?
   c) What **growth medium** is used to identify this organism and how does it **appear** on culture?
3. A 60-year-old fell and sustained a deep wound from a rusty nail that penetrated his leg. Although the wound was cleaned, the next morning he developed fever of 102F. his thigh became painful and swollen. In the surgical emergency, he developed crepitus (gas in tissue). The exudates of the wound area revealed gram-positive rods. Q20, Pg 423
   a) What is the most likely causative **organism**? Name three other **members** of clostridium species. **(2)**
   b) Give:
      i) the **disease** caused
      ii) **transmission/predisposing factors**
      iii) **action of toxin** of different members of clostridium species. **[Supple 2010]**
4. A 50-year-old male meets a road side automobile accident resulting in compound fracture of his femur. He was brought to the hospital from his village. Next day he developed foul smelling discharge from the wound, crepitations in the subcutaneous tissue occur. He developed high grade fever and went into shock. **[Supple 2012, Supple 2011]** Q29, Pg 428
   a) Name the **organism** responsible for this condition. **(1)**
   b) Give an account of **pathogenesis** and **toxins** produced by this organism. **(4)**
5. A young hostel student was used to eating home canned food. She presented at emergency department complaining of difficulty in speech and swallowing. She soon developed visual problems with diplopia and went into complete bulbar paralysis. Her respiration also became labored. She admitted eating rancid canned food without heating. **[Annual 2012]**
   a) What is the **pathogenesis** of her illness? **(3.5)** Q31, Pg 429
   b) Name the other pathogens belonging to this **species** of bacteria and **diseases** produced by each in humans. **(1.5)**
6. An unimmunized child of remote village develops fever, sore throat and cervical lymphadenopathy. On examination there is a thick adherent membrane over the tonsils and throat. A provisional diagnosis of sore diphtheria was made. Q44, Pg 434
   a) Name the **causative organism** of this child's illness? What is its **appearance** on gram staining? **(2)**
   b) Briefly describe the **mechanism of action** of toxin produced by this organism. What is the role of **antitoxin** in the treatment of diphtheria? **(3) [Supple 2013]**

7. A patient of acute lymphocytic leukemia with fever and neutropenia develops diarrhea after administration of amoxicillin therapy. What is the most probable **causative agent** and what **disease** it can cause? **(5) [Supple 2014]** Q47 · Pg 436
8. A 4 years old girl is brought to the emergency with history of fever and sore throat for 4 days. Her immunization status is unknown. On examination, she is anxious, tachypneic and ill looking. Her temperature is 38.6° C and her voice is hoarse. Examination of pharynx revealed tonsillar and pharyngeal edema with gray membrane coating the tonsils extending over uvula and soft palate. There is cervical lymphadenopathy and lungs are clear. **[Annual 2015]** Q54, Pg 439
   a) What is most likely **diagnosis**? Name the causative **organism**. **(1+1)**
   b) Give the **characteristics** of causative organism and its **toxin**. **(1+2)**
9. A young male develops a large area of gangrenous necrosis on lateral aspect of leg following wound contamination associated with compound fracture during a road traffic accident. A large gas and fluid filled bulla appears producing subcutaneous crepitations close to the area of gangrene. **[Annual 2016]** Q62 Pg 443 ·
   a) Name the **causative organism** and give the most likely **diagnosis**. **(2)**
   b) Briefly discuss the **pathogenesis** of this organism. **(3)**
10. A 10-year-old male child is brought to the hospital emergency with high grade fever and chills, sore throat, headache, dysplasia and dyspnea. On examination, cervical lymphadenopathy is noted along with greyish white adherent pseudo-membrane over the tonsils. **[Supple 2018 held in 2019]**
    a) What is the most likely **diagnosis**? Give the **mechanism** of disease development by this pathogen.
    b) Enlist three **complications** associated with this disease.
11. A 5 years old boy presents with a severe sore throat & fever in the pediatric OPD. On examination, a grayish exudate (pseudo-membrane) is seen over the tonsils & pharynx. Gram smear show Gram-positive club shaped rods. **[Annual 2020]** Q73) Pg 450
    a) What is the likely **cause** of the boy's pharyngitis? Give two important complications of this disease. **(1+2)**
    b) Name two Gram-positive **exotoxin-producing bacilli** & their **mode of action. (2)**

15 GRAM – NEGATIVE RODS RELATED TO ENTERIC TRACT

GRAM-NEGATIVE RODS

WITHIN & OUTSIDE THE ENTERIC TRACT	PRIMARILY WITHIN THE ENTERIC TRACT	OUTSIDE THE ENTERIC TRACT
• ESCHERICHIA • SALMONELLA	• SHIGELLA • VIBRIO • CAMPYLOBACTER • HELICOBACTER	• KLEBSIELLA-ENTEROBACTER-SERRATIA GROUP • PROTEUS-PROVIDENCIA-MORGANELLA GROUP • PSEUDOMONAS • BACTEROIDES

ESHERICHIA *COLI*

Disease:
- Urinary Tract Infections
- Hemolytic uremic syndrome
- Traveler's diarrhea
- Bloody diarrhea
- Neonatal meningitis
- Sepsis

Important Properties:
- Gram -ve rods
- Facultative anaerobe
- Ferments lactose
- Motile
- Three antigens;
  i. O or cell wall antigen,
  ii. H or flagellar antigen,
  iii. K or capsular antigen

Normal Flora: Human Colon

Transmission:

Neonatal meningitis	Acquired during birth
Traveler's diarrhea	Ingestion of contaminated food

Pathogenesis:
Virulence factors;
- Pili
- Capsule
- Endotoxin
- Three exotoxins
  - ✓ Two exotoxins cause watery diarrhea.
  - ✓ One exotoxin causes bloody diarrhea & Hemolytic-uremic syndrome.

Intestinal Tract Infection

➢ Attachment of organism to the cells of jejunum & ileum by pili.

➢ Once attached, the bacteria synthesize enterotoxin which act to cause diarrhea.

Enterotoxigenic strains of E. coli

Produces two enterotoxins;
i. Heat-labile toxin ⇒ stimulate adenylate cyclase
ii. Heat-stable toxin ⇒ stimulate guanylate cyclase

Adenylate cyclase → ↑cAMP → cAMP **phosphorylates** ion transporter in the membrane → Export ions → Outpouring of fluid → Diarrhea

Entero-invasive strains of E. coli

➢ Invasion of the epithelium of large intestine, causing bloody diarrhea accompanied by inflammatory cells in stools

Enterohemorrhagic strains of E. coli **(O157:H17)**

Shiga toxin → removal of adenine from ribosomal (28S) RNA → Protein synthesis stopped

Hemolytic Uremic Syndrome

Occur when Shiga toxin enters the bloodstream
- Hemolytic anemia → Death of endothelial cells of small blood vessels in which RBCs passing through the damaged area distort & lyse.
- Thrombocytopenia → Platelets adhere to the damaged endothelial surface.
- Renal failure → Death of kidney epithelial cells.

Urinary Tract Infections

➢ O serotypes of E. coli cause UTIs.
➢ Pili have adhesin proteins that bind to specific receptors on the urinary tract epithelium.

Systemic Infections

- Capsule ⇒ Neonatal meningitis by K1 antigen
- Endotoxin ⇒ Sepsis such as fever, hypotension & DIC

Laboratory Diagnosis:
- Gram stained smear ⇒ Gram -ve rods
- Blood agar ⇒ Round colonies
- MacConkey's agar ⇒ Pink colonies
- Lactose fermentation ⇒ +ve
- AMB agar ⇒ Green sheen colonies

Features that distinguish E. coli from other lactose fermenting gram -ve rods;
- ✓ Produces indole from tryptophan
- ✓ Decarboxylates lysin
- ✓ Uses acetate as its only source of carbon
- ✓ Motile

SALMONELLA

Species:

Typhoidal species	*S. typhi, S. paratyphi*
Non-typhoidal species	*S. enterica*

Disease:
- Enterocolitis
- Septicemia
- Typhoid fever
- Osteomyelitis

Important Properties:

- Gram -ve rods
- Non-spore forming
- Do not ferment lactose
- Motile
- Produce H_2S

Transmission: Fecal-oral route

Pathogenesis:

- Three antigens;
  - i. O or cell wall antigen,
  - ii. H or flagellar antigen,
  - iii. Capsular Vi antigen (virulence)

Enterocolitis

- **Invasion** of epithelial & sub-epithelial tissues of small & large intestine
- Organism **penetrate** into lamina propria → Inflammation & Diarrhea
- Neutrophils limit the infection to the gut & the adjacent mesenteric lymph nodes.
- Gastric acid is an important host defense.

Typhoid fever

- Infection begins in small intestine.
- The organism enter & multiply in the mononuclear phagocytes of Payer's patches
- Spread to the phagocytes of liver, gallbladder & spleen.
- Leads to the bacteremia associated with the onset of fever & other symptoms.

Septicemia

Occur in;

- Children with enterocolitis
- Patients with chronic disease

Clinical Finding:

- Diarrhea (with or without blood)
- **Rose spots** (rose colored macules on abdomen)
- Fever
- Nausea
- Vomiting
- Abdominal pain
- Enlarged spleen
- Leukopenia
- Anemia

Laboratory Diagnosis:

- Gram stained smear ⟹ Gram -ve rods
- TSI agar ⟹ Alkaline slant & acid butt with gas & H_2S
- MacConkey's agar ⟹ Non-lactose fermenting colonies
- Bone marrow cultures ⟹ +ve
- Stool cultures ⟹ +ve
- Agglutination test
- Only *S. typhi* produces H_2S
- Widal Test ⟹ Detection of rise in antibody titer in patient's serum (gold standard laboratory test)

WITHIN ENTERIC TRACT

SHIGELLA

Disease: Enterocolitis (Bacillary dysentery)

Important Properties:

- Gram -ve rods
- Do not produce H_2S
- Non-lactose fermenting
- Non-motile
- O antigen in the cell wall

Habitat: Human Colon

Transmission: Fecal-oral route

Pathogenesis:

- Have very low ID-50, ingestion of only 100 organism cause disease.
- Shigella → **invade** the cells of mucosa of distal ileum & colon → Diarrhea
- Local inflammation accompanied by ulceration.

Clinical Finding:

- Fever
- Abdominal Cramps
- Diarrhea (first watery, then bloody)

Laboratory Diagnosis:

- Gram stained smear ⟹ Gram -ve rods
- TSI agar ⟹ Alkaline slant & acid butt with no gas & no H_2S
- MacConkey's agar or EMB agar ⟹ Non-lactose fermenting colonies
- Slide agglutination
- Methylene blue stain of fecal sample

Comparison of Salmonella & Shigella

Salmonella	Shigella
Gas production from glucose fermentation	No gas production
H_2S production	No H_2S production
Motile	Non-motile

VIBRIO *CHOLERA*

Disease: Cholera

Important Properties:

- Gram -ve rods
- Curved, coma shaped
- Oxidase +ve

Habitat: Human Colon, Shellfish

Transmission: Fecal-oral route

Pathogenesis:

- Colonization of small intestine by organism & secretion of enterotoxin.
- Adherence to the cells of brush border of gut by secretion of bacterial enzyme **mucinase**.

V. cholera → Adenylate cyclase → ↑cAMP → Protein kinase → **phosphorylates** ion transporter in the membrane → export ions → outpouring of fluid → Diarrhea

Clinical Finding:

- Watery Diarrhea
- **Rice-water stool**
- Cardiac failure
- Acidosis
- Renal failure
- Hypokalemia

Laboratory Diagnosis:

- Gram stained smear ⟹ Gram -ve rods

- TSI agar ⟹ Acidic slant & acid butt with no gas & no H_2S
- MacConkey's agar ⟹ colorless colonies
- Agglutination with known anti-sera
- Oxidase test ⟹ +ve
- **Slow lactose fermentation**
- Rise in antibody titer in acute- & convalescent-phase sera

CAMPYLOBACTER *JEJUNI*

Disease:
- Enterocolitis
- Guillain-Barre syndrome

Important Properties:
- Gram -ve rods
- Curved, coma or S shaped
- **Microaerophilic** (grow best in 5% oxygen)
- Oxidase +ve

Habitat: Human & animal feces

Transmission: Fecal-oral route

Pathogenesis:
- **Invasion** of mucosa of colon by organism, but does not penetrate.
- **Inflammation** of intestinal mucosa.

Clinical Finding:
- Watery foul-smelling diarrhea
- Bloody stools
- Reactive arthritis
- Guillain-Barre syndrome
- Abdominal pain
- Reiter's syndrome

Laboratory Diagnosis:
- Gram stained smear ⟹ Gram -ve rods
- Oxidase test ⟹ +ve
- Urease test ⟹ -ve
- Sensitive to nalidixic acid
- Incubate at 42°C in microaerophilic atmosphere

HELICOBACTER *PYLORI*

Disease:
- Gastritis
- Peptic ulcer
- Mucosal associated lymphoid tissue (**MALT**)

Important Properties:
- Gram -ve rods
- Curved
- Urease +ve

Habitat: Human stomach

Transmission: Ingestion

Pathogenesis:
- Attachment of H. *pylori* to mucus secreting cells of gastric mucosa.
- Production of large amount of ammonia from urea by the **urease** coupled with an inflammatory response.

- Damage to mucosa → loss of protective mucous coating → Gastritis & peptic ulcer.

Clinical Finding:
- Recurrent pain in the upper abdomen
- Bleeding into GIT

Laboratory Diagnosis:
- Gram stained smear ⟹ Gram -ve rods
- Urease test ⟹ +ve
- **Urea breath test**
- H. *pylori* in the stool
- Serological tests
- Radioactive Urea ingested → if H. *pylori* present → urease will cleave the ingested urea

KLABSIELLA

Disease:
- Pneumonia
- UTIs

Important Properties:
- Gram -ve rods
- Large polysaccharide capsule
- Striking mucoid appearance.

Habitat: Human Respiratory tract; Enteric tract

Transmission:
- Aspiration
- Respiratory droplets
- Ascending spread of fecal flora

Pathogenesis: Virulence factor → antiphagocytic capsule

Clinical Finding:
Klebsiella → produces a thick, mucoid, bloody sputum (**"currant-jelly" sputum**).

Laboratory Diagnosis:
- MacConkey's agar or EMB agar ⟹ Lactose fermenting colonies

PROTEUS

Disease: UTIs

Important Properties:
- Gram -ve rods
- **Motile**
- Produce enzyme phenylalanine
- Urease production
- **Striking swarming effect**

Habitat: Human Colon, Water, Soil

Transmission: Ascending spread of fecal flora

Pathogenesis: Virulence factor; **Urease** → degrades urea to produce ammonia → raises pH → alkaline urine → stone formation

Clinical Finding:
- Pneumonia
- Wound infections
- Septicemia

Laboratory Diagnosis:
- MacConkey's agar or EMB agar → Non-lactose fermenting colonies
- Blood agar → swarming effect
- Produce H_2S gas

PSEUDOMONAS (Aerobic)

Disease:
- Malignant otitis externa
- Ventilator associated pneumonia
- Lower Respiratory Tract infections
- Sepsis
- Pneumonia
- Cellulitis
- Melioidosis
- Urinary tract infections

Important Properties:
- Gram -ve rods
- Aerobes
- Non-fermenters
- Oxidase +ve
- Able to grow in water
- Withstand disinfectants
- Mucoid appearance due to glycocalyx

Pigments;
- *Pyocyanin* Color the pus in a wound blue
- *Pyoverdine* Yellow green pigment; used in early detection

Habitat:
- Soil & water
- Human colon
- Upper respiratory tract
- Skin

Transmission:
- Aspiration
- Fecal contamination
- Water aerosols

Pathogenesis: Components involved;
- Endotoxin: Fever & shock associated with sepsis
- Exotoxin A: ADP-ribosylation of **Elongation Factor-2** → Inhibition of Protein synthesis
- Enzymes: Elastase & Protease → Histotoxic → Invasion of organism into bloodstream
- Pyocyanin: Damages the cilia & mucosal cells of respiratory tract.
- Type III secretion system: Transfer exotoxin from bacterium directly into adjacent human cell

Clinical Finding:
- Pneumonia (in **cystic fibrosis patients**)
- UTIs
- Wound Infections
- Ecthyma gangrenosum
- Endocarditis
- Malignant otitis media
- Hot tub folliculitis
- Corneal infections
- Chronic lung infections
- Ventilator associated pneumonia

Laboratory Diagnosis:
- Gram stained smear → Gram -ve rods
- Oxidase test → +ve
- EMB agar → Non-lactose fermenting colonies
- TSI agar → Typical metallic sheen

BACTEROIDES (Anaerobic)

Disease:
- Sepsis
- Abscesses
- Peritonitis

Important Properties:
- Gram -ve rods
- Non-Sporing forming
- Anaerobes

Habitat: Human Colon

Transmission: Spread from the colon to the blood or peritoneum

Pathogenesis: Components involved;
- Polysaccharide capsule: Virulence factor
- Endotoxin: Les active than typical endotoxin
- Enzymes: Hyaluronidase, Collagenase, Phospholipids

Clinical Finding:
- Peritonitis
- Pelvic abscess
- Localized abscess
- Bacteremia
- Necrotizing fasciitis

Laboratory Diagnosis:
- Sugar fermentation
- Gas chromatography

UNIVERSITY QUESTIONS

1. After recent flooding in a slum area of Faisalabad, there is large influx of patients in the emergency department of DHQ hospital with specimens sent to lab being classical rice water stools.
   a) Name the most likely etiological **agent**.
   b) Give the **pathogenesis** and lab **diagnosis** of this. **[Annual 2007]**
2. Women of child bearing age are far more prone to UTIs than men because of shortened urethra.
   a) Enlist 2 **bacteria** in order of frequency which cause UTI in women of child bearing age.
   b) Give 4 **risk factors** predisposing to UTI. **[Annual 2008]**
3. A foreign journalist who recently returned home from trip to Pakistan after preparing report on IDPs of Pakistan goes to physician complaining persistent high fever, malaise, constipation that presided over a week. She recalls that the fever began lowly and then went up to 41° C. PE reveals enlarged spleen and tenderness of abdomen with rose colored spots. **[Supple 2009]**
   a) Which **organism** is most likely to cause this condition? Name any 3 **method to diagnose** infection caused by this organism
   b) Associated with this organism, give any 2 **virulence factors** and their **effects.**
4. A case of acute abdomen was brought to emergency department and was diagnosed as a case of perforated intestine. Partial resection and ileostomy were carried out. Following that the patient developed peritonitis, high grade fever and went into shock. Discuss **pathogenesis** of both E. coli and Bacteroides in causing peritonitis after abdominal surgery. **[Annual 2009]**
5. A female infant was delivered by a midwife at home. Within a day she developed meningitis and dies the next day. **[Supple 2010]**
   a) Name two common organisms which are likely to cause this disease. **(2)**
   b) Name three risk factors which increase the chances of a new born acquiring this infection. **(1.5)**
   c) Name three laboratory tests that are helpful in identifying the causative organism. **(1.5)**

6. A previously healthy 12-year-old boy came to the emergency room complaining of worsening bloody diarrhea and abdominal pain for the past 24 hours. He was anuric for 12 hours. His physical examination was unremarkable except for dehydration. His mother does admit to cooking his son a hamburger using meat that has been sitting on the kitchen counter for 'sometime'. Blood examination showed evidence of reduced renal function and lysed blood cells. **[Annual 2010]**
   a) Based on the symptoms and family activities, what **organism** & **strain** is the cause of this disease? **(2)**
   b) What unique **complications** can be caused by this organism? **(3)**
7. A villager was brought to the emergency department in the state of severe dehydration. He gave history of developing profuse diarrhea without blood, nausea and vomiting followed by features of fluid loss. Doctor on duty quickly examined his stool specimen microscopically and found curved organisms with classical darting motility. **[Annual 2011]**
   a) Describe the lab diagnosis of this disease in a systematic stepwise manner. **(3)**
   b) What is the pathogenesis of this disease? **(2)**
8. A young female with cystic fibrosis gets exacerbation of her bronchitis with cough. Abundant mucoid colonies were grown from sputum after 24 hours of incubation which were gram -ve bacilli, oxidase positive and motile. **[Supple 2012, Supple 2011]**
   a) Describe growth and cultural **characteristics** of this microorganism. **(2)**
   b) Give the clinical **spectrum** of infection with this microorganism. **(3)**
9. A 30-year-old male comes to hospital with complaints of severe headache, high-grade fever that followed a step ladder pattern. He is constipated. On examination, he has tender abdomen with rose spots. Relative bradycardia is also present.
   a) Name the **bacterium** responsible for this condition.
   b) What is **pathogenesis** of this disease? **[Annual 2014]**
10. You are a medical officer at camp for IDPs where an outbreak of diarrhea occurred. Patients complain of excessive watery stools with no bleeding. Gram stain of stool showed curved gram-negative rods.
   a) Name the most likely **diagnosis**. Name the causative **bacteria**.
   b) How it can be further confirmed in **lab**?
   c) Briefly discuss its **pathogenicity**. **[Supple 2015]**
11. Several young children in a refugee camp presented with fever, gripping pain with repeated passage of blood and mucous containing stools. The crowded living conditions of the camp suggest person to person contact. Non-motile, gram-negative bacilli producing non-lactose fermenting colonies are isolated on selective media. Based on these findings,
   a) Which **disease** are these children suffering from? Name the etiological **agent**. **(2)**
   b) Briefly discuss the **pathogenesis** of this infection. **(3) [Supple 2015 held in 2016]**
12. A young male is admitted with a 7-day history of 'step ladder pattern fever' and generalized weakness. Blood culture showed gram negative motile rods. Typhoid fever is suspected. Discuss the **laboratory procedures** employed in diagnosing this disease. **[Annual 2016]**
13. Nine people from a village in the province of Punjab presents with sudden onset of vomiting and massive watery diarrhea. They observed rice water stools with flecks of mucous. Culture reveals motile gram-negative curved bacilli. **[Supple 2016 held in 2017]**
   a) Name the most likely **pathogen** and discuss the **mode of action** of its enterotoxin (exotoxin) leading to watery diarrhea. **(1+2)**
   b) Give the **laboratory procedures** employed in diagnosing this infection. **(2)**
14. A 22-year-old male develops high-grade fever with persistent headache &constipation, hepatosplenomegaly, lymphadenopathy &rose spots on abdomen are observed in the second week of infection.
   a) Briefly discuss the **pathogenesis** of this infection. What can be the possible **complications** if this patient is left untreated? **(2+0.5)**

   b) Name the **gold standard laboratory test** for diagnosis of this disease and reaction of this pathogenesis on **TSI agar**. **(2.5) [Annual 2017]**
15. A patient was admitted in a hospital with high grade fever, malaise and constipation. He had bradycardia and hepatosplenomegaly. There was maculopapular rash 'rose spots' on his chest and abdomen. His CBC revealed leukopenia. **[Supple 2017 held in 2018]**
   a) What is the most likely **diagnosis**? **(1)**
   b) Give the **laboratory diagnosis** of this disease. Mention the specimens more likely to give positive results in the first and second week. Give the **serologic tests** available to diagnose the cases that present late to the hospital. **(4)**
16. A boy has cramping abdominal pain. He is passing stool containing pus cells and red blood cells.
   a) Name three **microorganisms** responsible for exudative disease resulting in the appearance of blood in stool. **(2)**
   b) Give the **mechanism of action** of Shiga toxin. **(3) [Supple 2017 held in 2018]**
17. A 65-year-old female presents with dysuria and hematuria. Culture of the urine sample reveals lactose-fermenting gram-negative motile rods. What will be the **pathogenesis** of urinary tract infection by this organism? What is **Hemolytic Uremic syndrome**? **(2+1.5) [Annual 2018]**
18. a) How will you diagnose a case of bacillary dysentery in the laboratory?
   b) Enlist four other dysentery causing organisms. **[Supple 2018 held in 2019]**
19. A 50-year-old man develops intense rice water diarrhea 24 hours after leaving the village. The stools are thin & watery, containing flakes of mucous, no pus or blood cells. Stool culture reveals rapid growth at the surface of alkaline peptone water. Name the most likely **microorganism** responsible for the diarrhea. What is the **mode of action** of enterotoxin of this pathogen? **(1+2) [Annual 2020]**

16 GRAM – NEGATIVE RODS RELATED TO RESPIRATORY TRACT

HAEMOPHILUS *INFLUENZA*

Disease:
- Meningitis
- Pneumonia
- Sepsis
- Upper respiratory tract infections

URTs Infections;
- Otitis media
- Conjunctivitis
- Sinusitis
- Epiglottitis

Important Properties:
- Gram -ve rods
- Polysaccharide capsule
- Growth on laboratory media requires addition of **heme** (factor X) & **NAD** (factor V) for adequate energy production

Transmission: Respiratory droplets

Pathogenesis: Virulence factors;
- Antiphagocytic capsule
- Endotoxin
- IgA protease

➢ H. *Influenza* → IgA protease → degrades secretory IgA → facilitates attachment to respiratory mucosa.

➢ After becoming established in URT, H. *Influenza* can enter bloodstream (**bacteremia**) & spread to the meninges.

