Microbiology

Second Year Students
UJ-GIG GI Booklet

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The University of Jordan
Gastroenterology Interest Group (UJ-GIG)
Booklet

Micobiology

Human Microbiota/Mucosal Immunity

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[Overview]

- The gastrointestinal (GI) tract represents the largest surface area exposed to the external environment in the human body and is continuously challenged by ingested food antigens, commensal microorganisms, and potential pathogens. Despite this constant exposure, the gut must maintain a delicate balance between tolerance to beneficial microbiota and effective defense against harmful microbes. This complex task is carried out by the **mucosal immune system**, which is uniquely adapted to **coexist** with the microbiome while simultaneously preventing microbial breach across a single layer of epithelial cells.

- Diarrheal diseases caused by enteric pathogens remain a major cause of morbidity and mortality worldwide, especially in children. It is both preventable and treatable.

[Levels of Natural Defense in the GIT]

- Protection against pathogens in the gastrointestinal tract relies on multiple integrated levels of defense:

➔ *Anatomical and Physiological Barriers*

- The GI epithelium consists of a single layer of tightly joined **epithelial cells** connected by **tight junctions**.
- This epithelial layer forms a physical barrier that **prevents microbial invasion**.
- The mucosal surface is covered by mucus, which traps microbes and limits their contact with epithelial cells.

➔ *Chemical Barriers*

Several chemical factors contribute to antimicrobial defense:

1) **Gastric acidity** destroys many ingested microorganisms.
2) **Bile salts and pancreatic enzymes** inhibit bacterial growth.
3) **Complement proteins** participate in microbial killing.
4) **Saliva** contains numerous hydrolytic enzymes (secretory phospholipase A2)

➔ *Antibacterial enzymes*

These include:

1) **Lysozymes**
2) **Secretory phospholipase A_2**: produced by Paneth cells.

Paneth cells are specialized epithelial cells found in the small intestine, mainly at the base of the crypts of Lieberkühn, especially in the ileum.

➔ *Antimicrobial peptides*

These are small proteins that are part of the **innate immune system**, such as:

1) Defensins
2) Cathelicidins
3) Histatins

➔ *Immunological Barriers*

1) Antibody production and secretion of **secretory Immunoglobulin A (IgA)**.
2) Presence of **immune cells within the epithelium and lamina propria**.
3) **Specialized lymphoid structures** that coordinate immune responses.

Type	Components	Main Function
Anatomical	Epithelium, Tight junctions, and Mucus	Physical barrier to pathogens
Chemical	Gastric acid, Complement	Kills/inhibits microbes
Enzymes	Lysozyme, Phospholipase A_2	Destroy bacterial cell walls/membranes
Antimicrobial peptides	Defensins, Cathelicidins, Histatins	Direct microbial killing
Immunological	Secretory IgA	Neutralizes pathogens, prevents epithelial adhesion

[Protection Against Pathogens: Levels of Defense]

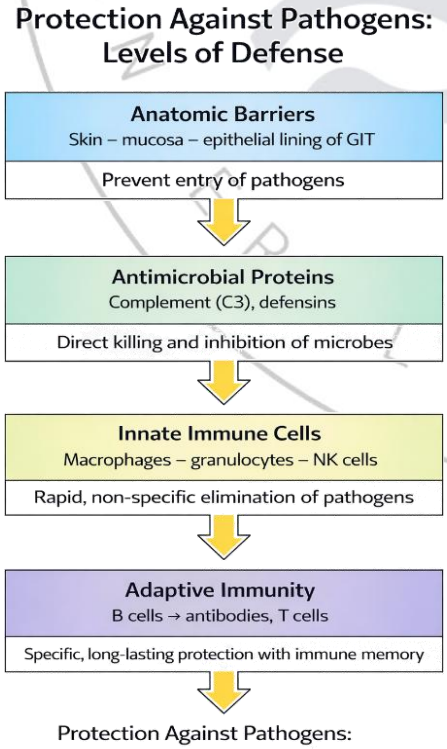
Protection against pathogens in the human body depends on **several coordinated levels of defense**, which act sequentially to prevent infection.

➔ **First, anatomical barriers** such as the **skin** and **mucosal epithelial surfaces** of the oral cavity, respiratory tract, and intestine **form the initial physical barrier** that **prevents pathogens from entering the body**.

➔ **Second, complement/antimicrobial proteins** provide **immediate chemical defense**. These include complement proteins (such as C3) and antimicrobial peptides like defensins, which **directly damage and kill microorganisms**.

➔ **Third, the innate immune system** responds rapidly once pathogens cross the barriers. Innate immune cells such as **macrophages, granulocytes, and natural killer (NK) cells recognize and eliminate pathogens in a non-specific manner**.

➔ **Finally, adaptive immunity** provides a highly **specific and long-lasting response**. B cells produce **antibodies**, while T cells coordinate and execute **cellular immune responses**, ensuring effective **pathogen clearance and immune memory**.



[How the Immune System Is Activated]

The immune system is activated when the body detects danger signals that indicate either:

1) The **presence of pathogens** (infection)
2) **Tissue damage** (injury, cell stress, or cell death)

This process happens in ordered steps:

1) ***Inflammatory Inducers***

These are substances that **signal danger and indicate that something is wrong** in the tissue.

Examples include:

- **Bacterial lipopolysaccharides (LPS)**: indicate the **presence of bacterial infection.**
- **ATP**: released from **damaged cells**.
- **Urate crystals**: released from **injured or dying cells**.

These molecules are collectively classified as:

- **PAMPs**
  Pathogen-Associated Molecular Patterns which indicate the presence of invading microorganisms.
- **DAMPs**
  Damage-Associated Molecular Patterns which indicate cell or tissue damage.