Clinical Finding:
- **Cherry-red epiglottis**
- Fever
- Stiff neck
- Drowsiness
- Headache
- Sinusitis
- Otitis media
- Pneumonia
- **Greenish** sputum
- Opacification of the infected sinus
- Redness with bulging of tympanic membrane

Laboratory Diagnosis:
- Isolation on heated **blood (chocolate) agar** enriched with factor **X** & factor **V**
- Quellung reaction
- Fluorescent antibody staining
- Latex agglutination tests

BORDETELLA *PERTUSSIS*

Disease: Whooping cough (Pertussis)

Important Properties:
- Coccobacillary
- Encapsulated

Transmission: Airborne droplets

Pathogenesis:

B. pertussis → attachment to ciliated epithelium of URT → decreased cilia activity → death of the ciliated epithelial cells

Virulence factors;

Filamentous hemagglutinin	Provide attachment
Pertussis toxin	Stimulate adenylate cyclase
Tracheal cytotoxin	Damages ciliated cells of respiratory tract

Clinical Finding:
- Acute tracheobronchitis
- CNS anoxia
- Leukocytosis
- Severe Paroxysmal cough
- Mild Upper Respiratory Tract symptoms

Laboratory Diagnosis:
- Isolation from **nasopharyngeal** swabs
- Bordet-Gengou medium
- Fluorescent antibody staining
- PCR

LEGIONELLA *PNEUMOPHILIA*

Disease: Pneumonia

Important Properties:
- Gram -ve rods
- Stain poorly with standard Gram stain
- Require increased iron and cysteine for growth in culture

Transmission: Environmental water sources such as air conditioners.

Pathogenesis: Virulence factors; Endotoxin (lipopolysaccharide)

➢ Portal of entry is respiratory tract

➢ Pathological changes primarily seen in lungs.

➢ In severe cases, bacteremia occurs, accompanied by damage to the vascular endothelium in multiple organs, especially in brain & kidney.

Clinical Finding:
- Vary from mild influenza-like symptoms to sever pneumonia
- Scanty & non-purulent cough
- Proteinuria
- Hematuria
- Pontiac fever
- Mental confusion
- Non-bloody diarrhea
- Atypical pneumonia

Laboratory Diagnosis:
- Charcoal yeast agar ⟹ supplemented with iron & yeast
- Silver impregnation stain
- Fluorescent antibody staining
- Urinary antigen

UNIVERSITY QUESTIONS

1. A 60-year-old woman has rapid onset of fever of 39° C & productive cough of greenish sputum. She is not immunocompromised. A chest x-ray reveals a left lobar infiltrate. **[Supple 2009]**
   a) Which **organism** is likely to be isolated on sputum culture? Give its **properties** on Gram staining and culture.
   b) Associated with this organism, give any 2 **virulence factors** and their effects.
2. A 60-year-old man is suffering from Legionnaires' disease after he returns from attending a convention. What is the **organism** involved and what **disease** it causes? **(5) [Supple 2014]**

17 GRAM – NEGATIVE RODS RELATED TO ANIMAL SOURCES

Species	Disease	Source of Human Infection	Mode of Transmission from Animals to Human	Diagnosis
Brucella species	Brucellosis	Pigs, cattle, goats, sheep	Dairy products; Contact with animal tissues	Serology or culture
Francisella tularensis	Tularemia	Rabbits, deer, ticks	Contact with animal tissues; Ticks	Serology
Yersinia pestis	Plague	Rodents	Flea bite	Immunofluorescence or culture
Pasteurella multocida	Cellulitis	Cats, dogs	Cat or dog bite	Wound culture
Bartonella henselae	Cat-scratch disease & bacillary angiomatosis	Cats	Cat scratch or bite; Flea or tick vector	Serology, staining, culture

18 MYCOBACTERIA (Rods)

Important Properties:
- Aerobes
- Acid-fast bacilli
- Retain carbol fuchsin stain
- High lipid content of cell wall

M. TUBERCULOSIS

Disease: Tuberculosis

Important Properties:
- Obligate aerobe
- Grows slowly → (DT - 18h)
- Acid-fast bacilli
- Grow on **Lowenstein-Jensen medium** (egg yolk + Malachite Green Dye)
- Catalase production
- Resistant to acids & alkalis

Cell Wall:

Lipids	Properties
Mycolic acid	1. Lend the organism increased resistance to; ▪ Chemical damage ▪ Dehydration ▪ Antibiotics 2. Allow organism to grow readily inside the macrophages, effectively hiding it from host's immunity. (Arbinogycan layer). 3. Acid fast property.
Phospholipids	Induce caseating necrosis
Wax-D	1. Increase antibody response to antigen. 2. Induce cellular immune response to bacteria.
Phosphatides	Enhance survival of phagocytosed mycobacteria.

Transmission: Respiratory aerosol

Factors predisposing:
▪ Poverty ▪ Over crowding ▪ Poor nutrition ▪ Low socioeconomic status

Pathogenesis: Virulence factors; Cord factor (**Trehalose dimycolate**).
- No exotoxin and endotoxin
- Organism primarily infects macrophages and other reticuloendothelial cells.
- Mycobacterium tuberculosis survives in phagosome
- Organism produces protein (**exported repetitive protein**) that prevent fusion of phagosome with lysosome. ERP.
- Escape from degradative enzymes.

Primary tuberculosis:
- Results in Ghon focus in **lower lobe** of lung.
- Heals by fibrosis
- Can lead to:
  - ✓ Progressive disease → death
  - ✓ Bacteremia
  - ✓ Miliary TB
  - ✓ Hematogenous dissemination (Lymphatic)

Dormant in organism → Reactivation in later life

Secondary tuberculosis:
- Cavity in the apices of upper lobes
- ✓ CNS lesions
- ✓ Vertebral osteomyelitis (Pott's disease)

Proteins:
- Tuberculosis necrotizing toxin (TNT)
- Early secreted antigen-6

Lesions: Lesions dependent on the presence of organism and host response

Exudative lesions: Acute inflammatory response and occur at the initial site of infection

Granulomatous lesion:
- Central caseation necrosis
- Epithelioid cells and Langhan's giant cells containing tubercle bacilli
- A tubercle heals by fibrosis and calcification

Ghon complex: Parenchymal exudative lesion along with draining lymph nodes.

Spread of organism: occurs by two phenomena;

Via tubercle bacilli:
- A tubercle can empty its caseous contents and spread the organism to other parts of lungs
- To GIT, if swallowed
- To other persons, if expectorated

Via bloodstream: Dissemination via bloodstream to internal organs, occurs when;
- Cell mediated immunity fails
- Immunocompromised

Clinical Finding:

Constitutional symptoms;
- Fever
- Fatigue
- Night sweats
- Weight loss

Pulmonary tuberculosis;
- Cough
- Hemoptysis

GIT tuberculosis;
- Abdominal pain
- Intestinal obstruction
- Diarrhea
- Fever
- Hemorrhage
- Weight loss

Oropharyngeal tuberculosis;
- Painless ulcer
- Local adenopathy

Renal tuberculosis;
- Dysuria
- Hematuria
- Flank pain
- Sterile pyuria
- WBCs in urine

Other;
- Scrofula
- Pott's disease
- Miliary TB
- Erythema nodosum
- Tuberculosis meningitis

Laboratory Diagnosis:

- Ziehl-Nelson staining ⟹ Acid-fast bacteria
- Lowenstein-Jensen agar ⟹ specimen treated with NaOH
- Auramine stain ⟹ visualized by fluorescence microscopy
- BACTEC medium ⟹ radioactive metabolites → production of CO_2

- Susceptibility & molecular tests ⟹ detect mutations in chromosomal genes
- Luciferase assay ⟹ detect drug resistant organisms
- Nucleic Acid Amplification test
- PPD skin test
- Interferon-gamma release assay (IGRA)

Tuberculin skin test:

Procedure:
- Antigens in the purified protein derivative injected intradermally on skin of forearm.
- Measure the diameter of induration (swelling) between 48-72 hours.

Interpretation:

Induration of 15 mm or more	No known risk factors
Induration of 10 mm or more	High risk factors
Induration of 5 mm or more	Deficient cell-mediated immunity

Pathogenetic phenomenon: Delayed Hypersensitivity reaction (Type IV)

Advantages of Direct Observed Therapy (DOT):

1. Useful in non-compliance patient.
2. Prevent development of MDR strains
3. Proper protocol of treatment observed by patient.
4. Cost effective.

Multidrug resistance (MDR) Tuberculosis

Development of resistance to at least two most powerful antituberculosis drugs;
- Isoniazid
- Rifampin

Extensively drug resistance (XDR) Tuberculosis

Resistance to isoniazid, rifampin plus any fluoroquinolones and at least one of three injectable second line drugs.

ATYPICAL MYCOBACTERIA

Mycobacteria other than tuberculosis (MOTTS)

- They are called atypical because they differ in certain aspects from prototype Mycobacterium tuberculosis.

Runyon classification: Basis;
- Rate of growth
- Production of pigment

Group	Growth rate	Pigment	Typical Species
I	Slow	yellow orange pigmented colony in light (photochromogens)	• *Mycobacterium kansasii* → lung disease similar to Mycobacterium tuberculosis • *Mycobacterium marinum* → swimming pool granuloma

II	Slow	pigment in dark (scotochromogen)	• **Mycobacterium *scrofulaceum*** → scrofula; granulomatous cervical lymphadenitis in children
III	Slow	little or no pigment irrespective of light or dark (non-chromogens)	• **Mycobacterium avium-intracellulare complex** → pulmonary disease in immunocompromised persons
IV	Rapid	-	• **Mycobacterium *fortuitum-chelonae* complex** → Importance in Immunocompromised patients & patients with prosthetic hip joints and indwelling catheters • **Mycobacterium *abscessus*** → Chronic lung infections & Infections of skin, bones and joints • **Mycobacterium *smegmatis*** → Part of normal flora of smegma

M. *LAPRAE*

Disease: Leprosy (Hansen's disease)

Important Properties:

- Not grown in laboratory
- Weak Acid-Fast Bacilli
- Optimum temperature for growth is 30ºC
- Grows in skin and superficial nerves

Transmission: Prolonged contact with patients of lepromatous leprosy

Pathogenesis:

- Organism replicates intracellularly with in;
  - Skin histiocytes
  - Endothelial cells
  - Schwann cells of nerves
- Nerve damage in leprosy is result of two processes
  - Damage caused by direct contact with the bacterium
  - Damage caused by cell mediated immune attack on nerves

Clinical Finding:

Tuberculoid leprosy:

- Hypopigmented macular or plaque-like skin lesions
- Thickened superficial nerves
- Loss of sensations in skin lesions

Lepromatous leprosy:

- Multiple nodular skin lesions
- Typical leonine (lionlike) facies
- Erythema nodosum lerposum

Forms of leprosy:

Feature	Tuberculoid leprosy (Paucibacillary)	Lepromatous leprosy (Multibacillary)
Type of lesion	One to five lesions with little tissue destruction, hypopigmented	More than five lesions with marked tissue destruction
Distribution	Asymmetric	Symmetrical
Nerve trunk	Only one nerve trunk	Many nerve trunks
Number of acid-fast bacilli	Few	Many
Likelihood of transmitting leprosy	Low	High
Cell mediated response to M leprae	Present	Reduced to present
Lepromin skin test	Positive	Negative
Type of IFN	Produce IFN gamma	Produce IFN beta

Laboratory Diagnosis:

- Diagnosis is usually on clinical signs and symptoms
- Specimen ⇒ Skin or nasal scrapings
- ZN stain ⇒ Lipid laden macrophages containing AFB
- Serology ⇒ IgM antibody in lepromatous type
- PCR

UNIVERSITY QUESTIONS

1. At present, TB is a global emergency acrid to WHO. There are 3 million cases in Pakistan.
   a) Mention 4 **factors** contributing to this recent disease.
   b) Mention 3 advantages of **Direct Observed Therapy (DOT)**. **[Annual 2007]**
2. M. tuberculosis has a complex cell wall conferring many properties to the organism.
   a) Why is heating required in the staining process of **ZN stain** method?
   b) Name 4 complex **lipids** and **properties** they confer to M. tuberculosis. **[Annual 2008]**
3. Each year 3 million people die of tuberculosis and 8 million new cases occur. Approximately one third of world's population is infected by Mycobacterium tuberculosis.
   a) Enumerate any 3 **components** of cell wall of Mycobacterium tuberculosis and give their **functions**.
   b) What do you understand by Multi-drug resistant (**MDR**) strains of M. tuberculosis? how do they **develop**? **[Supple 2009]**
4. A 32-year-old woman from Sindh presents to dermatologist with the history of 'white spots' on her body. PE reveals multiple, asymmetrically distributed, circular, hypopigmented lesions on her body. The lesions are sharply demarcated with raised erythematous borders and atrophic scaly centers. The lesions are anesthetic and hairless. Biopsy of lesion reveals granuloma formation within nerves. **[Annual 2009]**
   a) What is likely **diagnosis** and causative organism? Give its 3 **characteristics**.
   b) How does this patient's condition **differ** from the more serious 'distinct form of disease'?
5. A 37-year-old women presents with a two-month history of progressive cough, weight loss and fever. X-ray chest shows bilateral cavitary disease suggestive of tuberculosis. Sputum culture grows an acid-fast bacillus that is photochromogen (makes an orange pigment when exposed to light).
   a) I) Name mycobacterium other than tuberculosis producing such lesion. **(1)**

ii) Name another species of **photochromogens** with the lesion caused by it. (1)

b) How would you perform and interpret tuberculin skin test? **(3) [Supple 2010]**

6. A 34-year-old male arrived at the local health clinic, complaining of fever and weight loss greater than 10% of his body weight in the last month. He also had a cough that produced rust-colored sputum. The physician orders for X-ray chest, sputum examination and a tuberculin test. He was living with a roommate positive for tuberculosis 6 months ago. **[Annual 2010]**

a) Based on the symptoms and laboratory results, which infectious **disease** does the patient suffer from? What is the **agent**? **(2)**

b) What is **tuberculin skin test**? **(3)**

7. A 25-year-old female presents to the gynecology ward for work up on her infertility. Pelvic ultrasound & hysterosalpingography revealed bilateral fallopian tube blockade, small nodules on pelvic peritoneum. A laparoscopic biopsy was taken from peritoneal nodules. It showed caseating granulomatous lesion on histological exam. **[Supple 2011]**

a) What is the possible **cause** of her infertility based on histological results in biopsy?

b) What are the different **lab** investigations available for confirming its diagnosis?

8. A 20-year-old male presented with a history of low-grade fever with evening rise for the past few months, loss of weight and cough with expectoration. X-ray revealed a cavitary lesion in the apex of right lung. **[Annual 2011]**

a) Which **test** can be performed to screen this patient for tuberculosis? **(1)**

b) Describe the **procedure**, **interpretation** and underlying pathogenetic **phenomenon** in case of a positive test for tuberculosis. **(1+1+2)**

9. A 55-year-old man as 5-month history of productive weakness and weight loss. There is history of low-grade fever with evening rise in, night sweats and productive cough. X-ray chest reveals pulmonary opacities due to granuloma formation. A sputum specimen when examined contain numerous acid-fast bacilli (AFB).

a) What is the most probably **diagnosis**? The patient is most likely to be isolated from which **organism**?

b) Why certain mycobacteria are known as '**atypical**'? What is the basis for their **classification**? Classify them. **(2+3) [Supple 2013]**

10. A 60-year-old laborer presents with history of chronic productive cough with occasional bouts of hemoptysis. Chest X-ray reveals a cavitating lesion in the apical region of right lung. Considering it to be a mycobacterial lung infection, a skin test was carried out to support the diagnosis which was strongly positive.

a) What is the **technique** and **interpretation** of skin test? **(2.5)**

b) Describe the cultural **characteristics** of this organism. **(2.5) [Annual 2012]**

11. An 18-year-old boy presented in medical OPD with low grade intermittent fever for last several months. He also has malaise, anorexia, history of weight loss and night sweats. On examination swelling of 2-3 cm on right side of neck is visible. His recent investigations showed Hb: 9.5 g/dl ESR: 100 mm/hr. **[Annual 2014]**

a) What is your **diagnosis**?

b) Name its causative **agent** and also enlist tis other **types**.

c) What are the **investigations** used to diagnose this condition?

12. Write a short note on **tuberculin skin test**. **[Supple 2015]**

13.a) A 10-year-old child has a primary pulmonary infection with Mycobacterium tuberculosis. What would be the features of tuberculosis in this child? What is the characteristic lesion in the lymph nodes called? **(2)**

b) Enumerate the constituents of mycobacterial **cell wall.** Give the **importance** of each constituent. **(3) [Annual 2015]**

14. Multi-drug resistant strain of Mycobacterium tuberculosis (MTB) is diagnosed in sanitary worker of a public hospital. **[Supple 2016]**

a) What is **MDR** strain of Mycobacterium tuberculosis? Enlist four **clinical manifestations** associated with MTB strains. **(2)**

b) What are the different **laboratory** investigations available for confirming this diagnosis? **(2)**

15. A 34 years old male complains of cough with rusty colored sputum and low-grade evening fever. His chest X-ray reveals opacity on right upper lobe of lung. Routine serum culture reveals normal throat flora. However, acid fast bacilli were seen on ZN smear. **[Annual 2016]**

a) Briefly discuss two types of **lesions** produced by this organism. **(2)**

b) What are **MDR** (Multi-drug resistant) strains of Mycobacterium tuberculosis? **(1)**

16. An adult male is presenting with hypopigmented patches on forearm and partial loss of cutaneous sensation in affected areas, presence of thickened ulnar nerve and presence of acid-fast bacilli (AFB) in nasal smears. **[Supple 2016 held in 2017]**

a) What is your **diagnosis**? What is the causative **organism**? **(0.5+0.5)**

b) Name the **laboratory** diagnostic methods used for detecting Mycobacterium Tuberculosis. **(4)**

17. A 68 years old retired school teacher was diagnosed as a case of primary tuberculosis on the basis of a strongly positive tuberculin skin test and cavitation found on the apical region of his left lung on chest X-ray. **[Annual 2017]**

a) What is the **technique** and **interpretation** of tuberculin skin test? **(2)**

b) Describe the cultural **characteristics** of Mycobacterium Tuberculosis. **(2)**

c) Name **atypical** mycobacteria. **(1)**

18. A Pakistani American man visited Pakistan for the first time at 18 years of age. E is living with his grandmother who is on anti-tuberculosis therapy for three weeks. He has not been vaccinated with BCG. If he acquires infection from his grandmother, **[Supple 2017 held in 2018]**

a) Give the **pathogenesis** and possible **outcomes** of infection. **(3)**

b) Name two **tests** used to diagnose latent tuberculosis. **(2)**

19. A 34-year-old male presented in OPD with complaints of fever and weight loss over the last two months. He had cough that produced rust color sputum. He was living with roommate positive for tuberculosis about six months ago. **[Annual 2018]**

a) Depending on the host response, describe the two types of **lesions** produced by this organism. **(2)**

b) What are different **lab** investigations available for confirming the diagnosis? **(3)**

20. A 30-year-old destitute male has cough with blood-streaked sputum and 30° C temperature for the last one month. Sputum culture reveals acid fast bacilli and on culture buff-colored colonies are found after 21 days. **[Supple 2018 held in 2019]**

a) Briefly describe the **clinical spectrum of organs** involved in this condition.

b) In a tabulated form, **compare** tuberculoid from lepromatous leprosy.

21. An old sanitary worker presents with history of fatigue, low-grade fever, night sweats & weight loss. X-ray chest shows ill-defined nodular opacities in the right lung. ZN smear of sputum is strongly positive for acid-fast bacilli indicating presence of *Mycobacterium tuberculosis*.

a) What **laboratory investigations** would you carry out for confirmation of this diagnosis. **(2)**

b) What is the **interpretation** of positive tuberculin? **(1)**

c) Define **MDR** & **XDR** (drug resistance) that can be encountered in patients suffering from tuberculosis. **(2) [Annual 2019]**

22. A 54-year-old male has five months history of progressive weakness, low-grade fever & weight loss. X-ray chest shows pulmonary opacities. ZN smear of sputum is strongly positive for acid-fast bacilli & culture confirms the presence of *Mycobacterium tuberculosis*.

a) What **features** make the acid-fast? Briefly discuss the two types of **lesions** produced by this organism. **(1+2)**

b) Define Multi drug resistant (**MDR**) & extensively drug resistant (**XDR**) strains of *Mycobacterium tuberculosis*. **(2)**

19 ACTINOMYCETES

ACTINOMYCES *ISRAELII*

Disease: Actinomycosis

Important Properties:
- Gram +ve
- Anaerobe

Normal Flora: Oral cavity

Transmission: **Dental disease** or Trauma

Pathogenesis: Broken jaw or dental extraction → Invasion of tissues → Formation of filaments surrounded by areas of inflammation

Clinical Finding:
- Hard, non-tender swelling that drains pus through sinus tracts
- Hard, yellow granules (**sulfur granules**) composed of a mass of filaments

Laboratory Diagnosis:
- Gram stained smear ⟹ Gram +ve branching rods
- Presence of sulfur granules
- Blood agar ⟹ growth of culture under anaerobic condition
- Immunofluorescence

NOCARDIA *ASTEROIDES*

Disease: Nocardiosis

Important Properties:
- Aerobes
- Weakly acid-fast

Clinical Finding:
- Lung abscess with cavity formation
- Lung nodules
- Pneumonia
- Empyema
- Brain abscess

Laboratory Diagnosis:
- Gram stained smear ⟹ Gram +ve branching filamentous rods
- Modified Ziehl-Nelson staining ⟹ **Weakly acid-fast bacteria**
- Blood agar ⟹ growth of culture under aerobic condition

UNIVERSITY QUESTIONS

1. A microbiologist received an improperly labelled sample that resembles some kind of fluid and source is unknown. Gram staining reveals that the microorganism in fluid is filamentous, gram +ve rod, forming long branching filaments. **[Annual 2009]**
   a) What are the 2 most likely bacterial **microorganisms**?
   b) How are they microorganisms differentiated in **lab**?
   c) If sample was drained from **oral abscess**, which 2 organisms are most likely and why?

20 MYCOPLASMAS

Mycoplasma *pneumoniae*

Disease: Atypical pneumonia

Important Properties:
- Smallest free-living organisms
- Absence of cell wall
- Colony of M. *pneumoniae* has characteristic **fried-egg** shape
- Stain poorly with Gram-stain
- Cell membrane contain cholesterol

Transmission: Respiratory droplets

Pathogenesis:
- In the lungs, the organism is rod-shaped, with a **tapered tip** that contains specific proteins that serve as the point of **attachment** to the respiratory epithelium.
- The respiratory mucosa is not invaded, but **ciliary motion** is inhibited and **necrosis** of the epithelium occurs.
- It produces **hydrogen peroxide**, which contributes to the damage to the respiratory tract cells.
- During M. *pneumoniae* infection, **autoantibodies** are produced against red cells (**cold agglutinins**) and brain, lung, and liver cells.
- These antibodies may be involved in some of the extrapulmonary manifestations of infection.

Clinical Finding:

Atypical pneumonia:
- Non-productive cough
- Whitish non-bloody sputum
- Sore throat
- Fever
- Headache
- Malaise
- Myalgias
- Ear ache

Extrapulmonary manifestations:
- Stevens Johnson syndrome
- Raynaud's phenomenon
- Cardiac arrhythmias
- Erythema multiforme
- Arthralgias
- Hemolytic anemia

Neurologic manifestations: Guillain Barre syndrome

Laboratory Diagnosis:
- Cold Agglutination Test ⟹ **Cold agglutinins are IgM autoantibodies** against RBCs that agglutinate these cells at 4°C.

UNIVERSITY QUESTIONS

1. Tabulate differences between Mycoplasma, Chlamydia and Rickettsia. **[Supple 2013]**
2. A 50-year-old male presented with the history of fever and bilateral diffused interstitial pneumonia. He has history of renal transplantation two months back for end stage renal disease. What is most likely **pathogen** & **disease** it causes? **[Supple 2014]**

21 SPIROCHETES

- Motile
- Thin walled
- Flexible
- Spiral

TREPONEMA *PALLIDUM*

Disease: Syphilis

Important Properties:
- Not seen on Gram-stained smear
- Not cultured in vitro

Transmission:
- Sexual contact
- From mother to fetus across the placenta

Pathogenesis:
- Treponema *pallidum* produces no important toxins or enzymes.
- The organism often infects the endothelium of small blood vessels, causing endarteritis.

Clinical Finding:

Primary syphilis	The spirochetes multiply at the site of inoculation. A local, nontender ulcer (**chancre**) usually forms in 2 to 10 weeks. The ulcer heals spontaneously. But spirochetes spread widely via the bloodstream to many organs.
Secondary syphilis	May occur after one to 3 months later These lesions often appear as **maculopapular rash**, on the palms and soles, or as moist papules on skin & mucous membranes (mucous patches). Moist lesions on the genitals are called **condylomata lata.** Patchy alopecia also occurs. Sometimes latency is seen Constitutional symptoms; • Low-grade fever • Weight loss • Generalized lymphadenopathy • Pharyngitis • Malaise • Headache • Meningitis • Nephritis • Anorexia • Myalgias • Hepatitis
Tertiary syphilis	Show granulomas (**gummas**), especially of skin and bones CNS involvement, also known as neurosyphilis (e.g., tabes, paresis) Cardiovascular lesions (e.g., aortitis, aneurysm of the ascending aorta).
Congenital syphilis	The organism is transmitted across the placenta, fetal infection can occur. In the infected neonates, skin and bone lesions are common. • hepatosplenomegaly • Interstitial keratitis • **eighth nerve deafness** Fetal infection can also result in stillbirth.

Laboratory Diagnosis:
- **Dark field microscopy**
- Direct fluorescent antibody (DFA)
- Silver stain
- Serologic tests
  - Nonspecific tests ⟹ VDRL & RPR
  - Specific test ⟹ FTA-ABS

BORRELIA *BURGDOFERI*

Disease: Lyme disease
Main reservoir: White-footed mouse
Transmission: Tick bites
Pathogenesis:
- Organism invades skin, causing a rash called **erythema migrans.**
- It then spreads via the bloodstream to involve primarily the heart, joints, and CNS.

Clinical Finding:
- Erythema migrans
- Flu-like symptoms
- Macular rash (**bulls eye** appearance)

Laboratory Diagnosis:
- Serologic tests ⟹ Detecting IgM antibody
- PCR test
- Western Blot assay

LEPTOSPIRA *INTERROGANS*

Disease: Leptospirosis
Important Properties: Tightly coiled spirochetes
Main reservoir: Wild & domestic animals
Transmission: Via animal urine
Pathogenesis:
- Human infection results when leptospiras are **ingested** or pass-through mucous membranes or skin.
- They **circulate** in the blood and **multiply** in various organs.
- The illness is typically **biphasic**; an initial bacteremic phase and a subsequent immunopathologic phase with meningitis.

Clinical Finding:
- Fever
- Chills
- Aseptic meningitis
- Impaired kidney function
- Jaundice
- Intense headache

Laboratory Diagnosis:
- Dark field microscopy
- Serologic tests ⟹ Rise in IgM antibody titers

UNIVERSITY QUESTIONS

1. a) **Classify** spirochetes in 3 genera.
   b) Name the **organisms** and **disease** caused by each species of genera. **[Annual 2007]**
2. A 50-year-old man presents with a spreading, non-pruritic, painless, circular red rash with a clear center at the tick-bite site. The rash was accompanied by non-specific 'flu-like' symptoms such as fever, chills, fatigue and headache. He was treated and cured in a few days. Few weeks later, the patient presented with myocarditis, third degree heart block and Bell's palsy.
   a) What is the most probable **diagnosis**? **(2)**
   b) How will you diagnose the case in **laboratory**? **(3) [Annual 2013]**

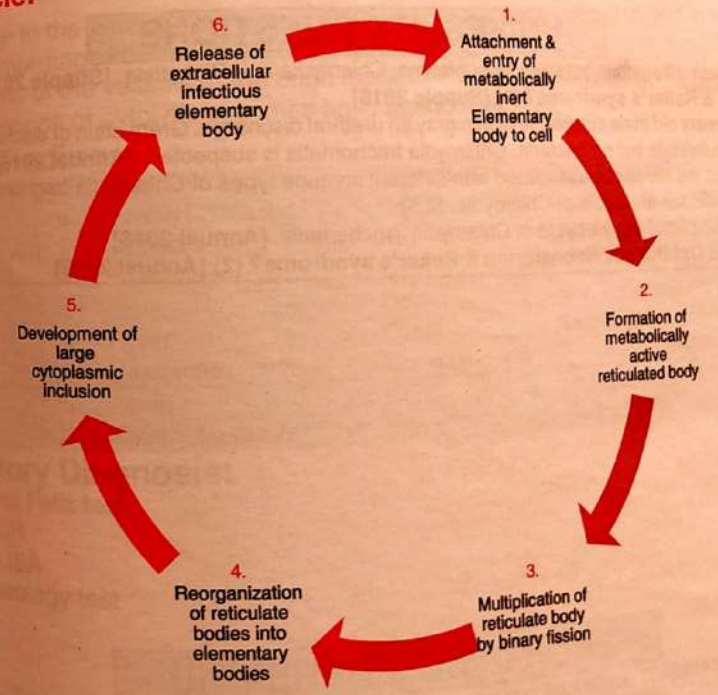
22 CHLAMYDIA

Species	Diseases	Transmission
C. trachomatis	• Eye infections • Respiratory infections • Reiter's syndrome • Genital tract infections	Sexual contact; Perinatal transmission
C. pneumoniae	Atypical pneumonia	Respiratory droplets
C. psittaci	Psittacosis	Inhalation of bird feces

Important Properties:

- Obligate intracellular bacteria
- Resembles gram -ve bacteria
- Lack muramic acid

Life cycle:



Pathogenesis: Infect the **epithelial cells** of the mucous membranes or the lungs.

Immune types of Chlamydia trachomatis: 15 immunotypes (A-L)

A, B, C	Trachoma (Chronic conjunctivitis)
D-K	Genital Tract Infections In males; Non-Gonococcal Urethritis (dysuria, non-purulent urethral discharge), may progress to epididymitis or prostatitis In females; Salpingitis & PID

Reiter's syndrome: characterized by Urethritis, Uveitis & Arthritis.

Laboratory Diagnosis:

- Cytoplasmic inclusion bodies ⟹ detected by GIEMSA stain or immunofluorescence.
- GIEMSA stain ⟹ Neutrophils in urethral discharge
- Nucleic Acid Amplification test
- ELISA

UNIVERSITY QUESTIONS

1. Tabulate differences between Mycoplasma, Chlamydia and Rickettsia. **[Supple 2013]**
2. What is **Reiter's syndrome? (1) [Supple 2016]**
3. A 24 years old male complains of thin grayish urethral discharge. Gram stain of discharge reveal many neutrophils but no bacteria. Chlamydia trachomatis is suspected. **[Annual 2016]**
   a) Enlist the **diseases** associated with different immune types of Chlamydia trachomatis. **(1.5)**
   b) Explain the **life cycle** of Chlamydia. **(2.5)**
4. Draw and label the **lifecycle** of Chlamydia Trachomatis. **[Annual 2018]**
5. What is **Ophthalmic Neonatorum & Reiter's syndrome? (2) [Annual 2019]**

23 RICKETTSIAE

Organism	Disease	Arthropod Vector
Rickettsia rickettsii	Rocky Mountain spotted fever	Dog ticks
Rickettsia prowazekii	Typhus	Human body louse
Coxiella burnetii	Q fever	-

Important Properties:

- Obligate intracellular bacteria
- Resembles gram -ve bacteria
- Very short rods cell
- Divide by binary fission

Pathogenesis:

- The typical lesion is a **vasculitis,** particularly in the endothelial lining of the vessel wall.
- Damage to the vessels of the skin results in the characteristic rash and in edema and hemorrhage caused by increased capillary permeability.
- Vasculitis of the vessels in the brain leads to the prominent headache.
- No exotoxins or cytolytic enzymes have been found.

Clinical Finding:

Rocky Mountain spotted fever	• Fever • Severe headache • Myalgias • Prostration	• Delirium • Coma • DIC • Edema	• Circulatory collapse • Typical rash, begins with macules that progress to petechiae
Typhus	• Chills • Fever • Headache	• Delirium • Coma • Collapse	• Maculopapular rash • Severe meningoencephalitis • Peripheral vascular
Q fever	• Fever • Severe headache	• Cough • Pneumonia	• Hepatitis • Endocarditis

Laboratory Diagnosis:

- Weil Felix test
- PCR
- ELISA
- Serology test

UNIVERSITY QUESTIONS

1. Tabulate differences between Mycoplasma, Chlamydia and Rickettsia. **[Supple 2013]**

Basic Virology

24 VIRUS

Viruses are not cells; they are not capable of independent replication, can synthesize neither their own energy nor their own proteins, and are too small to be seen in the light microscope.

Characteristics:

1. Viruses are particles composed of
   - an internal **core**
   - containing ***either*** DNA ***or*** RNA (but not both)
   - covered by a **protective protein coat**
   - some viruses have an outer lipoprotein membrane, called an **envelope**, external to the coat
   - Viruses do not have a nucleus, cytoplasm, mitochondria, or ribosomes.
2. Viruses must reproduce (replicate) within cells, because they cannot generate energy or synthesize proteins.
   - Because they can reproduce only within cells, viruses are **obligate intracellular parasites**.
3. Viruses replicate in a manner different from that of cells (i.e., viruses do not undergo binary fission or mitosis). One virus can replicate to produce hundreds of progeny viruses.

Comparison of Viruses and Cells

Property	Viruses	Cells
1. Type of nucleic acid	DNA or RNA but not both	DNA and RNA
2. Proteins	Few	Many
3. Lipoprotein membrane	Envelope present in some viruses	Cell membrane present in all cells
4. Ribosomes	Absent	Present
5. Mitochondria	Absent	Present in eukaryotic cells but not in prokaryotic cells
6. Enzymes	None or few	Many
7. Multiplication by binary fission or mitosis	No	Yes

25 STRUCTURE

Virus Size & Structure:

- Viruses range in size from that of large proteins (~20 nm) to that of the smallest cells (~300 nm).
- Most viruses appear as **spheres** or **rods** in the electron microscope.
- All viruses have a **protein coat called a capsid** that covers the genome.
- The capsid is composed of repeating subunits called **capsomers**.
- In some viruses, the capsid is the outer surface, but in other viruses, the capsid is covered with a lipoprotein **envelope** that becomes the outer surface.
- The structure composed of the nucleic acid genome and the capsid proteins is called the **nucleocapsid**.

Icosahedral symmetry	Either enveloped or naked	Poliovirus
Helical nucleocapsid	Enveloped	Rabies virus

Viral Nucleic Acids:

- The genome of some viruses is DNA, whereas the genome of others is RNA.
- These DNA and RNA genomes can be either **single-stranded** or **double-stranded**.
- Some RNA viruses, such as influenza virus and rotavirus, have a **segmented genome** (i.e., the genome is in several pieces).
- All viruses have one copy of their genome (haploid) except **retroviruses**, which have two copies (diploid).

Viral Proteins:

- Viral surface proteins mediate **attachment to host cell receptors**.
- This interaction determines **the host specificity and organ specificity** of the virus.
- The surface proteins are the **targets of antibody**.
- Viruses also have internal proteins, some of which are **DNA** or **RNA polymerases**.
- The **matrix protein** mediates the interaction between the viral nucleocapsid proteins and the envelope proteins.
- Some viruses produce **antigenic variants** of their surface proteins that allow the viruses to evade our host defenses.

Viral Envelope

The viral **envelope** consists of a membrane that contains lipid, derived from the host cell and proteins encoded by the virus.

Naked viruses	Enveloped viruses
Survive longer in the environment	Less stable (i.e., they are more easily inactivated)
Transmitted by indirect means such as the fecal–oral route.	Transmitted by direct contact via blood and body fluids.

Prions:

- **Prions** are infectious particles composed **entirely of protein**.
- They have **no DNA or RNA**.
- They cause diseases such as **Creutzfeldt-Jakob disease** and **kuru** in humans.
- These diseases are called transmissible **spongiform encephalopathies**.
- The term **spongiform** refers to the **spongelike appearance** of the brain seen in these diseases.
- Prion proteins are **encoded by a cellular gene**.
- When these proteins are in the normal, **alpha-helix configuration**, they are nonpathogenic.
- But when their configuration changes to a **beta-pleated sheet**, they aggregate into **filaments**, which disrupts **neuronal function** and results in the symptoms of disease.
- Prions are **highly resistant** to inactivation by ultraviolet light, heat, and other inactivating agents.
- Because they are **normal human proteins**, they do not elicit an inflammatory response or an antibody response in humans.

Comparison of Viruses & Prions

Viruses	Prions
An infective agent that typically consists of a nucleic acid molecule in a protein coat and is able to multiply only within the living cells of a host.	An infectious protein particle similar to a virus but lacking nucleic acid; thought to be the agent responsible for the degenerative diseases of nervous system.
Made up of nucleic acid molecule covered by a protein coat.	Made up of only proteins.
Can cause a variety of infections.	Mainly cause neurodegenerative diseases.
Inactivated rapidly by ultraviolet light or heat.	Resistant
Induces antibody.	Does not induce antibody.
Induces inflammation.	Does not induce inflammation.

UNIVERSITY QUESTIONS

1. a) What is the name given to the study of the most common infectious agents?
   b) Give three properties of the infectious agent which make it different from bacteria.
   c) Name infectious particle smaller than this particle. **[Annual 2008]**
2. Give two differences between viruses and bacteria. **[Annual 2010]**
3. a) What are the two forms of viral nucleocapsid **symmetries**? Give at least one **example** of each.
   b) Compare viruses and cells. **[Annual 2010]**
4. Draw and label the **structure** of an enveloped RNA virus with icosahedral symmetry. **[Supple 2015]**

26 REPLICATION

Stages of the Viral Growth Cycle:

Attachment and penetration by parental virion
↓
Uncoating of the viral genome
↓
Early viral mRNA synthesis
↓
Early viral protein synthesis
↓
Viral genome replication
↓
Late viral mRNA synthesis
↓
Late viral protein synthesis
↓
Progeny virion assembly
↓
Virion release from cell

Viral Growth Curve:

The **eclipse period** is the time when no virus particles are detected within the infected cell. It occurs soon after the cell is infected.

Cytopathic effect (CPE) is the term used to describe the damage, both morphologic and functional, inflicted on the cell by the virus. In the clinical laboratory, the presence of a virus in the patient's specimen is often detected by seeing a CPE in cell culture.

Polarity of viral genome RNA:

Positive polarity RNA	Genome RNA that has the same base sequence as the mRNA	No need of polymerase for translation except retroviruses.
Negative polarity RNA	Genome RNA that has a base sequence complementary to mRNA	Must have an RNA polymerase for translation.

Lysogeny:

Lysogeny	Process by which **viral DNA becomes integrated into host cell DNA**, replication stops, and no progeny virus is made. The integrated viral DNA is called a **prophage**.
Transduction	Process by which viruses carry genes from one cell to another.
Lysogenic conversion	Term used to indicate that the cell has acquired a new trait as a result of the integrated prophage.

27 CLASSIFICATION OF MEDICALLY IMPORTANT VIRUSES

Classification of RNA Viruses

Class	Example
Picornavirus	• Poliovirus • Rhinovirus • Hepatitis A virus
Hepevirus	• Hepatitis E virus
Calicivirus	• Norovirus
Reovirus	• Rotavirus
Flavivirus	• Yellow fever virus • Dengue virus • West Nile virus, • Hepatitis C virus
Togavirus	• Rubella virus
Retrovirus	• HIV • Human T-cell leukemia virus
Orthomyxovirus	• Influenza virus
Paramyxovirus	• Measles virus • Mumps virus • Respiratory syncytial virus
Rhabdovirus	• Rabies virus
Filovirus	• Ebola virus • Marburg virus
Coronavirus	• Corona Virus
Arenavirus	• Lassa fever virus • Lymphocytic choriomeningitis virus
Bunyavirus	• California encephalitis virus • Hantavirus
Delta virus	• Hepatitis delta virus

Classification of DNA Viruses

Class	Example
Parvovirus	• B19 virus
Polyomavirus	• JC virus • BK virus
Papillomavirus	• Human papillomavirus
Adenovirus	• Adenovirus
Hepadnavirus	• Hepatitis B virus
Herpesvirus	• Herpes simplex virus • Varicella zoster virus • Cytomegalovirus • Epstein–Barr virus
Poxvirus	• Smallpox virus • Molluscum • Contagiosum virus

UNIVERSITY QUESTIONS

1. Give four names of DNA and RNA viruses with example of one disease caused by each. **[Annual 2010]**

28 PATHOGENESIS

The Infected Cell:

- Death of infected cells is probably caused by inhibition of cellular protein synthesis.
- Translation of viral mRNA into viral proteins pre-empts the ribosomes preventing synthesis of cellular proteins.
- **Inclusion bodies** are aggregates of virions in specific locations in the cell that are useful for laboratory diagnosis.
- Two important examples are:
  - **Negri bodies** in the cytoplasm of rabies virus-infected cells.
  - **Owl's eye inclusions** in the nucleus of cytomegalovirus-infected cells.
- **Multinucleated giant cells** form when cells are infected with certain viruses, notably herpesviruses and paramyxoviruses such as respiratory syncytial virus.

The Infected Patient:

- Viral infection in the person typically has **four stages**:
  1. Incubation period
  2. Prodromal period
  3. Specific-illness period
  4. Recovery period
- The main **portals of entry** are the respiratory, gastrointestinal, and genital tracts, but through the skin, across the placenta, and via blood are important as well.
- Transmission from mother to offspring is called **vertical transmission.** (rubella, Cytomegalovirus, herpes simplex virus-2)
- All other modes of transmission (e.g., fecal–oral, respiratory aerosol, insect bite) are **horizontal transmission.**
- Most serious viral infection are systemic (i.e., the virus travels from the portal of entry via the blood to various organs).
- However, some are localized to the portal of entry, such as the common cold, which involves only the upper respiratory tract.

Pathogenesis:

Mechanism	Example
Death of the infected cells and a consequent loss of function.	**Poliovirus** kills neurons, resulting in paralysis.
Killing of virus-infected cell by the attack of cytotoxic T cells that recognize viral antigens on the cell surface.	Damage to the liver caused by **hepatitis viruses.**
Formation of virus–antibody complexes that are deposited in tissues.	Arthritis associated with **parvovirus B19** or **rubella virus** infection.

- **Immunopathogenesis** is the process by which the symptoms of viral diseases are caused by the immune system rather than by the killing of cells directly by the virus.
- Viruses can evade host defenses by producing **multiple antigens**, thereby avoiding inactivation by antibodies, and by **reducing the synthesis of class I MHC proteins**, thereby decreasing the ability of a cell to present viral antigens and blunting the ability of cytotoxic T cells to kill the virus-infected cells.
- Viruses also produce receptors for immune mediators, such as IL-1 and TNF, thereby preventing the ability of these mediators to activate antiviral processes.

Persistent Viral Infections:

Carrier state	People who produce virus for long periods of time and can serve as a source of infection for others.	• Hepatitis C virus
Latent infections	Infections that are not producing virus at the present time but can be reactivated at a subsequent time.	• Herpes simplex virus
Slow virus infections	Diseases with a long incubation period, often measured in years	• Creutzfeldt-Jakob disease

UNIVERSITY QUESTIONS

1. Name viruses that can be **vertically transmitted**. **[Annual 2014]**

29 HOST DEFENSES

Interferons

- Viruses and double-stranded RNA are the **most potent inducers** of interferons.
- Many viruses induce interferons, and many viruses are inhibited by interferons.
- Interferons act by binding to a receptor on the cell surface that signals the cell to **synthesize ribonuclease**, **protein kinase**, and **oligo A synthetase** in an inactive form.
- Double-stranded RNA made by the infecting virus activates these proteins.
- Interferons inhibit virus replication by blocking protein synthesis, primarily by **degrading mRNA** and by **inactivating elongation factor-2**.
- Alpha and beta interferons have a **stronger antiviral action** than gamma interferon.
- Gamma interferon acts primarily as an **interleukin** that activates macrophages.

Other Nonspecific Defenses

- **Natural killer (NK) cells** are lymphocytes that destroy cells infected by many different viruses (i.e., they are **non-specific**).
  - NK cells do not have an antigen receptor on their surface.
  - NK cells recognize and destroy cells that do not display class I MHC proteins on the surface.
  - They kill cells by the same mechanisms as do cytotoxic T cells.
- Phagocytosis by **macrophages** and the clearance of mucus by the cilia of the respiratory tract are also important defenses.
- **Increased corticosteroid levels** suppress various host defenses and predispose to severe viral infections.
- **Malnutrition** predisposes to severe **measles infections** in developing countries.

Specific Defenses

- **Active immunity** to viral infection is mediated by **both antibodies and cytotoxic T cells**. It can be elicited either by exposure to the virus or by immunization with a viral vaccine.
- **Passive immunity** consists of antibodies preformed in another person or animal.
- The duration of active immunity is much longer than that of passive immunity.
- Passive immunity is effective immediately, whereas it takes active immunity 7 to 10 days in the primary response (or 3–5 days in the secondary response) to stimulate detectable amounts of antibody.
- **Herd immunity** is the protection of an individual that results from immunity in many other members of the population (the herd) that interrupts transmission of the virus to the individual.

30 LABORATORY DIAGNOSIS

1. Identification in Cell Culture

- The presence of a virus in a patient's specimen can be detected by seeing a **"cytopathic effect" (CPE)** in cell culture
- A specific identification of the virus usually involves an antibody-based test such as fluorescent antibody, complement fixation, or enzyme-linked immunosorbent assay (ELISA).

2. Microscopic Identification

- ✓ **Inclusion bodies**, formed by aggregates of many virus particles, can be seen in either the nucleus or cytoplasm of infected cells.
- ✓ **Multinucleated giant cells** are formed by several viruses, notably certain herpes viruses, respiratory syncytial virus, and measles virus.
- ✓ Fluorescent antibody staining of cells obtained from the patient or of cells infected in culture can provide a rapid, specific diagnosis.
- ✓ Electron microscopy is not often used in clinical diagnosis but is useful in the diagnosis of certain viruses, such as Ebola virus.

3. Serologic Procedures

- The **presence of IgM** can be used to diagnose current infection.
- The **presence of IgG** cannot be used to diagnose current infection because the antibody may be due to an **infection in the past**.
- As a result, an acute and convalescent serum sample should be analyzed.
- An antibody titer that is fourfold or greater in the convalescent serum sample compared with the acute sample can be used to make a diagnosis.

4. Detection of Viral Antigens & Nucleic Acids

- ❖ The **presence of viral proteins**, such as p24 of HIV and hepatitis B surface antigen, is commonly used in diagnosis.
- ❖ The **presence of viral DNA or RNA** is increasingly becoming the "gold standard" in viral diagnosis.
- ❖ Labeled probes are highly specific, and results are rapidly obtained. Small amounts of viral nucleic acids can be amplified using reverse transcriptase to produce amounts detectable by the probes.
- ❖ An important example is the **"viral load" assay of HIV RNA**.

UNIVERSITY QUESTIONS

1. Enlist five approaches to the **diagnosis** of viral diseases by the use of any clinical specimens. **(2.5) [Annual 2016]**

Clinical Virology

31 DNA ENVELOPED VIRUSES

HERPES VIRUSES

These viruses are noted for their ability to cause **latent infections.**
This family includes:

Virus	Disease
Herpes simplex virus types 1	Painful vesicles on the face
Herpes simplex virus types 2	Painful vesicles on the genitals
Varicella-zoster virus	Varicella (chickenpox) typically in children and, when it recurs, zoster (shingles)
Cytomegalovirus	Congenital malformations
Epstein–Barr virus	Infectious mononucleosis (kissing disease)
Human herpesvirus 8	Kaposi's sarcoma

Structure: (enveloped or Naked).

- Has an icosahedral core
- Genome is linear double-stranded DNA
- Does not contain a polymerase
- Surrounded by a lipoprotein envelope
- Large (120–200 nm in diameter)

HERPES SIMPLEX VIRUSES

Important Properties:

- HSV-1 and HSV-2 are structurally and morphologically indistinguishable.
- They can be differentiated by:
  - **Restriction endonuclease** patterns of their genome DNA.
  - Type-specific monoclonal antisera against **glycoprotein G.**

Mode of Transmission:

- Saliva (HSV-1)
- Sexual Contact (HSV-2)

Pathogenesis:

Herpes Simplex Virus Type 1

- Initial **vesicular lesions** occur in **the mouth or on the face.**
- The virus then travels up the axon and becomes **latent in sensory (trigeminal) ganglia.**
- Recurrences occur in skin innervated by affected sensory nerve and are induced by fever, sunlight, stress, etc.
- Dissemination to internal organs occurs in patients with depressed **cell-mediated immunity** with life-threatening consequences.
- HSV-1 encephalitis often affects the **temporal lobe.**

Herpes Simplex Virus Type 2

- Initial **vesicular lesions** occur on **genitals**.
- The virus then travels up the axon and becomes **latent in sensory (lumbar or sacral) ganglion cells**.
- Recurrences are less severe than the primary infection.
- HSV-2 infections in neonate can be **life-threatening** because neonates have reduced cell-mediated immunity.
- Asymptomatic shedding of HSV-2 in the female genital tract is an important contributing factor to neonatal infections.

Clinical Findings:

Herpes Simplex Virus Type 1

- Gingivostomatitis
- Herpes Labialis
- Keratoconjunctivitis
- Encephalitis
- Herpetic Whitlow
- Herpes Gladiatorum
- Eczema Herpeticum
- Disseminated Disease
- Erythema Multiforme

Herpes Simplex Virus Type 2

- Genital Herpes
- Aseptic Meningitis
- Neonatal Herpes
- Erythema Multiforme

Laboratory Diagnosis:

- Cell culture ⇒ causes CPE and can be identified by *antibody neutralization* or *fluorescent antibody test*.
- *ELISA* ⇒ use Monoclonal Antibody against GpG to distinguish HSV 1 from HSV 2.
- Tzanck smear ⇒ multinucleated giant cells & intranuclear inclusion bodies
- *PCR* ⇒ Diagnosis of Herpes Encephalitis
- *Serological Test* ⇒ Diagnosis of Primary Infection.