2) ***Sensor Cells***

Special immune cells constantly **monitor tissues for danger signals**. These include:

- Macrophages
- Neutrophils
- Dendritic cells

These cells have **pattern recognition receptors** (PRRs) that **recognize inflammatory inducers**. Once activated, **sensor cells initiate the immune response**.

3) ***Mediators***

Activated sensor cells release mediators, such as:

- Cytokines (e.g., interleukins, TNF)
- Molecules that cause cytotoxic effects

They function to:

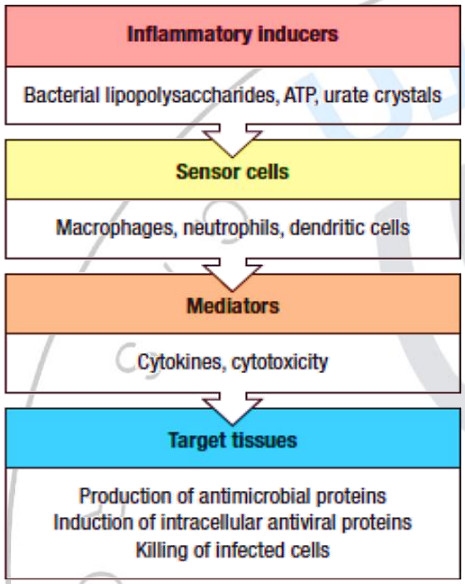
- **Communicate** between immune cells
- **Amplify inflammation**
- **Direct the immune response** to the site of infection or damage

4) *Target Tissues*

Mediators act on tissues to produce protective outcomes, including:

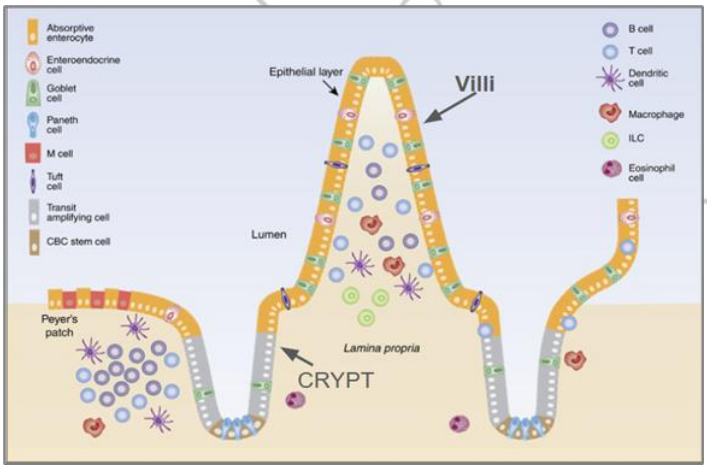
- **Production of antimicrobial proteins**
- **Induction of intracellular antiviral proteins**
- **Killing of infected or damaged cells**

This step eliminates pathogens and helps restore tissue integrity.



[Epithelial Surfaces as the First Barrier Against Infection]

The epithelial surfaces of the body form the **first line of defense against infection**. In the gastrointestinal tract, this barrier is especially important because it is constantly exposed to food, microbes, and potential pathogens.



Quick review of GIT histology

Topic	Key Points
Structure of the Small Intestinal Epithelium	• Most digestion and nutrient absorption occur in the small intestine • Intestinal surface is highly specialized to maximize absorption
Villi	• Finger-like projections lining the small intestine • Greatly increase surface area for absorption
Enterocytes (Intestinal epithelial cells IECs)	• Columnar epithelial cells covering villi • Form a tight, continuous lining • Act as a physical barrier while allowing nutrient absorption
Crypts of Lieberkühn	• Small invaginations between villi • Protect stem cells • Stem cells continuously renew the intestinal epithelium
Goblet Cells	• Found throughout the GI tract • Secretes mucus • Mucus traps microbes and prevents epithelial contact
Paneth Cells	• Located at the base of small intestinal crypts • Secrete antimicrobial molecules (defensins, lysozyme) • Control microbial growth and protect stem cells

[The Intestinal Epithelium as a Unique Immune Compartment]

The intestinal epithelium is not just a physical barrier; it is **an active and specialized part of the immune system**. It must perform a difficult task:

- Allow nutrient absorption
- Tolerate beneficial microbiota but still protect against pathogens.

➔ ***Peyer's Patches: Immune Surveillance Sites***

- Along the small intestine, the continuous surface of villi is interrupted by lymphoid nodules called **Peyer's patches**.
- Peyer's patches are part of the **Gut-Associated Lymphoid Tissue (GALT).**
- Their main function is **immune surveillance of luminal antigens.**

➔ ***Microfold (M) Cells: Antigen Sampling Cells***

The epithelium covering Peyer's patches contains microfold (M) cells. M cells are **specialized intestinal epithelial cells (IECs)**.

Their function is to:

- **Transport of luminal antigens**
- **Deliver them to antigen-presenting cells (APCs)** such as dendritic cells and macrophages below the epithelium

Clinical Importance of M Cells:

Because M cells allow the passage of luminal material, they also **increase susceptibility to infection**.

Several pathogens exploit M cells as an entry point, including:

- *Salmonella enterica*
- *Shigella*
- *Yersinia pestis*

This explains why some gut pathogens can cross the epithelial barrier despite intact mucosa.

➔ ***Intraepithelial Lymphocytes (IELs): Rapid Immune Responders***

- The intestinal epithelium contains many intraepithelial lymphocytes (IELs).
- More than 90% of IELs in the small intestine are T cells.
- About 80% of these T cells express CD8.