VARICELLA-ZOSTER VIRUS

Disease:

- **Varicella** (chickenpox) in children
- **Zoster** (shingles) in adults

Mode of Transmission:

- Varicella is transmitted primarily by **respiratory droplets**.
- Zoster is not transmitted; it is caused by a **reactivation of latent virus**.

Pathogenesis:

- VZV infects the mucosa of the upper respiratory tract.
- Spreads via the blood to the skin, where the typical **vesicular rash** occurs.
- **Multinucleated giant cells** are seen in the base of the lesions.
- The virus infects sensory neurons.
- Carried by retrograde axonal flow into the **dorsal root ganglia**, where the virus becomes **latent**.
- At times of reduced cell-mediated immunity or local trauma, the virus is activated and causes the vesicular skin lesions and **nerve pain** of **zoster**.

Clinical Findings:

Varicella: Incubation period 2-3 weeks

- Fever
- Malaise
- Pneumonia
- Papulo-vesicular Rashes
- Reye's syndrome

Zoster

- Painful vesicles along course of sensory Nerve on Head or Trunk
- Post-Zoster neuralgia **(Post-herpetic Neuralgia)**
- Pneumonia in Immunocompromised

Laboratory Diagnosis:

- Tzanck smear ⇒ multinucleated giant cells
- Cell culture ⇒ causes CPE and can be identified by *fluorescent antibody test*.
- A fourfold or greater rise in antibody titer in convalescent-phase serum

CYTOMEGALOVIRUS VIRUS

It causes **Cytomegalic Inclusion Disease** in neonates.

Important Properties:

Giant cells are formed, hence the name cytomegalo.

Mode of Transmission:

- Saliva
- Breast Milk
- Placenta
- Blood Transfusion
- Birth Canal
- Organ Transplant

Pathogenesis:

- Initial infection usually in the **oropharynx**.
- In fetal infections, the virus spreads to many organs (e.g., central nervous system and kidneys).
- In adults, **lymphocytes** are frequently involved.
- A latent state occurs in **monocytes**.
- **Disseminated infection** in immunocompromised patients can result from either a primary infection or reactivation of a latent infection.

Immunity:

- Assembly of CMV with **MHC I** is unstable.
- Hence virus is not presented on cell surface and hence cannot activate **CD8+ cells**.
- It also codes for **miRNA** which prevents translation of mRNA for MHC I protein.

Clinical Findings:

- Cytomegalic Inclusion disease
- Heterophil-Negative mononucleosis
- Irritable colitis with diarrhea
- Hepatosplenomegaly
- Mental Retardation
- Pneumonitis
- Esophagitis
- Hepatitis
- Rhinitis

Laboratory Diagnosis:

- Cell culture ⇒ causes CPE and can be identified by *fluorescent antibody test*.
- Histological staining ⇒ **Oval owl's eye** shaped intranuclear inclusion bodies.
- Immunofluorescence assay ⇒ Detect pp65 (Antigen in nucleocapsid) within blood lymphocytes.
- Shell vials coupled with the use of immunofluorescent antibody.
- A fourfold or greater rise in antibody titer in convalescent-phase serum

Infectious Mononucleosis

EPSTEIN-BARR VIRUS (Kissing Disease)

Important Properties: It contains;
- Viral Capsid Antigen (VCA)
- Early Antigen
- Viral Membrane Antigen (Neutralizing activity is directed against this antigen)
- Epstein-Barr nuclear antigen
- Lymphocyte-determined membrane antigen

Mode of Transmission: Saliva

Pathogenesis:
- Infection begins in the **pharyngeal** epithelium.
- Spreads to the **cervical** lymph nodes.
- Travels via the blood to the liver and spleen.
- EBV establishes latency in B lymphocytes.

Association with Cancer:
- EBV can induce malignant transformation in B lymphocytes in vitro.
- In Burkitt's lymphoma, oncogenesis is a function of the **translocation of the c-*myc* oncogene** to a site adjacent to immunoglobulin gene promoter.
- This enhances synthesis of the c-myc protein, a potent oncoprotein.

Clinical Findings:
- Infectious Mononucleosis
- Burkitt's lymphoma
- Hairy Leukoplakia
- Hodgkin's Lymphoma
- Nasopharyngeal Carcinoma
- Post-transplant lymphoproliferative disorder
- Post-transplant lymphoproliferative disorder

Laboratory Diagnosis:

Hematological Approach
- Smear shows enlarged **atypical lymphs** with expanded nucleus and vacuolated cytoplasm.

Immunological Approach
- Heterophil antibody test for early diagnosis.
- Monospot test to detect heterophile antibody.
- EBV-specific Antibody Test
  - ✓ IgM VCA antibody response show early infection
  - ✓ IgG VCA antibody response shows prior infection.
- Fluorescent antibody staining of nuclear antigen detection in cord lymphocytes.

POXVIRUS

The largest and most complex of the viruses.

SMALLPOX VIRUS

Important Properties:
- Brick-shaped
- Linear
- Double-stranded DNA
- Disk-shaped core within a double membrane
- Enveloped
- DNA-dependent RNA polymerase

Mode of Transmission:
- Respiratory droplets
- Direct contact with the virus from skin lesions

Pathogenesis:
- The virus infects the mucosal cells of the upper respiratory tract.
- Spread by viremia to the liver and spleen and later the skin.
- The rash is the result of virus replication in the skin.
- Followed by damage caused by cytotoxic T cells attacking virus-infected cells.

Clinical Findings: Incubation period 1-2 weeks
- Fever
- Malaise
- Rash

The rash evolves through stages from macules to papules, vesicles, pustules, and, finally, crusts in 2 to 3 weeks.

Laboratory Diagnosis:
- Cell culture
- Chick embryos
- Immunofluorescence

Comparison between Chicken Pox and Smallpox

Chicken Pox	Smallpox
Superficial lesions	Deep hard lesions
Lesions usually not umbilicated	Lesions often umbilicated (central depression)
Lesions at different stages of development	Lesions at the same stage of development
Lesions more common on the trunk	Lesions more common on the extremities

UNIVERSITY QUESTIONS

1. A 70-year-old woman c/o rash on left of her back. Rash is vesicular and restricted to one side of the back. She is being treated with chemotherapy for carcinoma of breast. **[Supple 2013]**
   a) What is the **diagnosis**?
   b) What is the other **disease** caused by this virus?
   c) How is this virus **transmitted**? What is its **pathogenesis**?
2. A 65 years old woman presents with painful vesicular rash on left side of her back. Smear from the base of vesicle reveals multinucleated giant cells with intracellular inclusions. Name the **etiological agent** of this disease. Name one other **disease** caused by this virus. **(1+1) [Annual 2018]**

32 RNA ENVELOPED VIRUSES

ORTHOMYXOVIRUSES

INFLUENZA VIRUS

Disease:

Influenza A virus	Worldwide epidemics of influenza
Influenza B virus	Major outbreaks of influenza
Influenza C virus	Mild respiratory tract infections

Important Properties:

- Single stranded (-) RNA
- Linear non-segmented
- Hemagglutinin and Neuraminidase on different spikes
- Polymerase present
- Enveloped
- Helical Symmetry
- Multiplies in Cytoplasm

Changes in the antigenicity:

Influenza viruses, especially influenza A virus, show changes in the antigenicity of their **hemagglutinin and neuraminidase proteins**.

This property contributes to their capacity to cause devastating **worldwide epidemics (pandemics)**.

There are two types of *antigenic changes*:

Antigenic drift	Antigenic shift
Involves the accumulation of mutations within the antigen-binding sites.	Results from the combination of two viral strains.
Minor antigenic change	Major antigenic change
Results in a new viral strain	Results in a new subtype of the virus
Easy to treat	Difficult to treat
Occurs in Influenza virus A, B and C	Occurs in Influenza virus A

Mode of Transmission: Respiratory droplets from human to human

Pathogenesis:

- After inhalation, **neuraminidase** degrades protective mucous and infect upper and lower respiratory tract.
- Infection is limited to respiratory tract because **hemagglutinin cleaving proteases** are located here.
- Systemic symptoms occur due to **cytokines**.
- **Necrosis** of superficial layers of the respiratory epithelium.

Immunity:

- Influenza virus has various antigenic types.
- Immunity produces against one type is not effective against other types.

- So, person immune system fails to protect when disease caused by antigenic type other than previous.

Clinical Findings: Incubation period of 24 to 48 hours

- Fever
- Headache
- Myalgias
- Sore throat
- Cough
- **Reye's syndrome**

Laboratory Diagnosis:

- ELISA ⟹ Detect influenza viral antigen in respiratory secretions.
- Cell culture and embryonated eggs ⟹ Detected by hemadsorption or hemagglutination.
- A fourfold or greater antibody titer rise in convalescent-phase serum.
- PCR

Vaccines:

1. **Killed** vaccine (containing purified **HA** and **NA**)
2. **Live, attenuated** vaccine (*temperature-sensitive mutant* of influenza virus)

Live Vaccine

- The virus in the live vaccine *replicates in cool nasal passages*, where it **induces secretory IgA**, but *not in warm lower respiratory tract.*

Killed Vaccine

- The killed vaccine is **not a good immunogen.**
- Because little IgA is made.
- Must be given annually.

Types of Influenza Vaccines

Vaccine made in Chicken Eggs	Vaccine not made in Chicken Eggs
Contains inactivated virus. (killed vaccine)	Virus grown in calf kidney cell culture then inactivated
Contains live, attenuated temperature-sensitive mutant virus (live vaccine)	Recombinant insect virus containing influenza virus **HA** grown in insect cells.

PARAMYXOVIRUSES

MEASLES VIRUS

Disease: Maculopapular rash.

Important Properties:

- Single stranded (-) RNA
- Linear non-segmented
- Hemagglutinin and Fusion proteins on spikes
- Polymerase present
- Enveloped
- Helical Symmetry
- Multiplies in Cytoplasm

Mode of Transmission: Respiratory droplets

Pathogenesis:

- Initial site of infection is the **upper respiratory tract**.
- Virus spreads to local lymph nodes.
- Then via the blood to other organs, including the **skin**.
- *Giant cell pneumonia* and *encephalitis* can occur.
- The *maculopapular rash* is due to **cell-mediated immune attack** by cytotoxic T cells on virus infected vascular endothelial cells in the skin.

Clinical Findings:

- Cough
- Coryza
- Conjunctivitis
- **Koplik's spots**
- Giant cell pneumonia (Warthin-Finkeldey cells)
- Maculopapular rash
- Photophobia

Complications:

- ❖ Encephalitis
- ❖ Bacterial otitis media
- ❖ Subacute sclerosing panencephalitis
- ❖ Increased risk of stillbirth in Pregnant woman

Laboratory Diagnosis:

- Rarely isolated
- Serological tests
- PCR

MUMPS VIRUS

Disease: Salivary gland swelling

Important Properties:

- Single stranded (-) RNA
- Linear non-segmented
- Polymerase present
- Enveloped
- Helical Symmetry
- Multiplies in Cytoplasm
- Hemagglutinin and Neuraminidase on same spikes
- Fusion proteins on different spikes

Mode of Transmission: Respiratory droplets

Pathogenesis:

- Initial site of infection is the **upper respiratory tract**.
- Virus spreads to local lymph nodes.
- Then via the blood to other organs, especially the parotid glands, testes, ovaries, meninges, and pancreas.

Clinical Findings:

- Fever
- Malaise
- Anorexia
- Parotid gland pain
- Swelling of salivary gland, either unilateral or bilateral

Complications:

- ❖ Orchitis
- ❖ Meningitis

Laboratory Diagnosis:

- Serological tests ⟹ Greater than fourfold rise in antibody titer
- PCR

TOGAVIRUSES

RUBELLA VIRUS

Disease: Characterized by a congenital malformation.

Important Properties:

- Single stranded (+) RNA
- Linear
- Hemagglutinin on spikes
- No polymerase
- Enveloped
- Icosahedral Symmetry
- Multiplies in Cytoplasm

Mode of Transmission:

- Respiratory droplets
- Transplacental

Pathogenesis:

- Initial site of infection is the **Nasopharynx**.
- Virus spreads to local lymph nodes.
- Then disseminate via the blood to the **internal organs** & **skin**.
- Leads to **vasculitis**.

During maternal infection,

- ❖ The virus replicates in the placenta
- ❖ Then spreads to fetal tissue.
- ❖ If infection occurs during the first trimester, a high frequency of congenital malformations occurs

Clinical Findings:

Rubella: Incubation period is 2-3 weeks.

- Fever
- Malaise
- Posterior lymphadenopathy
- Polyarthritis in Adult Women
- Maculopapular Rash on Face and Extremities.

Congenital Rubella Syndrome:

- Occur in Fetus in first Trimester and leads to heart, eye and brain malformation
- Infected Children continue to excrete Rubella virus for months after birth.

Laboratory Diagnosis:

- Fourfold or greater rise in antibody titer between acute-phase and convalescent-phase.
- Presence of **IgM** antibody in a single acute-phase serum sample.
- Amniocentesis
- PCR

RHABDOVIRUSES

RABIES VIRUS

(Negri Body)

Disease: Characterized by encephalitis → Inflammation of Active Brain Tissues

Important Properties:

- Single stranded (-) RNA
- Linear non-segmented
- Polymerase present
- Enveloped
- Helical Symmetry
- Multiplies in Cytoplasm

- Bullet shaped

Mode of Transmission: Animal bites

Pathogenesis: Viral receptor → Ach receptor

- The virus multiplies locally at the bite site.
- Infects the sensory neurons, and moves by **axonal transport** to the CNS.
- The virus multiplies in the central nervous system
- Then travels down the peripheral nerves to the **salivary glands** and other organs.
- From the salivary glands, it enters the saliva to be transmitted by the bite.
- Within the CNS, **encephalitis** develops, with the death of neurons and **demyelination.**
- Infected neurons contain an eosinophilic cytoplasmic inclusion called a **Negri body.**

Clinical Findings:

Incubation period is 2-16 weeks but depends on the site of bite. Closer the bite site to CNS, lesser will be incubation period.

- Anorexia
- Fever
- **Furious (Encephalitis)**
- Agitation (State of Anxiety OR Nervous excitement)
- Delirium
- **Dumb (Paralytic)**
- Ascending Paralysis
- Paresthesia (Abnormal sensation, tingling or Pricking)
- Hydrophobia

Laboratory Diagnosis:

- Isolation from saliva, spinal cord and brain tissue
- Fluorescent Antibody staining
- PCR
- Histological Staining ⟹ Negri bodies in cytoplasm of hippocampal neurons
- Rise in antibody titer

UNIVERSITY QUESTIONS

1. A 10-year-old boy is bitten by a wild dog while playing in the street.
   a) What **disease** is this boy at risk of contracting and which organism is the cause?
   b) What are the **characteristics** of this organism?
   c) How is the disease **diagnosed**? **[Supple 2009]**
2. A 50-year-old woman visits her physician complaining of feeling unwell with fever, chills, muscle aches, dry cough and sore throat. She has had these symptoms for several days with no significant improvement. There is H/O similar illness in other family members. Physical examination reveals small, tender cervical lymphadenopathy, swollen nasal mucosa and erythematous pharynx. **[Annual 2009]**
   a) What is the likely **diagnosis**? Describe the causative **agent**.
   b) Although the patient had similar infection in past, why isn't her **immune system** protecting her from this illness?
   c) What **characteristic** of this microorganism's genome makes deadly epidemic possible?
3. A 6-year-old adopted boy was brought to pediatrician with history of fever for few days, ear ache and swollen cheeks. His vaccination status is unknown. On examination, he had tender swelling of parotid glands bilaterally with bilateral cervical lymphadenopathy. Ear and throat examination were normal. **[Annual 2015]**
   a) What is the most likely causative **agent** of this child's disease? **(1)**

b) Briefly give **characteristics** of this virus and their impact on its **transmission**. **(4)**

4. In the month of January, many people developed high grade fever, severe headache and body aches. In the hospital they were diagnosed to have viral pneumonia. **[Supple 2017 held in 2018]**
   a) Name the **virus** responsible. **(1)**
   b) What do you understand by **antigenic drift** and **antigenic shift** in these viruses? **(4)**
5. While playing in the street a young boy is attacked by a stray dog and bitten repeatedly on the arms and face. Three weeks later, the boy develops a chance in behavior becoming irritable and agitated. He eventually became comatose and died from respiratory arrest. Give the **pathogenesis** of disease development in this case. **(1+2) [Supple 2018 held in 2019]**

33 CORONA VIRUS

ARNA VIRUS

LCG

Pepleomers

Disease:
- Common cold
- Gastroenteritis
- Lower Respiratory Tract Infections

Important Properties:
- Single stranded (+) RNA
- Linear
- Hemagglutinin molecules make up **peplomers** on virus surface, which give **shape like sun** with corona
- No Polymerase
- Enveloped
- Helical Symmetry
- Multiplies in Cytoplasm

Mode of Transmission: Respiratory droplets

Pathogenesis:
- Infection is typically limited to the **mucosal cells of the respiratory tract.**
- The binding of the virus to **angiotensin-converting enzyme-2 (ACE-2)** on the pneumocytes of the lungs
- After binding, the virus enters the pneumocytes & uses the cell's machinery for its replication & damages the pneumocytes.
- The presence of virus & damage to pneumocytes initiate a local inflammatory response that include **recruitment of macrophages & T cells** to that site.
- These inflammatory cells release various mediators that causes increase in vascular permeability, leading to fluid in the alveoli.
- Damaged pneumocytes and fluid in alveoli lead to the **respiratory distress**
- COVID-19 may lead to **hypercoagulable state of blood** leading to stroke, MI and kidney failure.

Clinical Findings:

❖ **Common Cold**
- Rhinorrhea
- Scratchy sore throat
- Pneumonia
- Low-grade fever

❖ **SARS**
- Fever
- Chills
- Malaise
- Headache
- Dizziness
- Cough
- Sore Throat
- Shortness of Breath
- Abnormal Chest Radiograph
- Elevated levels of Chemokines
- Death from Respiratory failure

❖ **MERS**
- Lymphopenia
- Leukopenia
- Thrombocytopenia
- Pneumonia
- Elevated Lactate Dehydrogenase

Laboratory Diagnosis:
- Antigen & Nucleic Acid Detection
  - PCR
  - ELISA
- Isolation & Identification of Virus ⇒ Vero Monkey Kidney cells
- Serology
  - ELISA
  - Hemagglutination test
  - Indirect Immunofluorescent Antibody test

34 RNA NON-ENVELOPED VIRUSES

PICORNAVIRUSES

POLIO VIRUS

Disease: Poliomyelitis

Important Properties:
- Single stranded (+) RNA
- Linear
- No polymerase
- Naked
- Icosahedral Symmetry
- Multiplies in Cytoplasm

Mode of Transmission: Fecal–oral route

Pathogenesis: Initial Site of Infection → GIT.

- The virus replicates in the **pharynx** and the **GI tract.**
- It can spread to the local lymph nodes.
- Then through the bloodstream to the **central nervous system.**
- Paralysis is the result of death of motor neurons, especially **anterior horn cells in the spinal cord.** Inflammation of meninges.

Aseptic meningitis is more frequent than paralytic polio.

Clinical Findings: The range of responses to poliovirus infection includes:
- *Inapparent, asymptomatic infection* (incubation period is 10 to 14 days.)
- *Abortive poliomyelitis* (mild, febrile illness) → Pt. with fever of 38°C or history of fever that persisted 2-7 days with
  - Headache
  - Vomiting
  - Nausea
  - Sore throat
- *Nonparalytic poliomyelitis*
  - Headache
  - Stiff neck
  - Fever
  - Aseptic meningitis No Localizing source
- *Paralytic poliomyelitis.*
  - Flaccid paralysis
  - Brainstem involvement can lead to respiratory paralysis.
  - Painful muscle spasms
  - The motor nerve damage is permanent
  - Both the meninges and the brain parenchyma involved (**meningoencephalitis**)
  - If the spinal cord is also involved (***meningomyeloencephalitis***)

Laboratory Diagnosis:
- **Cell culture** ⇒ causes CPE can be identified by *neutralization of CPE with specific antisera.*
- Rise in antibody titer
- Virus can be recovered from the throat, stool, or spinal fluid by inoculation of cell cultures.

Vaccines: Induce humoral antibodies
1. **Killed** vaccine (Salk vaccine, inactivated vaccine, **IPV**)
2. **Live, attenuated** vaccine (Sabin vaccine, oral vaccine, **OPV**)

REOVIRUSES

ROTA VIRUS

Gastroenteritis

Disease: Viral Gastroenteritis

Important Properties:

- Double stranded RNA
- Polymerase present
- Segmented
- Naked
- Icosahedral Symmetry

Mode of Transmission: Fecal–oral route

Pathogenesis:

- Rotavirus replicates in the mucosal cells of the small intestine.
- Excess secretion of fluids and electrolytes into the bowel lumen.
- Loss of salt, glucose, and water leads to diarrhea.

Clinical Findings:

- Nausea
- Vomiting
- Non-bloody diarrhea

Laboratory Diagnosis:

- Stool examination ⇒ Detection by using ELISA & radioimmunoassay

UNIVERSITY QUESTIONS

1. Briefly discuss the **pathogenesis** of poliomyelitis. **[Annual 2014]**
2. Poliomyelitis is diagnosed in three children in a village of Swat. Briefly discuss the **pathogenesis** and **mode of transmission** of polio virus. **(2.5) [Annual 2016]**

35 HEPATITIS VIRUSES

Main site of infection: Liver

HEPATITIS A VIRUS

RNA (+)

Virus Family: Picornavirus

Important Properties:

- Single stranded (+) RNA
- Linear
- No polymerase
- Naked (Naked)
- Icosahedral Symmetry
- Multiplies in Cytoplasm

Mode of Transmission: Fecal-Oral route (Indirect)

Pathogenesis:

- The virus replicates in the **GI tract.**
- Then spreads to the **liver** via blood.
- The virus is **not cytopathic** for the hepatocyte.
- Hepatocellular injury is caused by immune attack by **cytotoxic T cells.** (CD8 T-cells)

Clinical Findings: Incubation period (3-4 weeks)

- Fever
- Anorexia
- Nausea
- Jaundice
- Dark urine
- Pale feces
- Vomiting
- Elevated transaminase levels

Laboratory Diagnosis:

- Detection of **IgM** antibody.
- Fourfold rise in **IgG** antibody. (4 times)

HEPATITIS B VIRUS

dsDNA

Virus Family: Hepadnavirus

Important Properties:

- Partially **dsDNA**
- Circular
- **HBsAg** in Envelop
- Replicate in Nucleus
- DNA polymerase in virion

Mode of Transmission:

- Sexual intercourse
- Blood transfusion
- During birth

Pathogenesis:

- Hepatocellular injury due to immune attack by **cytotoxic (CD8) T cells**.
- **Chronic carrier state** occurs in 90% of neonatal infections because neonates have **poor cytotoxic T-cell activity**.
- Chronic carrier state can lead to *chronic hepatitis*, *cirrhosis*, and *hepatocellular carcinoma*.
- Hepatocellular carcinoma may be related to **the integration of part of the viral DNA into hepatocyte DNA** and subsequent synthesis of HBx protein.
- Antigen–antibody complexes cause arthritis, rash, and glomerulonephritis.

Clinical Findings: Incubation period (10-12 weeks)

- **Acute Hepatitis B** clinical appearance of is same as Hepatitis A, but more severe
  - Serum sickness like symptoms • Rash • Fever • Arthralgias
- **Chronic Hepatitis B** (asymptomatic)
  - Neuropathies • Glomerulonephritis • Polyarthritis nodosa

Laboratory Diagnosis:

- ELISA
- PCR
- Immunofluorescence assay

Serological Findings:

	Hepatitis B surface antigen HBsAg	Hepatitis B surface antibody HBsAb	Hepatitis B core antibody HBcAb
Acute Disease	Positive	Negative	Positive
Window Phase	Negative	Negative	Positive
Complete Recovery	Negative	Positive	Positive
Chronic carrier State	Positive	Negative	Positive

IgM is found in the acute stage; IgG is found in subsequent stages.

Terminologies:

HBsAg	Antigen found on surface of HBV; positive during acute disease; continued presence indicates carrier state.
HBsAb	Antibody to HBsAg; provides immunity to hepatitis B.
HBcAg	Antigen associated with core of HBV.
HBcAb	Antibody to HBcAg; positive during window phase.
HBeAg	Important indicator of transmissibility.
HBeAb	Antibody to e antigen; indicates low transmissibility.
Window period	Period between end of detection of HBsAg and beginning of detection HBsAb.

HEPATITIS C VIRUS

Virus Family: Flavivirus

Important Properties:

- Single stranded (+) RNA
- Linear
- No polymerase
- Enveloped
- Icosahedral Symmetry
- Multiplies in Cytoplasm

Mode of Transmission:

- Blood transfusion
- Perinatal
- Sexual intercourse (less common)

Pathogenesis:

- Hepatocellular injury caused by **cytotoxic T cells**.
- HCV does not cause a cytopathic effect.
- More than 50% of infections result in the **chronic carrier state**.
- The chronic carrier state predisposes to *chronic hepatitis* and to *hepatocellular carcinoma*.

Clinical Findings: Incubation period (8 weeks)

- **Acute Hepatitis C** (asymptomatic)
- **Chronic Hepatitis C**
- **Liver Cirrhosis** • Hepatocellular carcinoma

Laboratory Diagnosis:

- ELISA
- Antibody to HCV
- PCR

HEPATITIS D VIRUS

Important Properties:

- Single stranded (-) RNA
- Circular
- No polymerase
- Enveloped
- **Delta antigen**
- **Defective** virus (cannot replicate by itself)
- Uses hepatitis B surface antigen as its protein coat.

Mode of Transmission:

- Blood transfusion
- Perinatal
- Sexual intercourse (less common)

Pathogenesis:

- Hepatocellular injury due to immune attack by **cytotoxic (CD8) T cells**.
- Delta antigen is **cytopathic** for hepatocytes.
- Infect those cells which are already infected by HBV.

Laboratory Diagnosis:

- Detection of Delta Antigen.
- Detection of **IgM antibody** to Delta Antigen.

HEPATITIS E VIRUS

Virus Family: Hepevirus

Important Properties:

- Single stranded (+) RNA
- Linear
- No polymerase
- Naked
- Icosahedral Symmetry
- Multiplies in Cytoplasm

Mode of Transmission: Fecal-Oral route

Laboratory Diagnosis: Detection of **IgM** antibody.

UNIVERSITY QUESTIONS

Name 3 **RNA hepatitis viruses** and **route of their transmission**. **[Supple 2015]**

Name important **diagnostic tests** during various stages of Hepatitis B. What are **serological findings** in a patient with acute Hepatitis B? **(1.5+1.5) [Annual 2018]**

36 ARBOVIRUSES

DENGUE VIRUS

Dengue is the most common insect-borne viral disease in the world.

Virus Family: Flavivirus
Vector: *Aedes* mosquito
Animal Reservoir: Humans, Monkey(suspected)

Important Properties:

- Single stranded (+) RNA
- Linear
- No polymerase
- Enveloped
- Icosahedral Symmetry
- Multiplies in Cytoplasm

Classic dengue fever (breakbone fever):

- Begins suddenly with an influenza-like syndrome;
  - Fever
  - Malaise
  - Retro-orbital pain
  - Headache
- Pains in muscles (**myalgia**)
- Pains in joints (arthralgia, **breakbone**)
- Enlarged lymph nodes
- Maculopapular rash
- Facial flushing
- Leukopenia

After a week or so, the symptoms regress but weakness may persist.

Dengue hemorrhagic fever:

Much more severe disease.

- The initial picture is the same as classic dengue.
- Then shock and hemorrhage, especially into the gastrointestinal tract and skin, develop.

Pathophysiology of Dengue hemorrhagic fever:

Hemorrhagic shock syndrome is due to the production of large amounts of **cross-reacting antibody** at the time of a **second dengue infection**.

- The patient recovers from classic dengue caused by one of the four serotypes.
- Antibody against that serotype is produced.
- When the patient is infected with another serotype of dengue virus, an **anamnestic, heterotypic response** occurs. (Memomary B cells are responsible)
- Large amounts of cross-reacting antibody to the first serotype are produced.

Renewed production of an Ab

There are two **hypotheses** about what happens next.

I. Immune complexes composed of virus and antibody are formed that activate complement, causing **increased vascular permeability** and **thrombocytopenia**.

II. The antibodies increase the entry of virus into monocytes and macrophages, with the consequent liberation of a large amount of cytokines

In either scenario, shock and hemorrhage result.

M / Mo → Cytokines

Laboratory Diagnosis:

- ✓ Cell culture
- ✓ Serologic test ⇒ Presence of IgM antibody
- ✓ Fourfold or greater rise in antibody titer in acute and convalescent sera.
- ✓ PCR assay

UNIVERSITY QUESTIONS

1.a) What is pathophysiology of **Dengue shock syndrome**?
b) What are the **laboratory tests** for diagnosis of dengue fever? **[Annual 2011]**
2.a) Describe **clinical spectrum of infection** by dengue virus.
b) How will you confirm **diagnosis in laboratory**? **[Annual 2011]**
3. 10-year-old boy presents with flu like symptoms during recent dengue epidemic. With clinical suspicion of dengue fever, her lab investigations are ordered by house officer on urgent request. **[Annual 2012]**
a) Is the test request appropriate?
b) Give an account of **lab investigations** available for diagnosis in relation to timing of presentation.
c) How does the **host immune response** influence the severe form of this disease in the infected person?

37 TUMOR VIRUSES

DNA Tumor Human Viruses

Virus	Associated Tumors
Human papillomavirus	Papillomas
Epstein-Barr virus	Burkitt's lymphoma
Kaposi's sarcoma associated virus	Kaposi's sarcoma

RNA Tumor Human Viruses

Virus	Associated Tumors
Human T-cell lymphotropic virus	Adult T-cell lymphoma
Hepatitis C virus	Hepatocellular carcinoma

UNIVERSITY QUESTIONS

1. Name 4 viruses associated with development of human cancers. Name also the cancers caused by these viruses. **[Supple 2015]**
2. Enlist two RNA and two DNA human tumor viruses and their associated tumors. **(2) [Annual 2017]**
3. Enlist four human tumor viruses with their associated malignancies. **(2) [Supple 2018 held in 2019, Annual 2007]**

38 HUMAN IMMUNODEFICIENCY VIRUS

(Retroviruses)

Virus Family: Retrovirus

Disease: AIDS (Acquired Immunodeficiency Syndrome)

Important Properties:

- Diploid Single stranded (+) RNA
- Enveloped
- Icosahedral Symmetry
- RNA dependent DNA Polymerase
- Linear
- Multiplies in Nucleus
- **Virus-encoded protease** cleaves polypeptides to produce functional viral proteins.
- The ***tat* gene** encodes a protein that activates viral transcription.
- **Enzymes** within the nucleocapsid of the virion:
  - ✓ Reverse transcriptase
  - ✓ Integrase
  - ✓ Protease (RIP)

Mode of Transmission:

- Sexual contact
- Vertical transmission (transmission from mother to offspring)
- Bloodborne (transfusions, dirty needles)

Pathogenesis: Target Receptors → CD4, CCR5 or CXCR4

- HIV infects and kills helper T cells, which predisposes to opportunistic infections.
- The **NEF protein** is an important virulence factor.
- It reduces **class-I MHC protein synthesis**, thereby reducing the ability of cytotoxic T cells to kill HIV-infected cells.

Immunity: Cytotoxic T cells are the main host defense against HIV.

Replicative Cycle:

1. Surface **gp120** of HIV binds to **CD4** of T-helper cells, macrophages, microglia, and co-receptors (CCR5 and CXCR4) found on **macrophages and Th cells**, respectively.
2. HIV is taken into the cell, losing the envelope; the **RNA is uncoated**.
3. The RNA is copied using the **virion-associated reverse transcriptase**; ultimately dsDNA with long terminal repeats is made.
4. The DNA and integrase migrate to nucleus, and the **viral DNA is integrated into host DNA** forming the provirus. The provirus remains in the host DNA.
5. The rate of viral replication is regulated by the activity of the regulatory proteins (tat/rev, nef, etc.).
   - *Tat upregulates transcription.*
   - *Rev regulates transport of RNA to cytoplasm.*
6. Transcription produces ss (+) RNA, some **cleaved** and some remain **intact.**
   - Cleaved RNA will be used as mRNA.
   - Uncleaved RNA is used as genomic RNA.
7. Translation produces the proteins, some of which are polyproteins that are cleaved by the HIV protease.
8. Assembly
9. Maturation/release of virus

Types of HIV:

- HIV-1
- HIV-2

Clinical Findings:

STAGES

1) **Early, acute** *stage* (within 2-4 weeks after infection)
   - Flu-like illness
   - Fever
   - Chills
   - Night sweats
   - Sore throat
   - Muscle aches
   - Fatigue
   - Swollen lymph nodes
   - Mouth ulcers

2) **Middle, latent** *stage* (last for several years)
   - Asymptomatic

3) **Late, immunodeficiency** *stage* (CD4 cells count fall below 200 cells/mm^3)
   - AIDS
   - Rapid weight loss
   - Recurring fever
   - Depression
   - Pneumonia
   - Memory loss
   - Diarrhea
   - Profuse night sweats
   - Swelling of the lymph glands
   - Extreme tiredness
   - Blotches on or under the skin
   - Sores of the mouth or genitals

Laboratory Diagnosis:

- ELISA
- PCR
- Isolated from blood or serum
- Western Blot

UNIVERSITY QUESTIONS

1. What are the **types** of HIV virus and what **disease** it causes? **[Annual 2010]**
2. An HIV positive patient has progressed from fatigue, rash, nausea and night sweat symptoms to occasional but define opportunistic infections. **[Supple 2015 held in 2016]**
   a) Enlist two opportunistic fungal, 2 viral infections and one malignancy associated with AIDS.
   b) Give the **clinical course of disease development** in HIV infection.
3. Briefly discuss the three **sages** seen during the clinical course of HIV infection. **(3) [Annual 2017]**
4. A 32-year-old homosexual, infected with HIV for the last 8 years, now presents with signs and symptoms of immunodeficiency stage. **[Supple 2017 held in 2018, Supple 2016 held in 2017]**
   a) Give three main **mechanisms** by which HIV can invade the immune system. **(1.5)**
   b) Name two opportunistic fungal infections associated with AIDS. **(1)**
   c) Enlist the specific tests used in **laboratory diagnosis** of HIV infection. **(2.5)**
5. a) Define **AIDS**. Enlist the three main **mechanisms** by which HIV evades the immune system. **(1+1.5)**
   b) Briefly discuss the **acute & latent stages** observed in the clinical course of HIV infection. **(2.5) [Annual 2020]**

MYCOLOGY

39 BASIC MYCOLOGY

Mycology is the study of fungi (molds, yeasts, and mushrooms).

Characteristics

1. Eukaryotic.
2. Cell wall made of **chitin**, glucan, and mannan.
3. **Ergosterol** is major membrane sterol.
4. Most fungi are **obligate aerobes** and some are **facultative anaerobes**.
5. Thermally **dimorphic** (different structure at different temperature).
6. Some reproduce **sexually** by mating and forming sexual spores.
7. Most propagate **asexually** by forming **conidia** (asexual spores).

Comparison of Fungi and Bacteria

Property	Fungi	Bacteria
Nucleus	Eukaryotic	Prokaryotic
Endoplasmic Reticulum	Present	Absent
Cell membrane	Sterols present	Sterols absent (except *Mycoplasma*)
Cell Wall	Chitin	Peptidoglycan
Mitochondria	Present	Absent
Spores	Sexual and asexual spores for reproduction	Endospores for survival
Thermal Dimorphism	Yes (some)	No
Ribosome	80S	70S

Types of Fungi

- Yeasts (grow as single cells that reproduce by asexual budding)
- Molds (grow as long filaments (**hyphae**) and form a mat (**mycelium**)).

Hyphae

Hyphae are **filamentous (tubelike) cells** of molds. Hyphae grow at the tips (apical growth).

a) **Septae** are transverse walls of hyphae and occur in the hyphae of the great majority of the disease-causing fungi. They are referred to as septate.
b) **Non-septae** or aseptate hyphae lack regularly occurring transverse walls. These cells are multinucleated and are also called **coenocytic**.
c) **Pseudo-hyphae** (Candida albicans) are hyphae with constrictions at each septum.

Conidia

i. Arthrospores (arise by fragmentation of the ends of hyphae)
ii. Clamydospores (thick-walled and quite resistant)
iii. Blastospores (formed by the budding process)
iv. Sporangiospores (formed within a sac)

form of discharge or puss.

Pathogenesis

- The response to infection with many fungi is the formation of **granulomas**.
- The **cell-mediated immune response** is involved in granuloma formation.
- **Acute suppuration**, characterized by the presence of neutrophils in the exudate, also occurs in certain fungal diseases such as *aspergillosis* and *sporotrichosis*.
- Activation of the cell-mediated immune system results in a **delayed hypersensitivity skin test** response to certain fungal antigens.

Fungi which cause granuloma formation

- Coccidioidomycosis
- Histoplasmosis
- Blastomycosis
- Aspergillus

Fungal toxins and allergies

- Mycotoxicosis (caused by ingested toxins)
- Allergies (to fungal spores)

Laboratory Diagnosis

- Direct microscopic examination (treated with **10% KOH**)
- Culture of the organism (**Subouraud's agar**)
- PCR
- Serological tests

Subouraud's agar

- Inhibits growth of bacteria in specimen.
- Facilitates the appearance of fungi on culture.
- Fungi are identified by appearance of **mycelium** and nature of **asexual spores**.

UNIVERSITY QUESTIONS

1. In a tabulated form, enlist four important **differences** between fungi and bacteria. **(2)** **[Annual 2018]**
2. a) What are four approaches to **lab diagnosis** of fungal diseases?
   b) How the **culture** of fungus is carried out? **[Annual 2014]**
3. Name the fungi which cause **granuloma formation**. **[Annual 2013]**
4. Nearly 300 of 10,000 to 200,000 species (depending on how they are classified) are thought to cause diseases.
   a) What is the study of these organisms known as?
   b) **Classify** mycoses in four groups. **[Annual 2008]**
5. Mention three **laboratory methods** for diagnosis of fungal diseases. **[Annual 2007]**

40 CUTANEOUS & SUBCUTANEOUS MYCOSES

CUTANEOUS MYCOSES

DERMATOPHYTES

Genera:
- Trichophyton
- Microsporum
- Epidermophyton

Anatomic Location:
- Epidermis
- Hair
- Nails

Disease:
Dermatophytic infections (tineas, also called ringworm).
Itching is the most common symptom of all tineas.
- ✓ *Tinea capitis* (scalp)
- ✓ *Tinea barbae* (beard)
- ✓ *Tinea corporis* (body)
- ✓ *Tinea cruris* (groin)
- ✓ *Tinea pedis* (athlete's foot)
- ✓ *Tinea unguium* (nails)

Important Properties:
- Filamentous
- Molds
- Use **keratin** as nutritional source

Mode of Transmission:
Direct contact from;
- Infected person (little inflammation)
- Animals (high inflammation)

Pathogenesis:
- These fungi grow only in the **superficial keratinized layer of the skin**.
- They **do not invade** underlying tissue.
- The lesions are due to the **inflammatory response** to the fungi.
- An important host defense is provided by the **fatty acids** produced by sebaceous glands.
- The **id reaction** is a hypersensitivity response in one skin location (e.g., fingers) to the presence of the organism in another (e.g., feet).

(Dermatophytid) = allergic response to circulating fungal antigens

Clinical Findings: → Itching Uncomfortable skin, makes you scratch it.
- Pruritic lesions
- Broken hairs and nails
- Rash with inflamed circular border

Laboratory Diagnosis:
- Microscopic examination with KOH ⇒ Presence of septate hyphae.
- **Sabouraud's agar** ⇒ Typical hyphae & conidia
- Wood's Lamp ⇒ Detect *Microsporum*
- Skin test

TINEA VERSICOLOR

Genera: Malassezia
Anatomic Location: Dead layer of skin
Disease:
- ✓ Scaly plaques on chest
- ✓ Hypopigmented area

Mode of Transmission: Direct contact from Infected person
Laboratory Diagnosis:
- Microscopic examination with KOH ⟹ Mixture of hyphae & yeast.

SUBCUTANEOUS MYCOSES

SPOROTRICHOSIS

Genera: *Sporothrix*
Anatomic Location: Subcutis
Disease:
- ✓ Local pustule
- ✓ Ulcer with nodules along draining lymphatics

Important Properties:
- Thermally dimorphic
- Mold in the soil
- Yeast in the body
- Cigar shaped yeast

Mode of Transmission:
- Trauma
- Rose thrones

Pathogenesis:
- This subcutaneous, nodular, fungal disease is generally **not painful**.
- Spreads via the lymphatics (**lymphocutaneous sporotrichosis**).
- Produces a chain of lesions on the extremities with the
  - Older (lower) lesions **ulcerating**.
  - Newer (upper) ones starting **nodular**.

Laboratory Diagnosis:
- **Sabouraud's agar** ⟹ Hyphae & oval conidia
- Cigar shaped budding yeasts

UNIVERSITY QUESTIONS

1. a) Enumerate the three **genera** of dermatophytes. What is the most likely **reason** that the infection with dermatophytes is restricted to nonviable skin, hair and nails? **(1+1)**
   b) Enumerate any three **skin diseases** caused by them, giving the **location** of lesions and their **clinical features. (3) [Annual 2015]**
2. An 11-year-old girl has pruritic rash on her chest for over 4 weeks. The lesions are round to oval with an inflamed border and clearing in center. **[Supple 2015]**
   a) What is most likely **diagnosis**?
   b) Name which **lab tests** can help in diagnosis.

a) Name 3 most important **genera** of dermatophytes.
A 15-year-old boy presents with several weeks of slowly worsening pruritus of both of his feet. He is otherwise well and taking no medication. On examination, he has bilateral erythematous, dry scaling lesions that are most obvious in the interdigital web spaces and on the soles. There is no bleeding or exudates.
a) What would most likely be found in a KOH mount of skin scrapings of the affected skin?
b) What other **lab tests** can be used to diagnose the case? **[Annual 2013]**

A. Examination between patient's toes shows severe inflammation and some tissue damage. Microscopic examination of this tissue shows hyphae, macroconidia and microconidia. **[Supple 2010]**
a) Name the **fungal disease** seen here and state the general descriptive name for this type of fungus.
b) Mention the two common fungi which can cause this disease.
c) Name two **laboratory tests** which can be used to confirm this diagnosis.

5. An 11-year-old football player complains of itching between his toes. Physical examination reveals pustules on the fingers of both hands and white macerated tissue between the toes. Pustules have been itchy and appeared about 10 days after infection between the toes began. **[Supple 2009]**
a) What is the most likely **diagnosis**? Name the 3 **genera** of Dermatophytes.
b) What **tests** can help confirm diagnosis?

41 SYSTEMIC MYCOSES

Genus	Form in tissue seen by microscope
Coccidioides	Spherule
Histoplasma	Yeasts within macrophages
Blastomyces	Yeasts with single broad-based bud
Paracoccidioides	Yeasts with multiple buds

HISTOPLASMA

Disease: Histoplasmosis

Important Properties:

- No capsule.
- Two kinds of asexual spores:
  - ❖ Tuberculate macroconidia (finger-like projections)
  - ❖ Microconidia (smooth walled spores)
- Thermally dimorphic

Habitat: Soil contaminated with bird or bat feces.

Mode of Transmission: Inhalation of airborne asexual spores (microconidia)

Pathogenesis:

- **Microconidia** enter the lung.
- Differentiate into yeast cells.
- The yeast cells are ingested by **alveolar macrophages** and multiply within them.
- The yeasts survive within the **phagolysosome** of the macrophage by producing **alkaline substances** which raise the pH and thereby **inactivate the degradative enzymes** of the phagolysosome.
- Spread throughout the body, especially liver & spleen.
- An immune response is mounted, and granulomas form (**calcified granulomas**).
- Suppression of **cell-mediated immunity** can lead to disseminated disease.

Clinical Findings:

- Cavitary lung lesions
- Tongue ulcer
- Pancytopenia
- Granulomas in liver and spleen

Laboratory Diagnosis:

- Microscopic examination ⇒ oval yeast cells within *macrophages*
- Sabouraud's agar ⇒ Hyphae with,
  - ✓ tuberculate macroconidia (at 25°C)
  - ✓ yeasts (at 37°C)
- ELISA
- Skin test (using **histoplasmin**)
- Increase in antibody titer
- **ID** test
- Urinary antigen
- Serological tests (for **IgM** & **IgG**)
  - ✓ Complement Fixation
  - ✓ Immunodiffusion

UNIVERSITY QUESTIONS

1. A 39 years old patient with AIDS, who had atypical tuberculosis infection a year back now develops ulcerating lesion on one side of his tongue. A Giemsa stain of biopsy specimen reveals budding yeasts within macrophages. **[Supple 2016 held in 2017]**
   a) Name the **causative agent** and give its **habitat**. **(2)**
   b) Briefly discuss the **pathogenesis** of this fungal infection. **(3)**
2. A patient with AIDS suddenly develops pancytopenia and ulcerative lesions on his tongue. Biopsy specimen of lesion reveals several budding intracellular yeast cells. **[Annual 2019]**
   a) Give the **habitat** and **pathogenesis** of fungal infection.
   b) Differentiate between the **yeast forms** of Cryptococcus, Blastomyces and Paracoccidioides.
3. Enlist two systemic fungi and its **form** in tissues seen by microscopy. **[Annual 2020]**

42 OPPORTUNISTIC MYCOSES

Genus	Form in tissue seen by microscope
Candida	Yeast form pseudo-hyphae (also hyphae)
Cryptococcus	Yeast with large capsule
Aspergillus	Mold with septate hyphae
Mucor & Rhizopus	Mold with non-septate hyphae

CANDIDA *ALBICANS*

Disease:
- Thrush
- Vaginitis
- Esophagitis
- Diaper rash
- Right-sided endocarditis
- Endophthalmitis
- Bloodstream infections
- Chronic mucocutaneous candidiasis

Important Properties:
- Oval yeast with a single bud
- Gram +ve yeast
- In tissues, appears as
  - Yeasts
  - Pseudo-hyphae

Normal Flora:
Mucous membrane of;
✓ Upper Respiratory Tract ✓ Female Genital Tract ✓ GIT

Predisposing factors:
- Reduced cell-mediated immunity
- Altered skin and mucous membrane
- Suppression of normal flora by antibiotics
- Presence of foreign bodies

Laboratory Diagnosis:
- Microscopic examination ⇒ yeast & pseudo-hyphae
- **Sabouraud's agar** ⇒ Typical yeast colonies
- Carbohydrate fermentation ⇒ differentiate C. albicans from other species
- Skin test
- **Chlamydospore** formation ⇒ grow at 42°C
- **Germ tube** formation ⇒ differentiate C. albicans from other species
- **Calcoflour white staining** ⇒ budding yeasts and pseudo-hyphae appear gram-positive

CRYPTOCOCCUS *NEOFORMANS*

Disease: Cryptococcal meningitis

Important Properties:
- Oval, budding yeast
- Narrow based bud
- Wide polysaccharide capsule

Habitat: Soil contaminated with pigeon feces.

Mode of Transmission: Inhalation of airborne yeast cells

Pathogenesis:
- Organisms cause **influenza-like syndrome** or pneumonia.
- They spread via the bloodstream to the meninges.
- **Subcutaneous nodules** are often seen in disseminated disease.
- Reduced **cell-mediated immunity** predisposes to severe disease.
- But some cases of cryptococcal meningitis occur in immunocompetent people who inhale a large dose of organisms.

Clinical Findings:
- Headache
- Fever
- Vomiting
- Neck stiffness
- Disorientation

Laboratory Diagnosis:
- **India ink preparation** ⇒ visualization of encapsulated yeast
- **Sabouraud's agar** ⇒ mucoid yeast colonies
- Cryptococcal Antigen test (**CRAG**) ⇒ detection of polysaccaride capsular antigen in spinal fluid
- PCR

ASPERGILLUS *FUMIGATUS*

Disease:
- Invasive aspergillosis
- Allergic bronchopulmonary aspergillosis
- *Fungus ball* in the lungs (aspergilloma)

Important Properties:
- Exist only as molds
- Septate hyphae
- V-shaped (**dichotomous**) branches
- Conidia form *radiating chains*

Habitat: Decaying vegetation

Mode of Transmission: Inhalation of airborne spores (**conidia**)

Pathogenesis:
- *Aspergillus* colonize and later invade abraded skin, wounds, burns, the cornea, the external ear, or paranasal sinuses.
- In immunocompromised persons, it can invade the **lungs** producing hemoptysis and the **brain** causing an abscess.
- Neutropenic patients are also predisposed to **intravenous catheter infections** caused by this organism.
- The organism invades blood vessels, causing *thrombosis* and *infarction*.
- A person with a lung cavity (e.g., from tuberculosis) may develop a **fungal ball (aspergilloma).**
- An allergic person is predisposed to allergic **bronchopulmonary aspergillosis** mediated by *IgE antibody*.