➔ **Why is this important?**

- $CD8^+$ IELs **provide rapid, localized immune defense**.
- They can quickly kill infected or stressed epithelial cells.
- This is very different from the lamina propria, where immune cells are more **diverse** and include more $CD4^+$ T cells and B cells.

- Villi → Absorption
- Peyer's patches → Immune surveillance
- M cells → Antigen transport
- IELs (mostly $CD8^+$) → Rapid epithelial defense

[Mucosal Tissues of the Human Body]

Mucosal tissues are specialized surfaces that line many internal body organs. These surfaces are in constant contact with the external environment, making them a major site for interaction with microorganisms. Because of this continuous exposure, **mucosal tissues are equipped with specialized immune structures that coordinate immune responses to environmental microbes** while maintaining tolerance to harmless antigens.

➔ **Why Are Mucosal Tissues Important?**

- Mucosal surfaces represent an enormous surface area that must be protected.
- They serve as **the main entry points** for pathogens.
- The immune system at these sites must balance:
  - **Defense against pathogens**
  - **Tolerance to food antigens and commensal microbiota**

This balance is achieved by the mucosal immune system.

➔ **What Is the Mucosal Immune System?**

The mucosal immune system consists of all internal body surfaces that are lined by a **mucus-secreting epithelium** and **their associated immune structures**.

Major Components of Mucosal Tissues

1) **Gastrointestinal Tract**

- Largest mucosal surface in the body
- Continuously exposed to food and microbes
- **Contains organized immune tissue (GALT, Peyer's patches)**

2) **Respiratory Tract**

- Includes both upper and lower respiratory tracts
- Exposed to airborne pathogens
- **Uses mucus, cilia, and immune cells for defense**

3) **Urogenital Tract**

- Exposed to the external environment
- **Protected by mucosal immunity and antimicrobial secretions**

4) **Middle Ear**

- Lined by mucosal epithelium
- Connected to the upper respiratory tract
- Prone to infections due to microbial exposure

5) **Associated Exocrine Glands**

The mucosal immune system also includes exocrine glands associated with mucosal surfaces, such as:

- Conjunctivae and lacrimal glands of the eye
- Salivary glands
- Lactating breast

These glands contribute to immunity by secreting:

- **Antibodies (especially IgA)**
- **Antimicrobial proteins**
- **Protective enzymes**

[Mucosal Tissues of the Human Body]

The mucosal immune system differs from the systemic immune system in that it must protect large, exposed surfaces without causing excessive inflammation. To achieve this, it has unique anatomical, functional, and regulatory features.

Distinctive features of the mucosal immune system	
Anatomical features	Intimate interactions between mucosal epithelia and lymphoid tissues
	Discrete compartments of diffuse lymphoid tissue and more organized structures such as Peyer's patches, isolated lymphoid follicles, and tonsils
	Specialized antigen-uptake mechanisms, e.g., M cells in Peyer's patches, adenoids, and tonsils
Effector mechanisms	Activated/memory T cells predominate even in the absence of infection
	Multiple activated 'natural' effector/regulatory T cells present
	Secretory IgA antibodies
	Presence of distinctive microbiota
Immunoregulatory environment	Active downregulation of immune responses (e.g., to food and other innocuous antigens) predominates
	Inhibitory macrophages and tolerance-inducing dendritic cells

[Mucosa-Associated Lymphoid Tissues (MALT)]

The mucosa-associated lymphoid tissues (MALT) represent the immune system components that **protect mucosal surfaces**, which are continuously exposed to environmental antigens such as food particles, microbes, and pathogens.

➔**Why Is MALT Important?**

1) The mucosal immune system contains as **many lymphocytes as the rest of the body combined**.

2) These lymphocytes form a **specialized immune population** adapted to life at mucosal surfaces.

3) Unlike lymphocytes in peripheral lymphoid organs (e.g., lymph nodes), MALT lymphocytes **follow distinct circulation and homing patterns**, allowing them to localize specifically to mucosal sites.

This specialization en**sures rapid and localized immune responses** where exposure is greatest.

➔ **Components of MALT**

It includes lymphoid tissues associated with different mucosal sites, the most important being **Gut-Associated Lymphoid Tissue (GALT)**.

GALT is the largest and best-studied component of MALT and includes:

1) **Tonsils and adenoids**, which together with surrounding lymphoid tissue form **Waldeyer's ring** at the entrance of the gut and airway
2) **Appendix**
3) **Peyer's patches**
4) **Isolated lymphoid follicles**

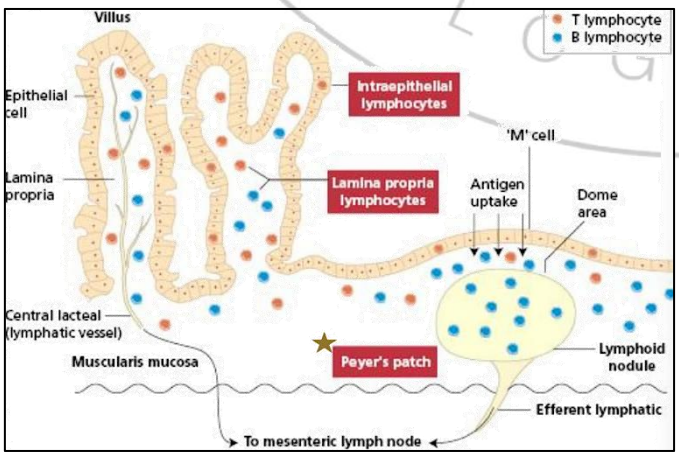
These structures are strategically located to **collect antigens from the epithelial surfaces** of the gastrointestinal tract.

➔ **Peyer's Patches: The Key Antigen-Sampling Sites**

- Peyer's patches are **highly organized lymphoid nodules** found mainly in the ileum.
- They are the **most important** organized structures within GALT.
- Their primary function is to **sample antigens from the gut lumen**.

➔ **Role of M Cells**

- The epithelium over Peyer's patches contains microfold (M) cells.
- M cells **transport antigens from the intestinal lumen to immune cells** located beneath the epithelium.
- This process allows antigen-presenting cells (APCs) to initiate immune responses.



[Mucus as a Key Protective Barrier in the Gut]

Mucus is a critical component of the intestinal defense system. It forms a physical, chemical, and immunological barrier between gut microbes and the epithelial surface.

➔ **Production of Mucus**

- **Goblet cells** in the intestinal epithelium secrete Mucins, which are large, heavily glycosylated proteins.
- Mucins oligomerize through disulfide bonds, forming a thick mucus gel.
- Mucins contain **PTS repeats (Proline-Threonine-Serine)** that undergo O-linked glycosylation**.**
- **Mucin Type II** is the major component of intestinal mucus.

➔ **Importance of O-linked glycosylation**

- **Provides structural stability**.
- **Confers resistance to enzymatic degradation**.
- **Maintains epithelial barrier integrity**.

➔ **Trapping of Microbes and Immune Molecules**

The glycan chains on mucins **create sticky binding sites** that trap:

- Bacteria
- Viruses
- Toxins
- Antibodies (especially **IgA**)
- Antimicrobial peptides
- Bacteriophages that kill trapped bacteria

This prevents microbes from reaching epithelial cells.

➔ **Barrier and Scaffolding Functions**

Mucus acts as:

- A **formidable physical barrier** that blocks microbial invasion.
- A **scaffold that retains immune molecules** secreted into the lumen, such as:
  - Secretory IgA
  - Antimicrobial peptides (defensins, lysozyme)

This allows immune defense to occur at a distance from epithelial cells, **reducing tissue inflammation**.

➔ **Role in Microbial Clearance**

- Mucus is slippery in nature.
- Trapped microbes and particles are moved along the gut.
- Normal peristaltic movements expel these materials.

This provides **continuous mechanical clearance** of pathogens.

[Uptake and Transport of Antigen by M Cells]

The intestine is constantly exposed to antigens from food and microorganisms. To generate appropriate immune responses, these antigens must be transported from the intestinal lumen across the epithelial barrier to immune cells located underneath.
This function is primarily performed by specialized epithelial cells known as **microfold (M) cells,** particularly within organized lymphoid tissues.

➔ **Why Are M Cells Needed?**

Most intestinal epithelial cells are designed for absorption, rather than immune surveillance. However, the mucosal immune system requires a mechanism to:

- Sample luminal antigens
- Deliver them to the underlying immune cells

M cells provide this specialized antigen-uptake pathway.

➔ **Location and Structure of M Cells**

- M cells are located in the epithelium overlying **Peyer's patches**.

- Unlike enterocytes:
  - M cells have few microvilli.
  - Lack a thick glycocalyx, making them more accessible to antigens and microbes.

- The basal surface of M cells forms a pocket containing:
  1) Dendritic cells
  2) Macrophages
  3) Lymphocytes

➔ **Mechanism of Antigen Uptake (Step-by-Step)**

Step 1: ***Antigen Recognition***

- Certain bacteria bind specifically to M cells.
- Example:
  The **FimH protein** on type 1 pili of bacteria is recognized by **glycoprotein 2 (GP2)** on the M-cell surface

Step 2: ***Transcytosis***

- After binding, the antigen is:
  - **Endocytosed** by the M cell
  - **Transported** through the cytoplasm in membrane-bound vesicles

Step 3: ***Delivery to Immune Cells***

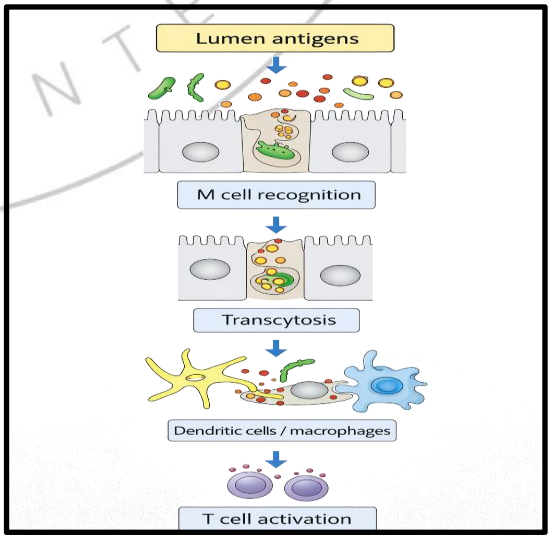
- Antigens are released **at the basal surface of the M cell**.
- They are captured by:
  - **Dendritic cells**
  - **Macrophages**

These **antigen-presenting cells (APCs)** process and present antigens to T cells, initiating immune responses.

➔ **Functional Importance of M Cells**

M cells enable:

- **Efficient sampling** of luminal antigens.
- **Activation of mucosal immune responses**.
- **Development of immune tolerance to harmless antigens** under non-inflammatory conditions.



[Transcytosis of Secretory IgA (sIgA)]

IgA is the predominant antibody class in the gastrointestinal tract and plays a central role in protecting mucosal surfaces without inducing inflammation. To exert its function, IgA must be **transported from immune cells in the lamina propria to the gut lumen** through a specialized process known as **transcytosis**.

➔ IgA form depends on the location

- In blood → IgA is mainly a **monomer**
- In mucosal tissues → IgA is predominantly a **dimer**, composed of:
  - Two IgA molecules
  - Linked by a **J (joining) chain**

The dimeric form is essential for epithelial transport.