Laboratory Diagnosis:

- Microscopic examination ⇒ septate hyphae invading tissue
- **Sabouraud's agar** ⇒ colonies with *radiating chains* of conidia
- High titers of **galactomannan antigen** ⇒ Invasive aspergillosis
- High levels of **IgE** ⇒ Allergic bronchopulmonary aspergillosis
- **IgG** precipitins ⇒ aspergilloma

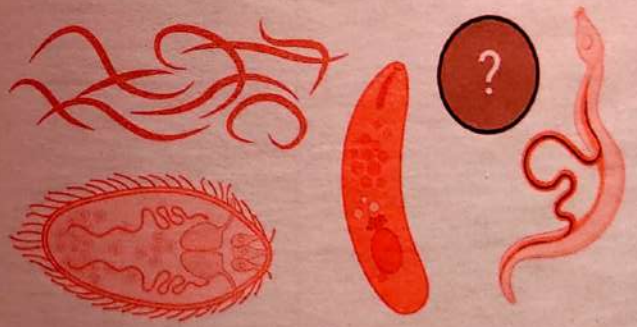
UNIVERSITY QUESTIONS

1. A 15-year-old girl has a 3 days history of severe headache, fever and neck rigidity. Lumber puncture done reveals markedly raised lymphocytes and a budding fungal organism with a thick gelatinous capsule. **[2018 Supple held in 2019]**
   a) What is your **diagnosis**? What steps are taken to reach this diagnosis in the **laboratory**?
   b) Enlist four opportunistic fungi with one important clinical disease by each.
2. How will you confirm the diagnosis of oropharyngeal candidiasis (thrush) in the **laboratory**? Enlist two other opportunistic fungi. **(2+1) [Annual 2018]**
3. A female presented to OPD of a hospital. She had white patches on her tongue. It was thought to be due to fungal disease. **[Supple 2017 held in 2018]**
   a) What is the patient suffering from? **(1)**
   b) Enumerate the **conditions pre-disposing** to such a disease. **(2)**
   c) Give the **findings** on Gram-Stained smear of the specimen from her tongue. **(2)**
4. A 30-year-old known asthmatic patient has started to work in a food industry where molds are used for making bread and fermentation process. After few months, he develops progressive worsening of his asthma symptoms. The sputum examination reveals septate hyphae with dichotomous branching.
   a) What is the most likely **diagnosis**? Name the two other clinical presentation of this pathogen. **(1+1)**
   b) Briefly describe the **pathogenesis** of the disease. **(1)**
   c) How will you diagnose this case in the **laboratory**? **(2) [Annual 2017]**
5. Cryptococcus neoformans is suspected in known HIV positive patient presenting with severe vomiting, headache, neck stiffness and delirium. **[Annual 2016]**
   a) How will you diagnose this case in **laboratory**? **(3)**
   b) Enlist the two other opportunistic fungi and one disease caused by each. **(2)**
6. An elderly poultry worker presents with severe headache, vomiting, neck stiffness and disorientation. Meningitis is suspected. CSF sample reveals several round budding encapsulated yeast cells with a distinct halo around them. **[Supple 2016]**
   a) Name the **causative agent** and give tis **habitat**. **(2)**
   b) What **laboratory tests** can help confirm the diagnosis? **(3)**
7. A 70-year-old male with uncontrolled Diabetes presents with right sided chest pain, fever and cough with purulent sputum, streaked with blood. He also complains of pneumonia. Chest X-ray is suggestive of necrotizing pneumonia. Sputum smear revealed fungal hyphae which were septate and of uniform width with dichotomous branching. **[Annual 2012]**
   a) What will be the **morphology of affected lung lobe** with this fungal infection?
   b) Describe the **pathogenesis** of invasive form of this fungal infection.
8. A 50-year-old post-renal transplant patient receiving steroid and immunosuppressive therapy suddenly developed acute pneumonic features with cough, dyspnea, hemoptysis and fever. He was suspected to have developed invasive Aspergillosis. **[Annual 2011]**
   a) What is the **pathogenesis**?
   b) Give an account of verities of **clinical presentations** with Aspergillus infection.

9. A 50-year-old patient suffering from AIDS presented with headache, neck stiffness and disorientation. His CSF examination revealed a high lymphocytic count, an increased protein and low glucose level. An India ink preparation of CSF mount was +ve for fungal infection.
   a) How will you identify this fungus in **lab**? **[Supple 2011]**
   b) Describe the **pathogenesis** of development of meningitis with this fungal infection.
10. A 35-year-old man who is HIV +ve has had a persistent headache and a low-grade fever (100° F) for past 2 weeks. Budding yeasts with a wide capsule in India ink preparation of spinal fluid are seen. **[Annual 2010]**
    a) What is the most likely **diagnosis**?
    b) What is the **mode of transmission**?
    c) What is the importance of **India ink preparation**?
11. A patient with diabetes presents in medical OPD with an adherent, white flaky substance on the skin under her breasts. Another female patient who has completed a course of oral antibiotics presents with itching and copious white vaginal discharge, while a third patient with AIDS presents with white exudates on his oral mucosa and soft palate. **[Annual 2009]**
    a) What is likely **diagnosis** and **causative organism** in all cases?
    b) Where is the microorganism normally found?
    c) What **laboratory tests** can help confirm diagnosis?
12. Mention four opportunistic fungi. **[Annual 2007]**
13. A 43-year-old HIV positive sex worker complains of headache and blurring of vision. Physical examination reveals papilledema and ataxia. The CSF examination show encapsulated budding organisms visible by India ink. What is likely **causative organism**? Give its **laboratory diagnosis**. **[Annual 2020]**

- E. Histolytica
- G. Lamblia
- Malaria.

PARASITOLOGY



43 PARASITOLOGY

asitology is the study of parasites, their hosts, and the relationship between them.

LASSIFICATION OF PARASITES:

asites are classified as protozoans or metazoans.

	Protozoa	Metazoa
Complexity	Single-celled	Multicellular
Onset of clinical symptoms	Days to weeks	More than 1 month
Diagnostic forms	Cysts and trophozoites	Eggs
Elevated immune levels	Neutrophils	Eosinophils

sts: The infected host is classified as:

ermediate host Host in which larval or asexual stages develop

Definitive host Host in which adult or sexual stages occur

ctors → living transmitters (e.g., a fly) of disease.

e Cycle Forms:

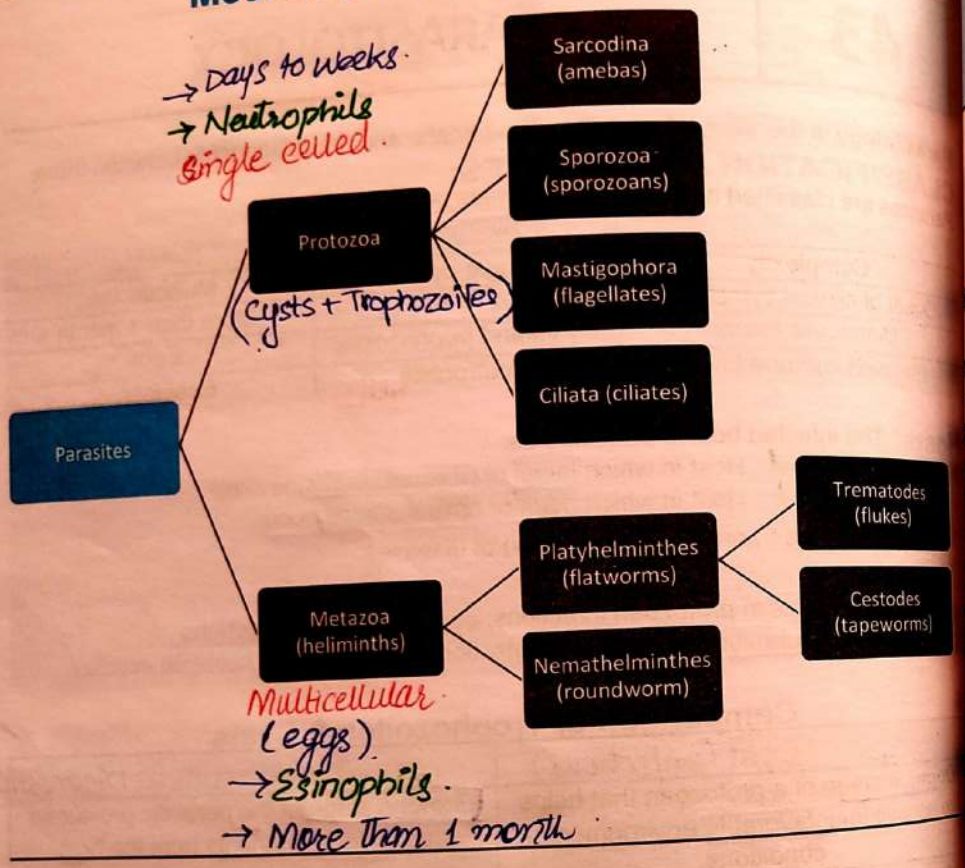
Infectious cysts in protozoan infections; eggs in helminth infections

Diagnostic trophozoite in protozoan infections; eggs/worm in helminth infection

Comparison of Trophozoites & Cysts

Cyst	Trophozoites
rmant stage of a protozoan that helps survive in unfavorable environmental conditions.	Growing stage of the parasitic protozoan that absorbs nutrients from the host.
rmed in a process called **encystation**.	Formed in a process called **excystation**
Dormant stage	Active and reproductive stage
Transmission state	Disease-causing state
Non-motile	Motile
Infective	Non-infective
Resistant to water & desiccation.	Consists of labile cell membrane, and are not resistant structures
Can survive outside the host	Cannot survive outside the host

Medically Important Parasites



→ Days to weeks.
→ Neutrophils
Single celled.
(cysts + Trophozoites)
Multicellular
(eggs)
→ Esinophils.
→ More than 1 month

Diarrhea containing
'BLOOD OR MUCOUS'
'Diarrhea' → Loose watery - more frequent Bowel movements.

44 INTESTINAL & UROGENITAL – PRATOZOA (Neutrophils) (cysts + Trophozoites)

INTESTINAL PROTOZOA

ENTAMOEBA *HISTOLYTICA* (4)

sease:
- Amebic dysentery (LAO)
- Liver abscess

portant Properties:
- Cyst has 4 nuclei
- Mature **trophozoite** has single nucleus (1)

de of Transmission: Fecal-oral transmission

thogenesis:
The ingested cysts **differentiate** into trophozoites in the ileum.
Colonize the cecum and colon.
The trophozoites **invade** the colonic epithelium and secrete enzymes that cause localized necrosis.
The lesion reaches the muscularis layer, a typical **flask-shaped ulcer** form.
Progression into the submucosa leads to invasion of the portal circulation by the trophozoites.
The most frequent site of systemic disease is the **liver,** where abscesses containing trophozoites form. (painful collection of pus)

inical Findings:
- **Acute Intestinal amebiasis** (feeling to pass stool)
  - Dysentery • Flatulence • Tenesmus • Lower abdominal discomfort
- **Chronic Intestinal amebiasis**
  - Diarrhea • Weight loss • Fatigue
- Granulomatous lesion (**ameboma**)
- **Liver Abscess**
  - Right-upper quadrant pain • Weight loss • Fever • Enlarged liver

boratory Diagnosis:
- Stool exaination ⇒ Detection of trophozoites or cyst
- Indirect hemagglutination ⇒ Invasive amebiasis

E. histolytica (4)	*E. coli* (8)
Cyst has 4 nuclei	Cyst has 8 nuclei

GIARDIA LAMBLIA

Disease: Giardiasis

Important Properties:

Cyst	• 4 nuclei	• Thick-walled
Trophozoite	• 2 nuclei	• 4 pairs of flagella
	• Pear-shaped	• Suction disk

Mode of Transmission: Fecal-oral transmission

Pathogenesis:

- The organism is acquired by ingestion of cysts.
- The ingested cysts **differentiate** into trophozoites in the duodenum.
- The trophozoites attaches to the gut wall.
- Does not invade the mucosa.
- Does not enter the bloodstream.
- Causes **inflammation** of the duodenum.
- Leads to **malabsorption** of protein & fat.

Clinical Findings:

- Foul-smelling diarrhea
- Watery diarrhea
- Nausea
- Anorexia
- Flatulence
- Abdominal cramps

Laboratory Diagnosis:

- Stool exaination ⟹ Detection of trophozoites or cyst
- ELISA
- String test

CRYPTOSPORIDIUM

Disease: Cryptosporidiosis

Mode of Transmission: Fecal-oral transmission

Pathogenesis:

- The organism is acquired by ingestion of oocysts.
- The ingested oocysts **differentiate** into trophozoites in the small intestine.
- The trophozoites attaches to the gut wall.
- Does not invade the mucosa.
- **Jejunum** is the site most heavily infested.

Clinical Findings:

- Watery diarrhea
- Fluid loss

Laboratory Diagnosis:

- Kinyoun acid-fast stain ⟹ Detection of oocysts

UROGENITAL PROTOZOA

TRICHOMONAS VAGINALIS

Disease: Trichomoniasis

Important Properties:

- Pear-shaped
- Central nucleus
- 4 flagella
- Exist only as **trophozoite.**

Mode of Transmission: Sexual contact

Pathogenesis:

- Trophozoites attach to wall of vagina.
- Cause inflammation and discharge.

Clinical Findings:

- **Vaginal discharge**
  - Watery
  - Foul-smelling
  - Greenish

Laboratory Diagnosis:

- Wet mount of vaginal discharge ⟹ Pear-shaped trophozoites
- Nucleic acid amplification tests (NAATs)

UNIVERSITY QUESTIONS

1.a) Name 3 protozoan causing intestinal infections
b) Give **pathogenesis** and **lab diagnosis** of Entamoeba Histolytica. **[Annual 2008]**

2. After one week camping in Murree, a 20-year-old medical student presents with abdominal pain, nausea, bloody diarrhea and fever. Stool specimens are sent to laboratory. Bacterial cultures are negative for intestinal pathogenesis but stool examination shows organisms with red blood cells inside them.
a) What is likely **diagnosis**? Name the **causative agent**.
b) Give the **lifecycle** of this intestinal protozoa. **[Annual 2009]**

3. Name diseases caused by each of the following protozoan and how are they transmitted to humans:
a) Entamoeba Histolytica
b) Giardia. **[Supple 2010]**

4. A young male presents with severe cramping, abdominal pain, fever and passage of scanty stool containing blood and mucus. A parasitic infection is suspected.
a) What will be **microscopic findings** of his fresh stool?
b) Briefly describe the **lifecycle** of this parasite. **[Supple 2011]**

5. Enumerate 2 parasites which infest human beings through orofecal route of transmission. **[Annual 2014]**

6. After one-week hiking trip in 'Nathia Gali', a third-year medical student presents I medical OPD with severe crampy abdominal pain, fever and passage of scanty stools containing blood and mucous. Stool specimen sent to microbiology laboratory is negative for bacterial pathogens on culture but microscopic examination reveals parasitic organisms with red blood cells inside them.
a) What is the most likely **diagnosis**? Name the **causative organism**. **(1+1)**
b) Give the **pathogenesis** and the **infective** form of this protozoan parasite. **(2+1)**
[Annual 2018]

45 BLOOD & TISSUE PROTOZOA

PLASMODIUM

Disease: Malaria

Species:

Species	Blood smears	Fever spikes
P. vivax	Enlarged host cells; Ameboid trophozoites	48-hour
P. oval	Oval, jagged, infected RBCs	48-hour
P. malariae	Rosette schizonts RS	72-hour
P. falciparum	Multiple ring forms crescent-shaped gametes	Irregular

Cell Cycle:

Phases of cell cycle

Sexual Cycle	Sporogony (sporozoites are produced)	Occurs in mosquito
Asexual Cycle	Schizogony (schizonts are produced)	Occurs in human

Vector: Female *Anopheles* mosquito

Pathogenesis:

Asexual Schizogony in humans

Exoerythrocytic phase:

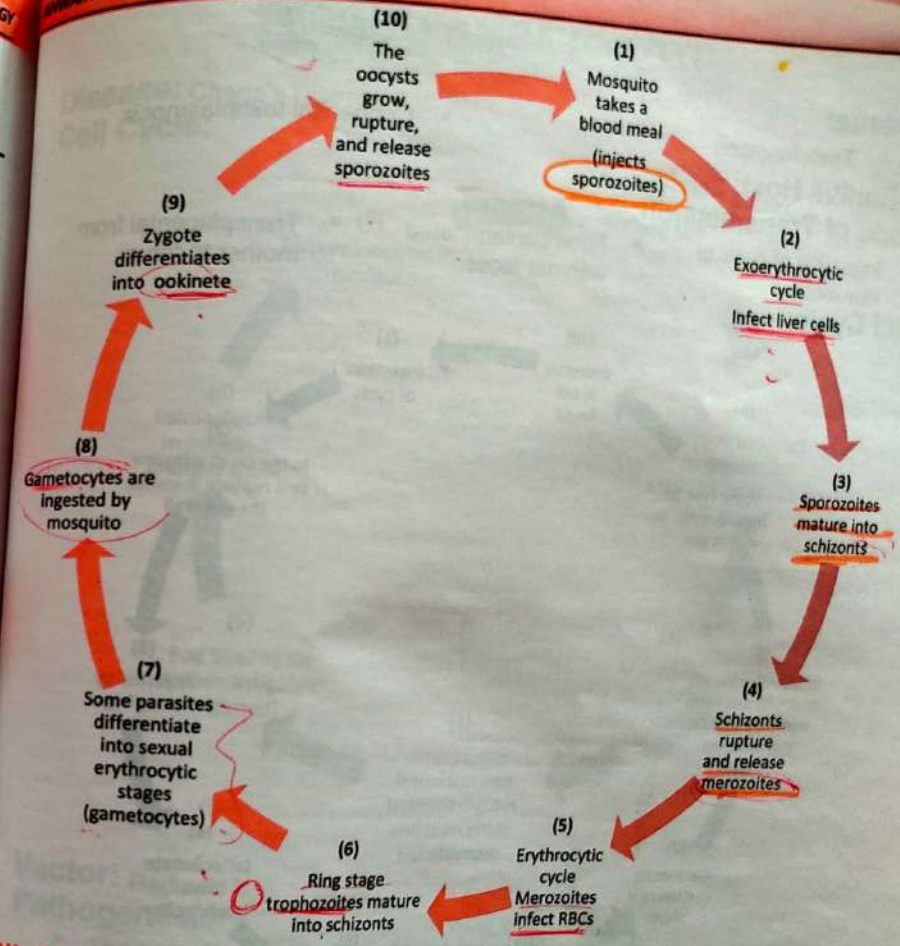
- Mosquito injects **sporozoites** which attack **hepatocytes.**
- Sporozoites replicate & differentiate into **merozoites**
- Merozoites infect RBCs.

Erythrocytic phase:

- Merozoites differentiate into **ring shaped** trophozoites in RBCs.
- Develop into **schizonts** filled with merozoites.
- Merozoites lyse RBCs at regular intervals and further infect RBCs.

Sexual Sporogony in mosquitoes

- Blood merozoites develop into male/female **gametocytes** in RBCs.
- Female mosquito eats these RBCs.
- Form one female **macrogamete** or 8 sperm-like **microgametes** in gut.
- **Diploid zygote** differentiates into **motile ookinete** which burrows through gut wall.
- **Oocyst** with haploid **sporozoites** form on stomach wall
- Sporozoites released and migrate to mosquito **salivary glands.**
- Female mosquito injects **sporozoites** into next human victim



Clinical Findings:

- Fever
- Chills
- Myalgias
- Headache
- Arthralgias
- Nausea
- Vomiting
- Abdominal pain
- Drenching sweats
- Splenomegaly
- Hepatomegaly
- **Blackwater fever**

Complications:

- Cerebral malaria
- Hemolysis
- Acute Renal Failure

Laboratory Diagnosis:

- Giemsa-stained smears
  - Thick smear → Presence of organism
  - Thin smear → Species identification
- ELISA
- PCR

TOXOPLASMA GONDII

Disease:

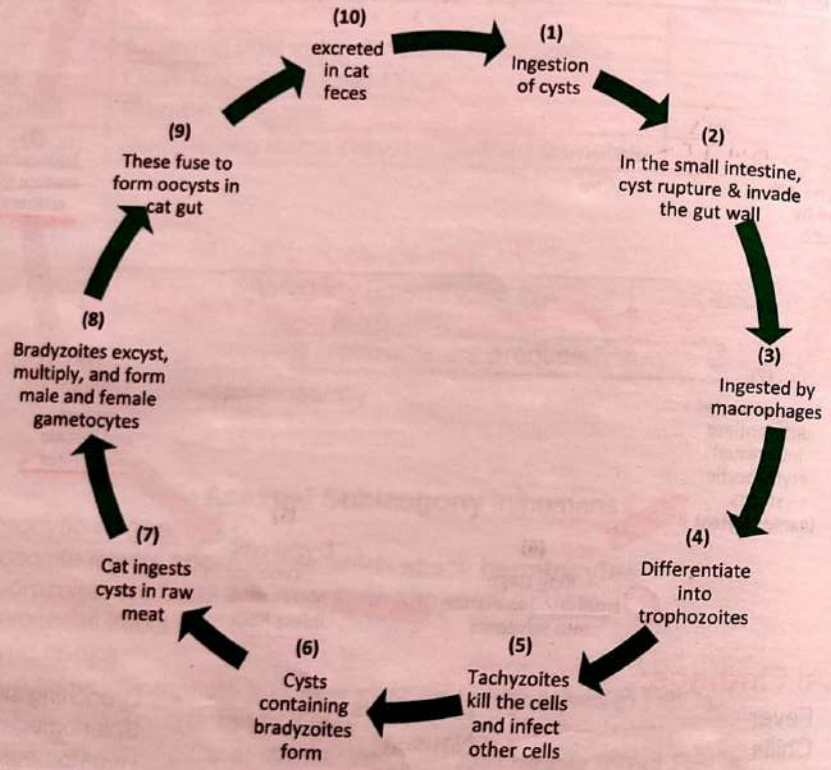
- Toxoplasmosis
- Congenital toxoplasmosis

Definitive Host: Cat

Mode of Transmission:

- Ingestion of cysts in raw meat
- Food contaminated with cat feces.
- Transplacental from mother to fetus

Cell Cycle:



Clinical Findings:

- Encephalitis
- Chorioretinitis
- Hepatosplenomegaly
- Fever
- Jaundice
- Blindness
- Intracranial calcification
- Mental Retardation

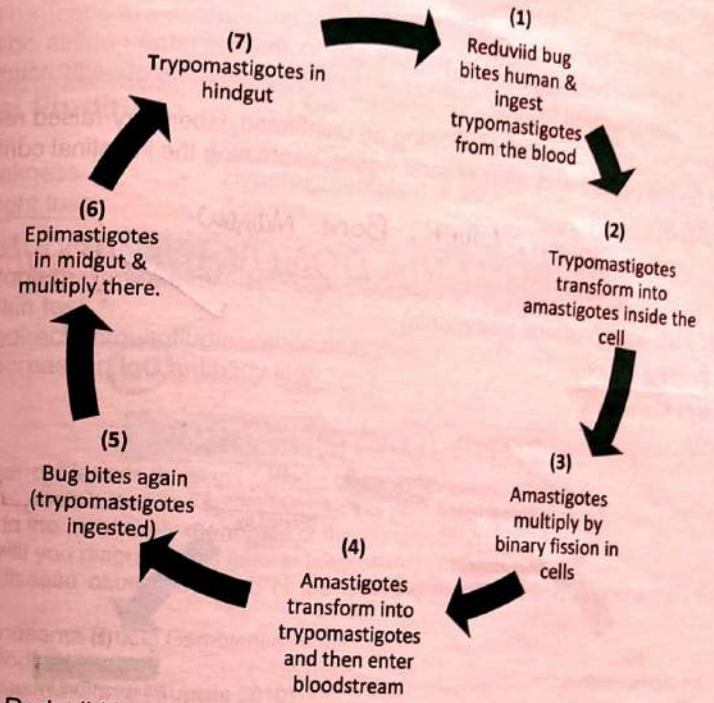
Laboratory Diagnosis:

- Giemsa-stained smears ⇒ Crescent-shaped trophozoites
- Immunofluorescence assay ⇒ **IgM** antibody
- Increase in **IgG** antibody titer ⇒ Diagnosis of acute infections

TRYPANOSOMA CRUZI

Disease: Chaga's Disease

Cell Cycle:



Vector: Reduviid bug

Pathogenesis:

- The **amastigotes** can kill cells and cause inflammation.
- **Cardiac muscle** is the most frequently and severely affected tissue.
- In addition, **neuronal damage** leads to cardiac arrhythmias and loss of tone in the colon (**megacolon**) & esophagus (**megaesophagus**).
- During the acute phase, there are both trypomastigotes in the blood and amastigotes intracellularly in the tissues.
- In the chronic phase, the organism persists in the amastigote form.

Clinical Findings:

Acute Phase	• Edematous nodule (**Chagoma**)	• Lymphadenopathy • Hepatosplenomegaly • Fever	• Unilateral palpebral swelling (Romaña's sign)
Chronic Phase	• Myocarditis • Arrythmia	• Dilated cardiomyopathy • CHF	• Megacolon • Megaesophagus

Laboratory Diagnosis:

- Thick or thin blood smears ⟹ Trypomastigotes
- Bone marrow biopsy
- Culture in vitro
- Xenodiagnosis
- Serologic tests
  - ✓ Indirect fluorescent antibody test
  - ✓ Indirect hemagglutination
  - ✓ Complement fixation

Xenodiagnosis → Consists of allowing an uninfected, laboratory-raised reduviid bug to feed on the patient and, after several weeks, examining the intestinal contents of the bug for the organism.