➔ Production of IgA

- IgA is **produced by plasma cells** in the lamina propria.
- These plasma cells secrete **dimeric** IgA containing a J chain.

➔ Binding to Polymeric Immunoglobulin Receptor (pIgR)

- Intestinal epithelial cells **express the polymeric immunoglobulin receptor** (pIgR) on their **basolateral** surface.
- The **J chain** of dimeric IgA binds specifically to pIgR.

This binding is **the key signal for transport**.

➔ Endocytosis and Transcytosis

The IgA–pIgR complex is:

- Endocytosed into the epithelial cell
- Transported across the cell in vesicles

This **vesicular transport across the cell** is called **transcytosis**.

➔ Release at the Apical Surface

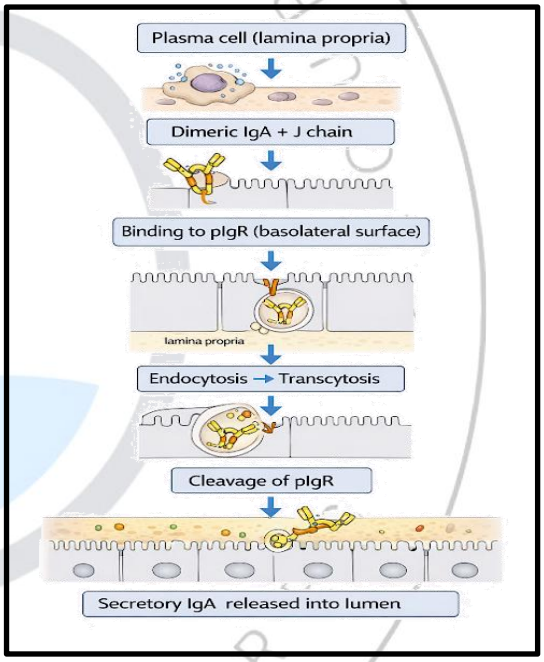
- At the **apical** (luminal) surface, pIgR is cleaved.
- A portion of the receptor remains attached to IgA, forming **the secretory component**.
- The final product released into the gut lumen is **secretory IgA (sIgA)**.

➔ Why Is the Secretory Component Important?

- **Protects IgA from proteolytic degradation**.
- **Allows IgA to function effectively** in the harsh gut environment.

➔ Function of Secretory IgA in the Gut

- **Binds pathogens and toxins**.
- **Prevents microbial adhesion to epithelial cells**.
- **Neutralizes pathogens**
- **Does not activate complement**, thereby providing protection while avoiding inflammation.



[Microbiota: Agents of Health and Disease]

The microbiota refers to **the community of microorganisms that live in and on the human body**. These organisms are not merely passive passengers; they play essential roles in maintaining health, but may also contribute to disease under certain conditions

➔ Overview of the Microbiota

- Not all microbes are pathogens.
- Many body sites are normally colonized by microorganisms from early life.

- These microbial communities form a relatively stable ecosystem known as the microbiota.
- The term “normal flora” was previously used, but **microbiota** is now preferred.
- Although the microbiota consists of many thousands of microbial species, only a small fraction (approximately 100 species) is known to be pathogenic.

➔ What Is the Microbiome?

The microbiome refers to:

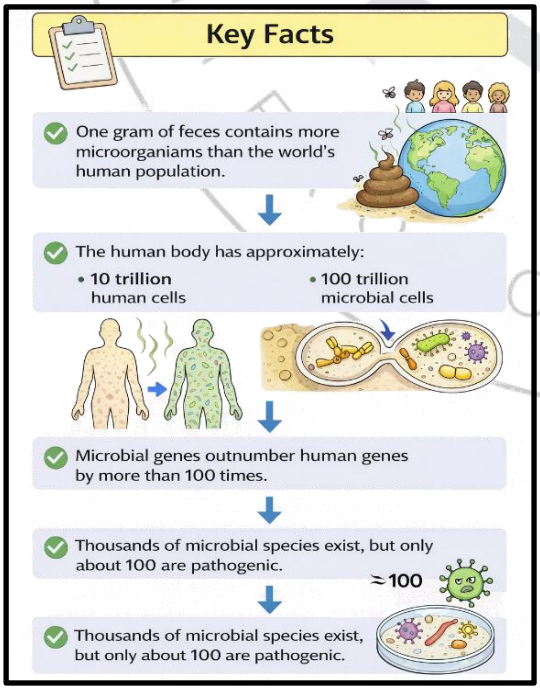
- The **microbiota** (all microorganisms)
- Plus, their **collective genetic material** which includes Bacteria, Viruses, Fungi, Protozoa and other eukaryotic microbes.

➔ Scale of the Human Microbiota

The human body harbors vast numbers of microorganisms:

- Approximately 10 trillion human cells
- Approximately 100 trillion microbial cells
- This gives a ratio of roughly 10:1 (microbial cells: human cells)

In addition, the collective genetic material of the microbiota exceeds that of the human genome by more than 100-fold, leading to the notion that humans are, in many ways, “more microbial than human.”



➔ Where Are Microbiota Found?

Microbiota are found mainly on body surfaces exposed to the environment, including:

- Skin
- Oral mucosa
- Conjunctiva
- Gastrointestinal tract

They are **NOT** normally present in:

- Blood
- Deep tissues
- Other sterile body sites

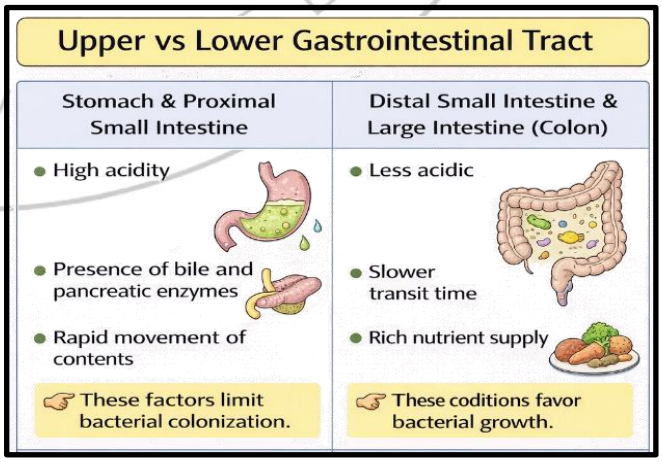
The gastrointestinal tract **contains the largest and most diverse microbial population**. The majority of microbiota reside in the gut, with microbial density increasing progressively toward the distal intestine, reaching its highest levels in the large intestine (colon).

Notably, one gram of feces contains more microorganisms than the total human population worldwide.

➔ Why Does Microbiota Distribution Differ Along the GI Tract?

Microbial composition varies along the gastrointestinal tract **due to differences in local environmental conditions**, including:

- **pH**
- **Oxygen availability**
- **Bile salts**
- **Digestive enzymes**
- **Peristalsis**



➔ **Relationship with the Human Host**

The microbiota exists in a symbiotic relationship with the host.
This relationship is mainly **mutualistic**, meaning:

- The host **provides nutrients and a habitat**.
- Microbes **provide protective and metabolic benefits**.

➔ **Why Is the Microbiota Important?**

1) ***Contribution to Health***

- **Prevents colonization by pathogens** (colonization resistance)
- **Aids digestion and nutrient metabolism**
- **Produces vitamins** (e.g., vitamin K)
- **Essential for normal immune system development**

2) ***Contribution to Disease***

The microbiota can contribute to disease when:

- Normal balance is disrupted (**Dysbiosis**)
- Host immune defenses are **weakened**
- Microorganisms **translocate** to normally sterile sites

3) ***Interaction with the Immune System***

- Plays a critical role in immune system maturation
- Promotes the development of:

1) Innate immune responses
2) Adaptive immune tolerance

[Who Are the Human Microbiota?]

The human microbiota is composed of a large and diverse community of microorganisms, mainly bacteria, that live in symbiosis with the host.

➔ **Major Bacterial Groups in the Intestine**

Although thousands of species exist, the intestinal microbiota is dominated by **two** main bacterial phyla:

1- Firmicutes

- **Gram-positive bacteria** include genera such as:
  1) *Clostridium*
  2) *Lactobacillus*
  3) *Enterococcus*

2- Bacteroidetes

- **Gram-negative bacteria** include:

1) *Bacteroides* species

Firmicutes + Bacteroidetes together make up more than **90%** of the gut microbiota.

➔ **Similarity Across Species**

- At the phylum level, microbiota composition is **similar in humans and mice**.
- This is why animal models are useful in microbiome research.

➔ **Individual Variation**

There is a large individual variation in microbiota composition, meaning no two individuals have the same microbiota.

- Differences depend on:
  1) **Body site sampled** (skin, gut, mouth, etc.)
  2) **Diet**
  3) **Environment**
  4) **Age**
  5) **Antibiotic exposure**

Despite variation, humans share a **core** microbiome of approximately 130 species, along with many additional variable species.

➔ **Other Important Bacterial Groups (Minor but Relevant)**

- **Proteobacteria** (Gram-negative),
  - Examples: *E. coli*, *Proteus*.

- **Actinobacteria**:
  - Important **skin** microbiota
  - *Bifidobacterium* is an example, which is the **predominant gut bacterium in infants**

- Other minor phyla: **Cyanobacteria** and **Verrucomicrobia**

Together, these account for about 10% of the microbiota but can be clinically important.

[Methods to study bacterial microbiota]

Methods to Study Bacterial Microbiota

Method	What it Does	Key Points / Limitations
Selective plating (culture)	Grows bacteria on selective media	Many gut bacteria are strict anaerobes and require community → underestmates diversity
DNA staining (e g. Cybergreen)	Stains bacterial DNA to estimate total num-bers	Gives quantity, not species identification
PCR amplification of 16S rRNA	Identifies bacteria using conserved & variable regions of 16S rRNA	Culture-independent, widely used
Fluorescent DNA probes (FISH)	Labels specific 16S rRNA sequences	Identifies type + location of bacteria in samples

[Resident vs Transient Microbiota]

Microorganisms on the skin and mucous membranes are classified into:

1) **Resident microbiota:**
   - Fixed and stable
   - Specific to body site
   - Re-establish themselves after removal
2) **Transient microbiota:**
   - Temporary microorganisms acquired from the environment.
   - Present for hours to weeks
   - Usually nonpathogenic but more likely to cause infections if resident flora is disturbed

[What influences the dynamic microbiota?]

Essentially, it means that the collection of microbes in our body is not fixed; instead, it constantly changes and adapts in response to various internal and external factors.

- **Environment**: Way of birth, Temp and humidity.
  - Natural vaginal birth→ *lactobacilli* and *Bifidobacterium*
  - Caesarean section→ *S. aureus*
- **Nutrition**: meat, vegetables.
- **Hormones**: estrogen, insulin.
- **Genetic constitution**: receptors on mucosal surfaces.
- **Antibiotics**: eliminate some which permit others to thrive.
- **Foreign objects**: valves, catheters.