Spleen, LIVER, Bone Marrow.

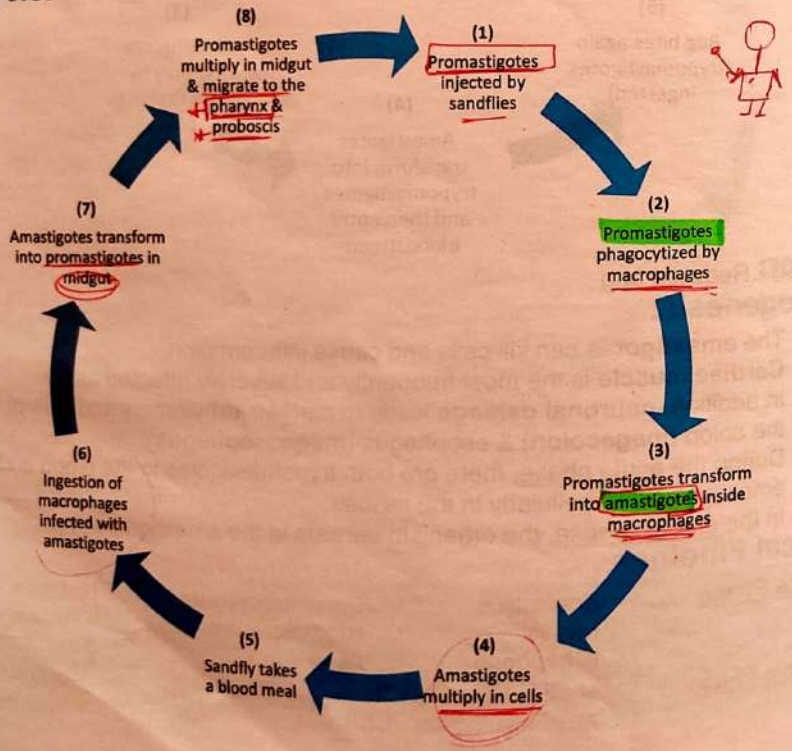
LEISHMANIA *DONOVANI* (PROTOZOA)

Disease: Kala-azar (visceral leishmania)

Vector: Female sandfly

Reservoir: Mammals

Cell Cycle:



Pathogenesis:

- In visceral leishmaniasis, the organs of the **reticuloendothelial** system (liver, spleen, and bone marrow) are the most severely affected.
- **Reduced bone marrow activity**, coupled with cellular destruction in the spleen, results in anemia, leukopenia and thrombocytopenia.
- This leads to secondary infections and a tendency to bleed.
- The striking **enlargement of the spleen** is due to a combination of proliferating macrophages and sequestered blood cells.

Clinical Findings:

- Fever
- Weakness
- Weight loss
- Splenomegaly (Giant)
- Hyperpigmentation of skin
- Anemia
- Leukopenia
- Thrombocytopenia
- GI bleeding

Laboratory Diagnosis:

- Bone marrow smear ⟹ Detection of amastigotes
- Skin test
- Indirect Immunofluorescence assay
- Increase in **IgG** antibody titer

UNIVERSITY QUESTIONS

1. A 20-year-old farmer develops periodic bouts of fever with chills & rigors occurring every 36-48 hours. He is anemic and has splenomegaly. His peripheral smear shows crescentic structures.
   a) What is the most likely **diagnosis** of this complication?
   b) How will you diagnose this case in **laboratory**? **[Annual 2007]**
2. Name disease caused by each of the following protozoan and how are they transmitted to humans:
   a) Trypanosoma Brucei Gambiense
   b) Plasmodium species
   c) Toxoplasma Gondi **[Supple 2010]**
3. A 15-year-old pathan boy presented with history of fever, weight loss, multiple nodules over forearm and dark discoloration of skin. On examination, he had mild splenomegaly and his CBC revealed anemia and thrombocytopenia.
   a) What is your most likely **diagnosis**?
   b) How will you **confirm** your diagnosis? **[Annual 2010]**
4. Describe **pathogenesis** of Plasmodium Falciparum infection. **[Annual 2011]**
5. Name hemo-flagellates infecting humans. **[Annual 2011]**
6. A 30-year-old female experienced sudden onset of fever, shaking chills and profuse sweating. She also has C/O headache and abdominal pain but no nausea, vomiting or diarrhea. There is no rash, neck stiffness or altered consciousness. Blood smear reveals thrombolites within the RBCs.
   a) What is the most likely **diagnosis**? Name the Plasmodium.
   b) Briefly describe the **lifecycle** of Plasmodium. **[Annual 2013]**
7. A 22-year-old watchman developed episodic spells of fever with chills and rigor occurring every 36-48 hours. On examination, he is anemic and has splenomegaly. His peripheral blood smears show crescentic structures. **[Annual 2017]**
   a) What is your likely **diagnosis**? **(1)**
   b) How will you diagnose this condition in **laboratory**? **(2)**
   c) What are the **complications** of this condition? **(2)**

Metazoa → Plathalmenthis (Flat worms)
(Helminths)

46 CESTODES

(Termatodes / flukes)

Tapeworms.
Multicellular.
eggs
More than 1 Month
Esinophilia.

- The cestodes are the tapeworms.
- For the most part, they have complex life cycles involving extraintestinal **larval forms** in intermediate hosts.
- When humans are the intermediate host, these infections are more serious than the intestinal infections with adult tapeworms.
- Diagnosed by finding eggs or proglottids in the feces.
- **Hermaphroditic**, with each proglottid developing both male and female reproductive organs, and mature eggs developing in the most distal proglottids

Structure:

Consists of two main parts;

- Scolex (rounded head)
  - ✓ Adhere to the mucosa
  - ✓ Knobby-looking
  - ✓ Has suckers or a sucking groove
- Proglottids (flat body consisting of multiple segments)

TAENIA *SOLIUM*

Pork Tapeworm

Disease:

- Taeniasis
- Cysticercosis

Important Properties:

- Scolex has four suckers and a **circle of hooks**.
- Gravid proglottids have **5 to 10** uterine branches.

Definitive Host: Humans

Intermediate Host: Pigs or Humans

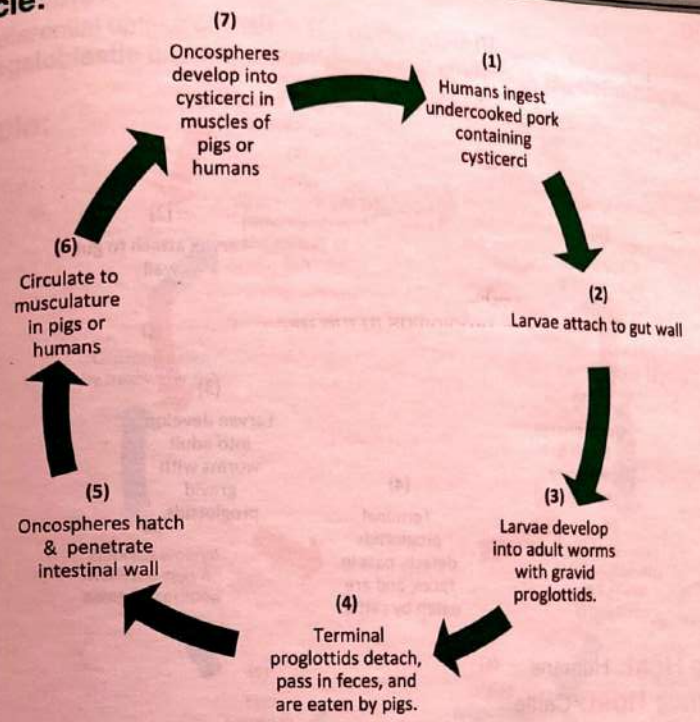
Mode of Transmission

- Ingestion of raw or uncooked pork (Taeniasis)
- Food contaminated with feces (Cysticercosis)

Pathogenesis:

- The adult tapeworm attached to the intestinal wall causes little damage.
- The **cysticerci**, on the other hand, can become very large, especially in the **brain.**
- They manifest as a **space-occupying lesion.**
- Living cysticerci do not cause inflammation.
- When they die, they can release substances that provoke an inflammatory response.

Cell Cycle:



Clinical Findings:

- Anorexia
- Diarrhea
- Retinitis
- Vomiting
- Headache
- Seizures
- Uveitis

Laboratory Diagnosis:

- Stool examination ⟹ Detection of gravid proglottids
- ELISA
- CT scan

TAENIA *SAGINATA*

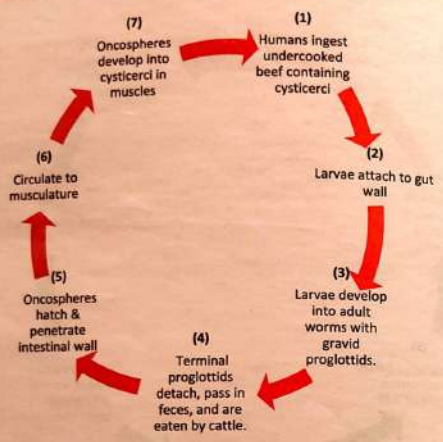
Beef Tapeworm

Disease: Taeniasis

Important Properties:

- Scolex has four suckers and a **no hooklets**.
- Gravid proglottids have **15 to 25** uterine branches.

Cell Cycle:



Definitive Host: Humans
Intermediate Host: Cattle
Mode of Transmission: Ingestion of raw or undercooked beef
Clinical Findings:
- Malaise
- Mild cramps

Laboratory Diagnosis:
- Stool examination ⟹ Detection of gravid proglottids

DIPHYLLOBOTHRIUM *LATUM*

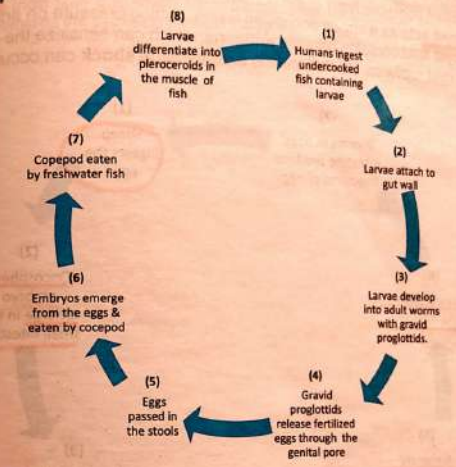
Fish Tapeworm

Disease: Diphyllobothriasis
Important Properties:
- Scolex has two elongated **sucking grooves** and **no hooks**.
- Gravid uterus in the form of **rosette.**
- Proglottids are wider.

Definitive Host: Humans
Intermediate Host: Copepods
Mode of Transmission: Ingestion of raw or uncooked freshwater fish

Pathogenesis:
- Preferential uptake of vitamin B_{12} by the worm.
- **Megaloblastic anemia** occurs as a result of **vitamin B_{12} deficiency.**

Cell Cycle:



Clinical Findings:
- Abdominal discomfort
- Diarrhea

Laboratory Diagnosis:
- Stool examination ⟹ Detection of eggs

ECHINOCOCCUS *GRANULOSA*

Dog Tapeworm

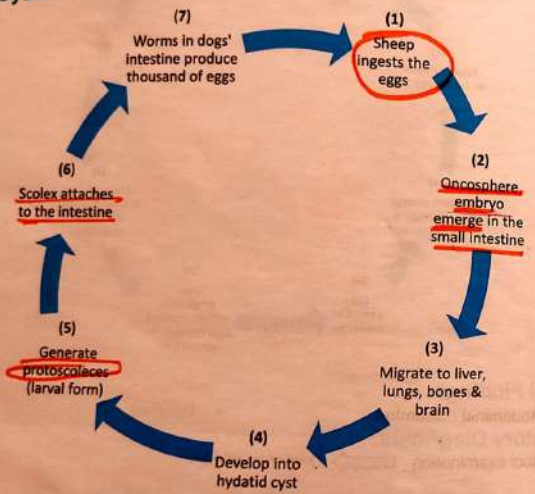
Disease: Unilocular hydatid cyst disease
Important Properties:
- Scolex has four suckers and a **circle of hooks.**
- **Three** proglottids

Definitive Host: Dogs
Intermediate Host: Sheep or Humans
Mode of Transmission: Food contaminated with dogs' feces

Pathogenesis:

- *Echinococcus granulosus* usually forms one large fluid-filled cyst (**unilocular**) that contains thousands of individual scoleces as well as many daughter cysts within the large cyst.
- Individual scoleces lying at the bottom of the large cyst are called **hydatid sand.**
- The cyst acts as a space occupying lesion, putting pressure on adjacent tissue.
- The cyst fluid contains parasite **antigens**, which can sensitize the host.
- If the cyst ruptures, life-threatening **anaphylactic shock** can occur.

Cell Cycle:



Clinical Findings:

- Hepatic dysfunction (liver cyst)
- Bloody sputum (lung cyst)
- Headache (brain cyst)
- Focal neurological signs
- Anaphylactic shock (rupture of cyst)

Laboratory Diagnosis:

- Microscopic examination ⟹ Brood capsules containing multiple protoscoleces
- Indirect Hemagglutination test

UNIVERSITY QUESTIONS

1. A 40 years old shepherd of sheep presents with upper right quadrant pain and appears slightly jaundiced. A stool exam was negative for ova and parasites but a CT scan reveals a large 14 cm cyst that appears to contain fluid, in the right lobe of the liver.
   a) What is the most likely **diagnosis**? Name the **parasites** responsible for this lesion. (2)
   b) Draw and label its **life cycle**. (3) **[Annual 2016]**

47 (eggs) → TREMATODES → flukes

→ Detection of Ova
→ Esinophilia

SCHISTOSOMA
Blood Flukes

Disease: Schistosomiasis

Species: mi-Haema

S. mansoni	Large lateral spine	affects veins of colon
S. japonicum	Small lateral spine	affects veins of small intestine & liver
S. haematobium	Large terminal spine	affects veins of urinary bladder

Important Properties:
- Either male or female.
- Eggs are distinguished by spines.

Definitive Host: Humans

Intermediate Host: Snails (a free Swimming Larval Stage in which parasitic fluke passes from Intermediate host to another Intermediate Host or to final vertebrate host.)

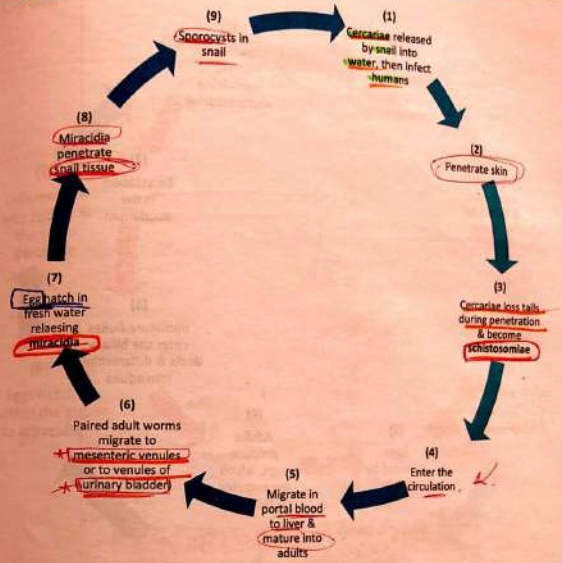
Mode of Transmission: Penetration of skin by cercariae

Pathogenesis:
- Eggs in the liver induce **granulomas**, which lead to fibrosis, hepatomegaly, and portal hypertension.
- Portal hypertension leads to **splenomegaly**.

Clinical Findings:
- Itching
- Dermatitis
- Fever
- Chills
- Diarrhea
- Lymphadenopathy
- Hepatosplenomegaly

Laboratory Diagnosis:
- Stool & urine examination ⟹ Detection of ova.
- Moderate eosinophilia

Cell Cycle:



CLONORCHIS
Liver Fluke

Disease: Clonorchiasis

Important Properties: Hermaphrodite

Definitive Host: Humans

Intermediate Host: Snails & Fish

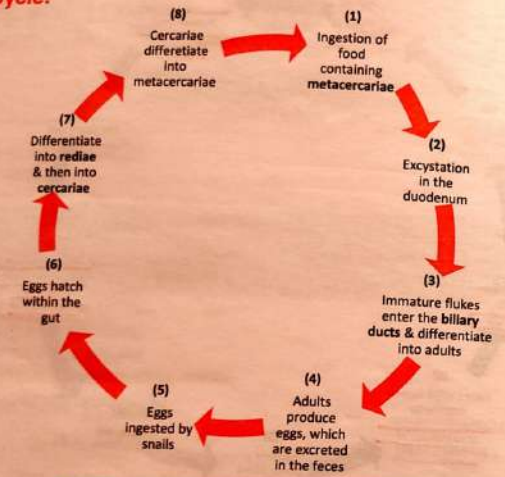
Mode of Transmission: Ingestion of raw or undercooked freshwater fish.

Pathogenesis:
- Inflammatory response can cause hyperplasia & fibrosis of the biliary duct.

Clinical Findings:
- Upper abdominal pain
- Anorexia
- Hepatomegaly
- Eosinophilia

Laboratory Diagnosis:
Stool examination ⟹ Detection of ova.

Cell Cycle:



PARAGONIMUS

Lung Fluke

Disease: Paragonimiasis
Important Properties: Hermaphrodite
Definitive Host: Humans
Intermediate Host: Snails & Crab
Mode of Transmission: Ingestion of raw or undercooked crab meat.
Pathogenesis:

- Within the lung, the worms exist in a fibrous capsule that communicates with a bronchiole.
- Secondary bacterial infection frequently occurs, resulting in **bloody sputum**.

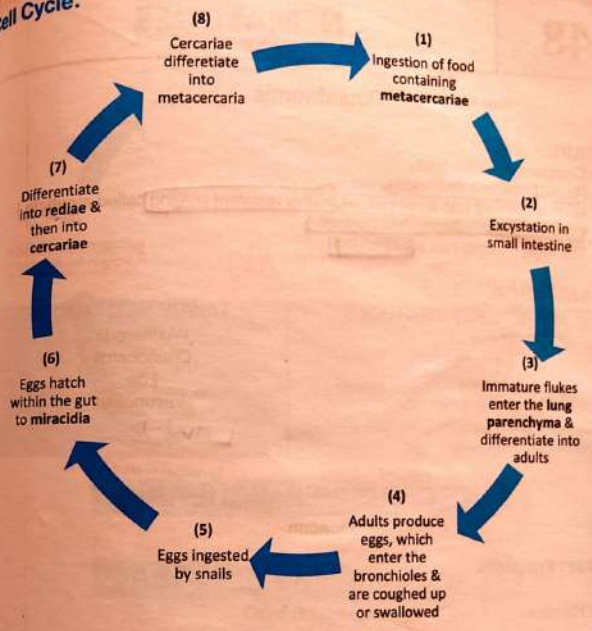
Clinical Findings:

- Chronic cough
- Bloody sputum
- Dyspnea
- Pleuritic chest pain
- Resemble tuberculosis

Laboratory Diagnosis:

- Stool & Sputum examination ⟹ Detection of operculated ova.

Cell Cycle:



UNIVERSITY QUESTIONS

1. A 25-year-old Egyptian living in Cairo complains of episodes of passing blood in urine. There is no pain or urethral discharge. Physician examination reveals no penile lesions. Urine analysis shows many red cells, no white cells and several large eggs with terminal spine. **[Supple 2015]**
a) What is the **diagnosis** and name of causative **agent**?
b) Give **pathogenesis** and **pathology** of this condition.
c) How humans acquire this infection?

48 NEMATODES

Roundworms

Structure:

- Complete digestive tract.
- Round unsegmented bodies.
- Body is covered with a noncellular, highly resistant coating called a cuticle.
- Female is usually larger than the male.
- The male typically has a coiled tail.

Classification:

INTESTINAL NEMATODES	TISSUE NEMATODES
Enterobius	*Wuchereria*
Trichuris	*Onchocerca*
Ascaris	*Loa*
Ancylostoma & Necator	*Dracunculus*
Strongyloides	
Trichinella	

ENTROBIUS VERMICULARIS

Pinworm

Disease: Entrobiasis

Host: Humans

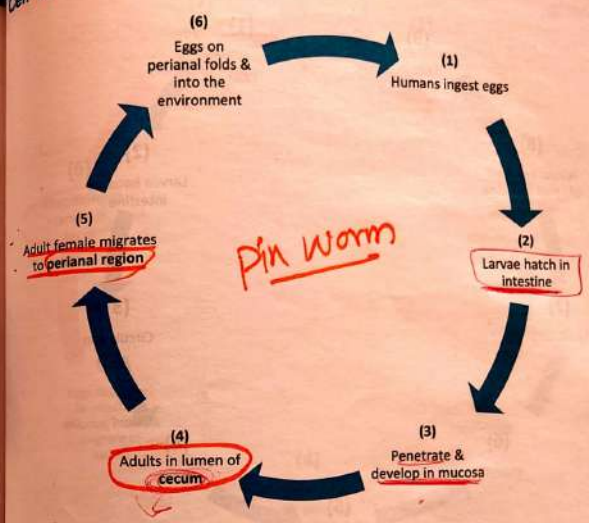
Mode of Transmission: Ingestion of worm eggs.

Clinical Findings: Perianal pruritis

Laboratory Diagnosis:

- Stool examination ⟹ Detection of small, whitish, adult worms.
- Scotch tape technique

Cell Cycle:



ASCARIS *LUMBRICOIDES*

Giant Roundworm

Disease: Ascariasis

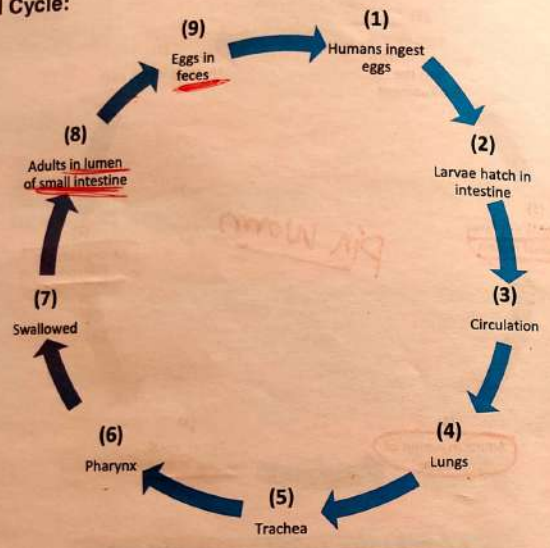
Mode of Transmission: Ingestion of contaminated food

Clinical Findings: Pneumonia

Laboratory Diagnosis:

- Stool examination ⟹ Detection of eggs.
- Eosinophilia occurs

Cell Cycle:



STRONGYLOIDS

Threadworm

Disease: Strongyloidiasis

Mode of Transmission: Skin penetration

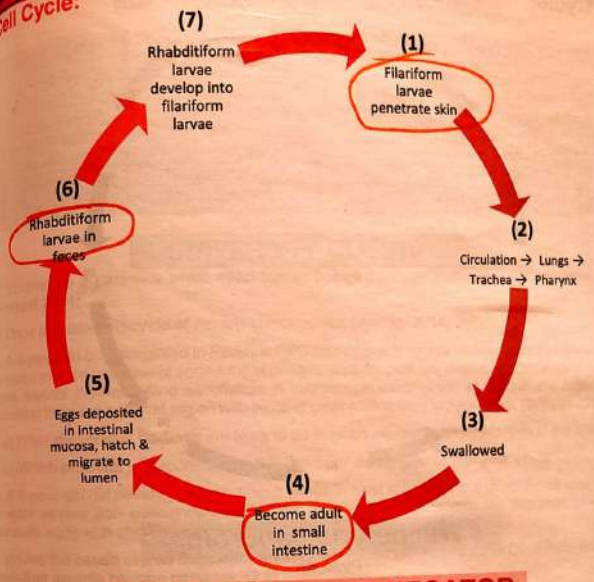
Clinical Findings:

- Pneumonia
- Watery diarrhea
- Pruritis
- Abdominal pain
- Fluctuating rashes

Laboratory Diagnosis:

- Stool examination ⟹ Detection of larvae.
- Eosinophilia occurs
- ELISA

Cell Cycle:



ANCYLOSTOMA & NECATOR

Hookworm

Mode of Transmission: Skin penetration

Pathogenesis:

- The major damage is due to the **loss of blood** at the site of attachment in the small intestine.
- Blood is consumed by the worm and oozes from the site in response to an **anticoagulant** made by the worm. M.C.Q.
- Weakness and pallor accompany the **microcytic anemia** caused by blood loss.

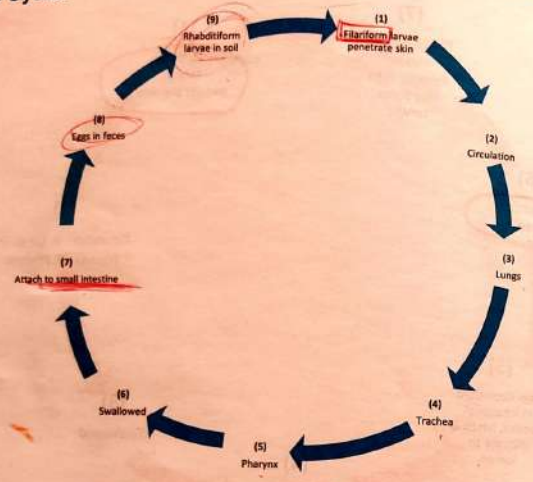
Clinical Findings:

- Pneumonia
- Weakness
- Pallor
- Microcytic anemia
- Pruritic papule

Laboratory Diagnosis:

- Stool examination ⟹ Detection of larvae.