Factors Influencing the Dynamic Microbiota

Factor	Effect on Microbiota	Examples / Notes
Environment	Determines initial and ongoing colonization	Mode of birth, temperature, humidity
Mode of birth	First major exposure to microbes	Vaginal birth → *Lactobacillus* *Bifidobacterium*
Nutrition	Shapes microbial composition	Meat vs. plant-based diet
Hormones	Influence microbial growth	Estrogen Insulin
Genetic constitution	Determines which microbes can colonize	Mucosal receptors, immune response
Antibiotics	Disrupt normal flora	Kill some microbes → others overgrow (dysbiosis)
Foreign objects	Provide surfaces for colonization	Catheters, valves → →biofilms

[Colonization of the Microbiota: Immediate and Lifelong]

➔ When does colonization start?

- At birth, the intestine is essentially **sterile**.
- Colonization begins **immediately after birth** through exposure to:
  - **Mother's vaginal microbiota** (vaginal delivery)
  - **Mother's skin and fecal microbiota**
  - **Food, fluids, and inhaled microbes**

These early exposures are major determinants of the initial microbial composition.

➔ How does colonization occur?

Microbes are acquired from the environment via:

- **Ingestion of food and fluids**
- **Inhalation**
- **Skin contact**

The microbiota is established rapidly in early life. Over time, it matures and stabilizes, forming a relatively stable adult microbiota.

➔ Effect of Food Consumption on Small Intestinal Microbiota

1) **Early life (Infancy)**

- Shortly after birth, ***Bifidobacterium* species dominate the gut microbiota**. These bacteria are:
    - **Anaerobic**, **Gram-positive**, **branched rod-shaped** bacteria
    - Specialized in **metabolizing breast milk components**
    - The most common bacteria in infants.

2) **Transition to Solid Food**

- As the child shifts from breast milk to solid food:
    - The gut environment changes
    - Microbial diversity increases
    - The microbiota shifts to a more **mixed** population, including other anaerobic bacteria Species such as *Clostridioides difficile*

[What do the Microbiota do for us?]

What Do the Microbiota Do for Us?

Role	Description	Examples

1 Microbial Antagonism (Colonization Resistance)	• Most important protective function – Prevents pathogen colonization and overgrowth	• Competes for space and nutrients, crowds out pathogens
		• Produces inhibitory substances
		  ◦ Bacteriocins
		  ◦ Lactic acid
		• Reduces oxygen, promoting anaerobes

2 Nutritional & Metabolic Benefits	• Contributes to host metabolism	• Produces vitamins (K, B12) • Breaks down bile acids, metabolizes steroids • Ferments dietary fibers into short-chain fatty acids (SCFAs)
3 Immune Development & Stimulation	• Essential for maturation of the immune system	• Stimulates mucosal immune system • Promotes immune balance • Germ-free animals have poorly developed immune systems

[What Are the Harmful Effects of Microbiota?]

Although beneficial, microbiota can also **cause disease under certain conditions**.

➔ Pathogenic Potential

Microbiota can cause disease if:

1) **Introduced into sterile sites** due to trauma or barrier disruption.
    - Examples: Urinary tract infections, Septic shock
2) **Host Status Changes**:
    - Disease risk increases in **immunocompromised** patients and **hospitalized** (nosocomial) patients

➔ Harmful Metabolic Byproducts (Gaseous & Fermentation Byproducts)

- Microbial fermentation produces gases like, Hydrogen sulfide and Methane.

- Up to 300 mL of gas per day can be produced.

➔ Dysbiosis (Very High-Yield Term)

Dysbiosis: Imbalance in microbiota composition.

- Common causes: **Antibiotic abuse**.
- Leads to: **Increased susceptibility to infection** and Conditions such as Pseudomembranous Colitis.

➔ Lifestyle and Disease Associations

Changes in microbiota composition have been linked to:

1) Cancer
2) Cardiovascular disease
3) Metabolic disorders (obesity, diabetes)
4) Allergies
5) Autoimmune diseases
6) Neurodevelopmental and mental health disorders (e.g., autism)

[What Is the Hygiene Theory?]

The hygiene theory proposes that **reduced exposure to microbes** in early childhood **impairs immune system developmen**t and increases susceptibility to allergic and autoimmune diseases later in life.

➔ Why Is Microbial Exposure Important?

During early life, exposure to microbes:

- **Trains the immune system**
- **Promotes immune tolerance**
- **Prevents excessive immune responses**

When this exposure is reduced, the immune system may:

- **Overreact to harmless antigens**
- **Shift toward allergic or autoimmune responses**

➔ Epidemiological Evidence (Very Important)

- **Infectious Diseases ↓**

Over the past 50 years, many infectious diseases have significantly **declined**:

- Rheumatic fever
- Hepatitis A
- Tuberculosis
- Mumps
- Measles

- Immune-Mediated Diseases ↑

At the same time, there has been a marked **increase** in immune-mediated diseases:

- Asthma
- Crohn's disease
- Multiple sclerosis
- Type 1 diabetes

This inverse relationship supports the hygiene theory.

[Microbiota and Disease]

Changes in the composition, diversity, or function of the microbiota (Dysbiosis) are associated with several common diseases:

➔ Obesity

- It is associated with an altered gut microbiota composition.
- An increased proportion of microbiota, especially **Firmicutes**, which **can extract energy more efficiently from food and produce more digestive enzymes**.
- This leads to:
    1) **Increased calorie harvesting**
    2) **Greater energy storage**

➔ Inflammatory Bowel Disease (IBD)

Includes **Crohn's disease** and **Ulcerative colitis**.