Cell Cycle:



Wuchereria bancrofti

Pinworm

Disease: Filariasis
Host: Humans
Mode of Transmission: Female mosquitoes bite (*Anopheles* and *Culex*)
Clinical Findings: Elephantiasis
Laboratory Diagnosis:
- Blood smear ⟹ Microfilariae

Parasites causing Anemia

Parasites	Type of anemia	Mechanism
P. falciparum	Normochromic	Hemolysis; Hypersplenism
L. donovani	Normochromic	Spleen & bone marrow involvement
T. gambiense	Normochromic	Hemolysis; reduced bone marrow activity
A. duodenale	Hypochromic microcytic	Hemorrhage
N. americanus		
S. haematobium	Normochromic	Hemorrhage
T. trichiura	Normochromic	Hemorrhage

UNIVERSITY QUESTIONS

1. Enumerate 2 parasites which infect human beings thorough **orofecal route** of transmission. **[Annual 2014]**

2. Draw and label **lifecycle** of Ascaris Lumbricoides. **[Annual 2014]**

3. A 4-year-old boy presented in Pediatric OPD with anal itching. His mother says that he is unable to sleep at night because of scratching of perianal area for past few days. On examination, perianal area reveals erythema and excoriation. A microscopic examination of the sample collected by touching from the perianal region with piece of clear scotch tape is performed. **[Supple 2016]**

a) What **diagnostic findings** are likely to be seen on microscopic examination of the sample?

b) Which **parasite** is responsible for this infection? Briefly give its **lifecycle**. **(1+3)**

4. A 9-year-old girl severely anemic from a village in Swat is brought to a clinic. Blood examination reveals severe iron deficiency anemia. Her stool examination shows the presence of several parasitic eggs with segmented ovum suggestive of Ancylostoma Duodenale.

a) Give the **cause of iron deficiency anemia** associated with Ancylostoma Duodenale and name two other anemia causing parasites. **(2.5)**

b) Draw and label in detail the **lifecycle** of this parasite. **(2.5) [Supple 2016]**

5. A 10-year-old malnourished boy is brought to pediatrician with complaints of persistent stomach ache for last two days. Stool examination reveals several typical eggs of Ascaris Lumbricoides. **[Supple 2016]**

a) Draw and label the **lifecycle** of this worm. **(2)**

b) Enlist the **pathogenic effects** produced by its larval and adult forms. **(3)**

6.a) Draw and label the **lifecycle** of Hookworm. **(3) [Supple 2017 held in 2018]**

b) What is the **cause of microcytic hypochromic anemia** in these patients? **(2)**

7.a) Diagrammatically explain the **lifecycle** of the largest intestinal nematode responsible for mechanical obstruction of the intestines together with its two important **clinical manifestations**.

b) Enlist two **tissue nematodes** with their vector. **[Supple 2018 held in 2019]**

8. An outbreak of mild intestinal distress, sleeplessness and perianal itching broke out among preschool children in a daycare center. Stool examination show several ova of enterobius vermicularis.

a) Diagrammatically explain the **life cycle** of this parasite enlist its different **mode of transmission. (3)**

b) Enlist two medically important **hemoparasite** and 2 tissue **nematodes. (2) [Annual 2020]**

Practice MCQs

1. A 65-year-old man undergoes cardiac bypass surgery and is placed on postoperative, broad-spectrum, antibiotic prophylaxis. Several days later, he develops fever, abdominal Pain, and bloody diarrhea. Colonoscopic biopsy demonstrates a thick mucopurulent exudate. Which of the following is the most likely etiology of this patient's gastrointestinal disorder?
a. Clostridium botulinum
b. Clostridium difficile
c. Clostridium perfringens
d. Clostridium tetani

Answer: B

2. A 33-year-old male in an underdeveloped country presents with a markedly edematous right foot that has multiple draining sinuses. A Gram stain from one of these draining sinuses reveals gram-positive filamentous bacteria that are partially acid-fast. The organism causing this abnormality is
a. Actinomyces israeli
b. Corynebacterium diphtheriae
c. Listeria monocytogenes
d. Nocardia asteroides

Answer: D

3. A 38-year-old male presents with right lower quadrant abdominal pain, fever, and a peripheral neutrophilia. An emergency appendectomy is performed, but the appendix is found to be grossly unremarkable. Instead, the lymph nodes surrounding the appendix are found to be enlarged, inflamed, and matted together. Which one of the listed organisms is the most likely cause of these abnormalities?
a. Enteropathic Escherichia coli
b. Enterobius vermicularis
c. Trichomonas hominis
d. Yersinia enterocolitica

Answer: D

4. Finding acid-fast bacilli within peripheral nerves is most suggestive of
a. Relapsing fever
b. Syphilis
c. Leprosy
d. Tuberculosis

Answer: C

5. An 8-year-old boy is found to have progressive corneal vascularization, deafness, notched incisors, and a flattened nose. The most likely cause of these changes is congenital infection by
a. Toxoplasma
b. Rubella
c. Cytomegalovirus
d. T. pallidum

Answer: D

6. Which of the following is associated with a deficiency of third component of complement C3:
a. Pyogenic infection
b. Immune complex disease
c. Systemic lupus erythematosus
d. Glomerulonephritis.

Answer: A

7. Which of the following components enhances the binding of antigen antibody complex to macrophages:
a. C1
b. C3a
c. C3b
d. C8

Answer: C

8. A secretary piece is attached to IgA:
a. In plasma cells
b. In epithelial cells
c. By T-cells
d. By macrophages.

Answer: B

9. The anti-phagocytic property of the group A streptococcus is associated with which of the following:
a. Hyaluronidase
b. Streptolysin S
c. M protein
d. Peptidoglycan.

Answer: C

10. Which of the following is correct sequence of steps in performing Gram Stain:
a. Safranin stain, crystal violet stain, iodine solution.
b. Crystal violet stain, decolorization, safranin stain, iodine solution.
c. Safranin stain, iodine solution, decolorization, crystal violet stain.
d. Crystal violet stain, iodine solution, decolorization, safranin stain

Answer: D

11. The most reliable method for diagnosis of primary syphilis is:

a. VDRL Test
b. FTA-ABS
c. Micro-hemagglutinin
d. Dark field examination of chancre material.

Answer: C

12. The pathogenesis of which of the following organisms is most likely to involve invasion of the intestinal mucosa:
a. Vibrio cholerae.
b. Enterotoxigenic E. coli.
c. Shigella somei
d. Clostridium botulinum.

Answer: C

13. Which of the following tests does not correspond with the respective disease:
a. Casoni's Test for Hydatid disease
b. Frei's Test in Infectious mononuclease
c. Schick's Test for Diphtheria
d. Wasserman's Test for syphilis.

Answer: B

14. Your patient is a 30-year-old woman with non-bloody diarrhea for the past 14 hours. Which one of the following organisms is LEAST likely to cause this illness?
a. Clostridium difficile
b. Streptococcus pyogenes
c. Shigella dysenteriae
d. Salmonella enteritidis

Answer: B

15. A 50-year-old homeless alcoholic has a fever and is coughing up 1 cup of green, foul-smelling sputum per day. You suspect that he may have a lung abscess. Which one of the following pairs of organisms is MOST likely to be the cause?
a. Listeria monocytogenes and Legionella pneumophila
b. Nocardia asteroides and Mycoplasma pneumoniae
c. Fusobacterium nucleatum and Peptostreptococcus intermedius
d. Clostridium perfringens and Chlamydia psittaci

Answer: C

16. Your patient has subacute bacterial endocarditis caused by a member of the viridans group of streptococci. Which one of the following sites is MOST likely to be the source of the organism?
a. Skin
b. Colon
c. Oropharynx
d. Urethra

Answer: C

17. A culture of skin lesions from a patient with pyoderma (impetigo) shows numerous colonies surrounded by a zone of beta hemolysis on a blood agar plate. A Gram-stained smear shows gram-positive cocci. If you found the catalase test to be negative, which one of the following organisms would you MOST probably have isolated?
a. Streptococcus pyogenes
b. Staphylococcus aureus
c. Staphylococcus epidermidis
d. Streptococcus pneumonia

Answer: A

18. Five hours after eating reheated rice at a restaurant, a 24-year-old woman and her husband both developed nausea, vomiting, and diarrhea. Which one of the following organisms is the MOST likely to be involved?
a. Clostridium perfringens
b. Escherichia coli
c. Bacillus cereus
d. Salmonella typhi

Answer: C

19. Your patient has subacute bacterial endocarditis caused by a member of the viridans group streptococci. Which one of the following sites is MOST likely to be the source of the organism?
a. Skin
b. Colon
c. Oropharynx
d. Urethra

Answer: C

20. Which one of the following is a virulence factor for Staphylococcus aureus?
a. A heat-labile toxin that inhibits glycine release at the internuncial neuron
b. An oxygen-labile hemolysin
c. Resistance to novobiocin
d. Protein A that binds to the Fc portion of IgG

Answer: D

21. A primary gram stain shows filamentous, branching gram positive rods. A modified acid-fast stain from the same specimen reveals that the bacteria are modified acid fast positive. The organism is most likely to be which of the following
a. Actinomyces
b. Streptomyces
c. Nocardia
d. Mycobacterium tuberculosis

Answer: C

22. Swimming pool granuloma is caused by of
a. M. tuberculosis
b. M. chelonei
c. M. ulceranes
d. M. marinum

Answer: D

23. Five hours after eating reheated rice at a restaurant, a 24-year-old woman and her husband both developed nausea, vomiting, and diarrhea. Which one of the following organisms is the MOST likely to be involved?
a. Clostridium perfringens
b. Escherichia coli
c. Bacillus cereus
d. Salmonella typhi

Answer: C

24. A 65-year-old man develops dysuria and hematuria. A Gram stain of a urine sample shows Gram negative rods. Culture of the urine on EMB agar reveals lactose-negative colonies without evidence of swarming motility. Which one of the following organisms is MOST likely to be the cause of his urinary tract Infection?
a. Enterococcus faecalis
b. Pseudomonas aeruginosa
c. Proteus vulgaris
d. Escherichia coli

Answer: B

25. A 25-year-old man complains of a urethral discharge. You perform a Gram stain on a specimen of the discharge and see neutrophils but no bacteria. Of the organisms listed, the one MOST likely to cause the discharge is:
a. Treponema pallidum
b. Chlamydia trachomatis
c. Candida albicans
d. Coxiella burnetii

Answer: B

26. Which of the following is a test used to differentiate between Streptococcus pneumonia and Viridans streptococci? '.
a. Bacitracin Susceptibility test
b. Optochin susceptibility test
c. Novobiocin susceptibility test
d. Vancomycin susceptibility test

Answer: B

27. A 30-year-old woman with systemic lupus erythematosus is found to have a positive serology test for syphilis (VORL test). She denies having had sexual contact with a Partner who had symptoms of a venereal disease. The next best step would be to:
a. Reassure her that the test is a false-positive reaction related to her autoimmune disorder
b. Trace her sexual contacts for serologic testing
c. Treat her with penicillin
d. Performa fluorescent treponemal antibody-absorbed (FTA-ABS) test on her serum

Answer: D

28. Which of the following bacterial substance binds to the Fc portion of immunoglobulin molecules:
a. Endotoxin
b. Coagulase
c. Lipoteichoic acid
d. Protein A.

Answer: D

29. An outbreak of sepsis caused by Staphylococcus aureus has occurred in the newborn nursery. You are called upon to investigate. According to your knowledge of the normal flora, what is the most likely source of the organism?
a. Nose
b. Colon
c. Vagina
d. Throat

Answer: B

30. Which of the following bacteria causing sexually transmitted disease cannot be grown on artificial media?
a. Neisseria gonorrhoeae
b. Chlamydia trachomatis
c. Treponema pallidum
d. Treponema pallidum and Chlamydia trachomatis

Answer: D

31. Patient presented to the OPD with clinical features of pneumonia. Sputum examination of the patient revealed a gram-positive-cocci with alpha hemolysis on sheep agar. Which test will you do to confirm the diagnosis?

a. Coagulase test
b. Bacitracin sensitivity
c. CAMP test
d. Bile solubility

Answer: D

32. The bacterial cells are at their metabolic peak during
a. Lag phase b. Log c. Stationary d. Decline

Answer: B

33. Which one of the following organisms is MOST likely to-be the cause of pneumonia in an immunocompetent young adult?
a. Nocardia asteroids
b. Serratia marcescens
c. Mycoplasma pneumonia
d. Legionella pneumophila

Answer: C

34. A 30-year-old male presents with multiple soft, raised, beefy-red superficial ulcers in his left groin. Physical examination reveals several enlarged left Inguinal lymph nodes. A histological section from an enlarged lymph node that Is stained with a silver, stain reveals characteristic Donovan bodies within macrophages. What Is the most likely diagnosis?
a. Chancroid
b. Gonorrhea
c. Granuloma inguinale
d. Lymphogranuloma venereum

Answer: C

35. A 35-year-old female who lives in the southeastern portion of the United States and likes to hike in the Great Smoky Mountains presents with a spotted rash that started on her extremities and spread to her trunk and face. A biopsy of one of these lesions reveals necrosis and reactive hyperplasia of blood vessels. What Is the most likely causative agent of her disease?
a. Bartonella henselae
b. Bartonella quintana
c. Coxiella burnetii
d. Rickettsia Quintana

Answer: D

36. Neisseria gonorrhoeae is able to ferment which of the following carbohydrates:
a. Glucose only
b. Glucose and maltose.
c. Glucose, maltose, and sucrose-
d. Glucose, maltose, sucrose, and lactose

Answer: A

Virology

1. General steps in viral multiplication cycle are
a. Adsorption, penetration, replication, maturation and release.
b. Endocytosis, uncoating, replication, assembly and budding.
c. Adsorption, uncoating, duplication, assembly and lysis.
d. Endocytosis, penetration, replication, maturation, exocytosis.

Answer: B

2. Two tests are used to detect the presence of HIV infections are:
a. Agglutination and neutralization reactions. 7
b. Compliment fixation and immunofluorescence tests.
c. ELIZA and Western Blotting.
d. Hemagglutination and Comb's Test.

Answer: C

3. A 23-year-old female presents with the recent onset of vaginal discharge. Physical examination reveals multiple clear vesicles on her vulva and vagina. A smear of material obtained from one of these vesicles reveals several multinucleated giant cells with intranuclear inclusions and ground glass nuclei. These vesicles are most likely the result of an infection with
a. Cytomegalovirus (CMV)
b. Herpes simplex virus (HSV)
c. Human papillomavirus (HPV)
d. Candida albicans

Answer: B

4. The typical clinical syndrome associated with rotavirus infection is:
a. Acute gastroenteritis of young adults.
b. Acute bronchitis of infants.
c. Nausea, vomiting and diarrhea in infants and very young children
d. Acute viral hepatitis.

Answer: C

5. A 40-year-old male Is diagnosed to have acute viral hepatitis B. He would be considered to be highly infectious if he Is positive for:
a. HBsAg
b. Anti-HBs

c. HBeAg
d. Anti-HBe

Answer: C

6. What Is the vector of Dengue Fever?
a. Aedes aegypti
b. Anopheles mosquito
c. Aedes albopictus
d. Deer fly

Answer: A

7. Virus belonging to which of the following groups, are likely to establish latent infections?
a. Herpes viruses
b. Poxviruses.
c. Filoviruses
d. Influenza viruses.

Answer: A

8. A 20-year-old medical student had acute onset of fever, nausea, and pain in right hypochondrium. He had jaundice and observed passing dark urine. On investigations he was positive for HAV IgM, but negative for other markers of viral hepatitis. The physician-can inform him that:
a. He acquired the infection from a recent injection for vaccination.
b. He is likely to develop chronic hepatitis.
c. He is at a risk of developing hepatocellular carcinoma.
d. He may transmit the infection to other students by person to person spread for up to 2weeks.

Answer: D

9. What is the most common cause of aseptic meningitis of viral etiology?
a. Enteroviruses
b. Herpesviruses
c. Arboviruses
d. Retroviruses

Answer: A

10. The finding of large, multinucleated, clumps of cells in the bronchial secretions of a 2-year-old girl with acute bronchopneumonia suggests that this infection is caused by:
a. Bordetella pertussis b. Epstein-Barr virus
c. Rhinovirus d. Respiratory syncytial virus (RSV)

Answer: D

11. Which of the following is not an RNA virus?
a. Retrovirus b. Enterovirus
c. Adenovirus d. Rubella virus

Answer: C

12. The following statements are true of Varicella-Zoster Virus
a. Causes a maculopapular rash
b. Respond to AZT therapy
c. Recurrent episodes of Shingles usually occur
d. Remains latent in sensory ganglia following Primary infection

Answer: D

13. Which one of the following immunizations should.be administered immediately after birth
a. Haemophilus influenzae type b vaccine
b. HIV Vaccine
c. Diphtheria-pertussis-tetanus (DPT) vaccine
d. Hepatitis B vaccine

Answer: D

14. A large number of patients presented to emergency departments of the Allied hospitals during an outbreak of dengue fever. Which of the following is the Most Rapid test for the diagnosis of this disease?
a. Determination of IgG-levels in the patient's blood.
b. Determination of IgM levels in the patient's blood.
c. Determination of Hemoglobin levels.
d. Determination of NS1 antigen in the blood.

Answer: D

15. A chronic carrier state may occur in the following:
a. Smallpox Infection
b. Hepatitis A
c. Hepatitis C
d. Hantavirus Infection

Answer: C

16. Which of the following conditions is not rightly against its causative agent:
a. Squamous cell carcinoma cervix = HPV (16, 18).
b. Nasopharyngeal carcinoma = EBV
c. Hepatocellular carcinoma = HDV.
d. Gastric lymphoma = H. Pylori.

Answer: D

17. Which of the following is an RNA virus?
a. Human papilloma virus.
b. Human T-cell leukemia virus.
c. Hepatitis B-virus.
d. Epstein Barr virus.

Answer: B

18. Which of the following serum component is an indicator of post infection and subsequent immunity to hepatitis B-viral infection:
a. HBS Ag b. HBC Ag
c. HBe Ag d. Anti HBS

Answer: D

19. Which one of the following forms of immunity to viruses would be LEAST likely to be lifelong?
a. Passive immunity
b. Passive—active immunity
c. Active immunity
d. Cell-mediated immunity

Answer: A

20. A 30-year-old man develops fever and jaundice. He consults a physician, who finds that blood tests for HBs antigen and anti-HBs antibody are negative. Which one of the following additional tests is MOST useful to establish that the hepatitis was indeed due to hepatitis B virus?
a. HBe antigen
b. Anti-HBc antibody
c. Anti-HBe antibody
d. Delta antigen

Answer: B

21. The routine screening of transfused blood for HBs antigen has not eliminated the problem of post-transfusion hepatitis. For which one of the following viruses has screening eliminated a large number of cases of post-transfusion hepatitis?
a. Hepatitis A virus
b. Hepatitis C virus
c. Cytomegalovirus
d. Epstein-Barr virus

Answer: B

22. Which one of the following is the BEST evidence on which to base a decisive diagnosis of acute mumps disease?
a. A positive skin test
b. A fourfold rise in antibody titer to mumps antigen
c. A history of exposure to a child with mumps
d. Orchitis in young adult male

Answer: B

Parasitology

1. Megaloblastic anemia is most commonly seen in infection with which of the following helminth?
a. Entamoeba histolytica
b. Leishmania donovani
c. Schistosoma haematobium
d. Diphyllobothrium latum

Answer: D

2. A 44-year-old female diabetic living on Martha's Vineyard develops the sudden onset of chills and fever. Her symptoms result from destruction of erythrocytes by a particular organism which was transmitted by the hard-shell tick (ixodid). What Is this organism?
a. Plasmodia vivax b. Plasmodia ovale
c. Leishmania donovani d. Babesia microti

Answer: D

3. Black water fever Is a special manifestation of malaria caused by:
a. P. falciparum b. P, malariae
c. P. ovale d. P. vivax

Answer: A

4. The main factor responsible for worldwide distribution of Entamoeba histolytica Is:
a. Extreme antigenic variation
b. Usual stability of its cysts in the environment
c. Wide spread distribution of mosquitoes
d. Poor hygienic conditions of individuals

Answer: B

5. All of the following characteristics are seen in the stool of Amoebic dysentery except one:
a. RBCs in clumps
b. Charcat Leyden crystals.
c. Eosinophils
d. Ghost cells.

Answer: D

6. Parasite Induced pernicious anemia Is caused by:
a. Taenia saginata
b. Taenia solium
c. Diphyllobothrium latum
d. Echinococcus granulosus

Answer: C

7. In malaria the form of plasmodium transmitted to man from mosquito is:
a. Sporozoites
b. Gametocytes
c. Merozoites.
d. Schizonts

Answer: B

8. The host that harbors the adult or sexually mature, parasite is called:
a. Intermediate host
b. Commensal host
c. Symbiotic host.
d. Definite host

Answer: D

9. Malaria 3. After sporozoite gain entrance to human body it undergoes developmental cycle-first in liver than.in RBC, only after which fever is seen. This incubation period varies between plasmodium species. Which species has longest incubation period.
a. P. falciparum b. P. malariae
c. P. ovale d. P. vivax

Answer: B

10. In malaria, the form of plasmodia that is transmitted from mosquito to human is the
a. Sporozoite b. Gametocyte
c. Merozoite d. Hypnozoite

Answer: A

11. If a human develops cysticercosis, by which means was the infection transmitted?
a. Eating beef
b. Eating pork
c. Fecal oral from cow feces
d. Fecal oral from human feces

Answer: D

12. Which infection predisposes to bladder cancer?
a. Schistosoma haematobium
b. Schistosoma mansoni
c. Schistosoma japonicum
d. Clonorchis sinensis

Answer: A

Mycology

1. A 10-year-old boy develops a dry, circular, scaly, pruritic lesion on his leg. Potassium hydroxide calcofluor white preparation of a scraping from the lesion shows branching, septate, non-pigmented hyphae. What is the diagnostic significance of these findings?
a. Chromomycosis
b. Dermatophytosis
c. Phaeohyphomycosis
d. Sporotrichosis

Answer: B

2. A 50-year-old diabetic developed white lesions in the mouth and on the surface of tongue diagnosed as oral thrush. The causative agent of this disorder is:
a. Aspergillus fumigatus
b. Candida albicans.
c. Haemophilus influenzae
d. Streptococcus pneumoniae

Answer: B

3. A 45-year-old man presents with chest pain, fever, productive cough, and rust-colored sputum. The patient was diagnosed with tuberculosis in his early 20s. A chest X-ray shows multiple, nodular, infiltrates and cavitary lesions. A lung biopsy reveals necrotizing inflammation and vascular thrombi with branching fungal hyphae. Which of the following is the most likely diagnosis?
a. Actinomycosis
b. Aspergillosis
c. Candidiasis
d. Cryptococcosis

Answer: B

4. Trichomonas vaginalis is an example of which type of protozoa:
a. Amoeba
b. Flagellate
c. Ciliate
d. Sporozoan

Answer: B

5. A 15-year-oldboy presents with circular bald patches on the head with short hair stubs and broken hair within hair follicles. Which of the following is responsible for his condition?
a. Tinea corporis
b. Tinea pedis.
c. Tinea capitis
d. Tinea cruris

Answer: C

6. Infection with dermatophyte is most often associated with
a. intravenous drug abuse.
b. inhalation of the organism from contaminated bird feces
c. adherence of the organism to perspiration moist skin
d. Fecal-oral transmission

Answer: C

7. A girl who pricked her finger while pruning some rose bushes develops a local pustule that

progresses to an ulcer. Several nodules then develop alone the. local lymphatic drainage. The most likely agent is
a. Aspergillus fumigatus
b. Sporothrix schenckii
c. Cryptococcus neoformans
d. Candida albicans a

Answer: B

8. You have made a clinical diagnosis of meningitis in a 50-year-old immunocompromised woman. A latex agglutination test on the spinal fluid for capsular polysaccharide antigen is: positive. Of the following organisms, which one Is the MOST likely cause?
a. Histoplasma capsulatum
b. Cryptococcus neoformans
c. Aspergillus fumigatus
d. Candida albicans

Answer: B

9. Aspergillosis is recognized in tissue by the presence of:
a. Budding cells
b. Septate-hyphae
c. Metachromatic granules
d. Pseudo hyphae

Answer: B

10. A patient presents. with a 1 slowly-developing pneumonia. Several opacities a are seen in a lung x-ray. In a stained smear of leukocytes from the buffy coat of blood tiny intracellular yeast forms are seen. When blood centrifuged a "buffy coat" Is formed. What organism is this most likely to
a. Coccidiodes immitis
b. Blastomyces dermatitidis
c. Histoplasma capsulatum
d. Cryptococcus neoformans

Answer: C

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