- Changes observed in IBD:
    - Increased **Proteobacteria**
    - Decreased **Firmicutes**
    - Decreased **Bacteroidetes**
- Why this matters:
    - Loss of protective commensal bacteria
    - Increase in inflammatory bacteria
    - Chronic intestinal inflammation

➔ Type 1 Diabetes

- There is an interaction between intestinal microbiota and the innate immune system.
- Dysbiosis may alter immune tolerance and promote autoimmune destruction of pancreatic **β-cells**.

➔ Gastrointestinal Cancers

Helicobacter pylori

- The only known colonizer of the stomach
- Associated with:
    - **Chronic gastritis**
    - **Peptic ulcer disease**
    - **Gastric cancer**

Colorectal Cancer

- Altered gut microbiota composition
- Certain bacterial species are associated with:
    - Increased inflammation
    - Tumor development

➔ **Oral Diseases**

Oral microbiota imbalance leads to:

- Cavities and gingivitis disease.
- Most common infectious disease worldwide.

➔ **Allergy-Like (Atopic) Diseases**

- Includes:
    1) Eczema
    2) Allergies
    3) Asthma

- Link to microbiota:

- Explained by the **Hygiene Hypothesis**
- Reduced microbial exposure early in life leads to:
    1) Poor immune tolerance
    2) Increased allergic responses

- Important contributing factors:

    - Early antibiotic use
    - Caesarean section
    - Lack of early microbial exposure

➔ **Pseudomembranous Colitis**

- Cause:
    - Occurs **after antibiotic treatment**
    - Antibiotics disrupt normal gut microbiota
    - Overgrowth of *Clostridium difficile*

- Key points:
    - Most common cause of diarrhea after antibiotic use

- Clinical features:
    - Severe diarrhea
    - Colitis

- Treatment:
    - Oral vancomycin or fidaxomicin(important exam point)
    - Fecal microbiota transplantation (FMT) improves outcomes

[Microbiota and the Immune System]

Human microbiota plays a critical role in the development, maturation, and regulation of the immune system. This relationship is bidirectional: the immune system shapes microbial composition, while the microbiota educates and modulates immune responses.

➔ **Evidence from Germ-Free Animals**

- Animals raised in germ-free (microbiota-free) conditions exhibit:

    1) Poorly developed lymphoid tissues
    2) Reduced immune cell numbers
    3) Impaired immune responses

These findings demonstrate that **normal immune system development requires microbial exposure**.

➔ **Role of Innate Immune Signaling**

- Microbial products interact with pattern recognition receptors (PRRs), especially **Toll-like receptors (TLRs)** on epithelial and immune cells.

Controlled activation of TLR signaling is essential for:

1) **Maturation of innate immunity**

2) **Proper development of adaptive immune responses**

➔ **Microbiota and T-Cell Differentiation**

Specific members of the microbiota influence the differentiation of key T-cell subsets:

Th17 Cells

Segmented Filamentous Bacteria (SFB) are essential for the induction of Th17 cells in the intestine.
Th17 cells are a critical T-cell lineage involved in:

1) Mucosal immunity
2) Defense against extracellular pathogens

- Germ-free mice lack intestinal Th17 cells.

- Antibiotic treatment can **reduce** Th17 cell levels by altering microbiota composition.

Regulatory T Cells (Treg)

- Certain commensal bacteria **promote the development and function of Treg cells**.
- Treg cells are essential for:
    1) Immune tolerance
    2) Prevention of excessive inflammation and autoimmunity
- Recent studies show that microbiota-derived signals directly influence Treg differentiation and maintenance.

[Common Methods to Manipulate Microbiota]

➔ Probiotics

- Live bacteria which provide health benefits when consumed in adequate amounts.
- Common examples:
    - *Lactobacillus*
    - *Bifidobacterium*
- How do they work?
    - **Restore or maintain normal gut flora**
    - **Compete with pathogenic bacteria**
    - **Modulate immune responses**
- Key point: Probiotics are generally safe, but their benefits can vary between individuals.

➔ Prebiotics

- **Non-digestible food components** (usually sugars or fibers)
- Promote the growth or activity of beneficial bacteria
- Examples: Certain carbohydrates and dietary fibers.
- Key idea: Prebiotics **feed good bacteria**, rather than adding bacteria directly.

➔ Immunomodulators

- **Modify immune responses that influence microbiota composition**
- Used mainly in inflammatory or immune-mediated diseases
- Examples:
    1) **Steroids**
    2) **Immunosuppressive drugs** (e.g., those used in cancer or autoimmune diseases)

These drugs indirectly affect microbiota by **altering the immune environment**.

➔ Antibiotics

- Kill or suppress bacteria
- Can be lifesaving
- Problem:
    - Excessive or inappropriate use:
        1) **Disrupts normal microbiota**
        2) **Leads to antibiotic resistance**
        3) **Causes dysbiosis**

➔ Phage Therapy

- Use of bacteriophages (viruses that infect bacteria)
- Targets specific bacterial populations
- Advantages:
    - Highly specific
    - Does not harm beneficial bacteria
- Limitation:
    - Bacteria can rapidly develop resistance to phages

➔ Fecal Transplantation

- Transfer of stool from a healthy donor to a patient
- Restores healthy gut microbiota
- Clinical use:
    - ***Clostridioides difficile* infection**
    - **Especially effective in recurrent cases**
- Important note: May require depletion of existing microbiota before transplantation.

➔ Use of Microbial Products

- Certain bacterial products can directly influence the immune system
- Example:
    - **Polysaccharide from *Bacteroides fragilis***

1) **Affects T-cell populations**
2) **Helps maintain Th1/Th2 balance**

[Questions]

Q1: All are considered a chemical barrier EXCEPT:
A. Skin
B. Acidity of the stomach
C. Antimicrobial proteins
D. Complement
E. B+C

Q2: What kind of cells are utilized by GI pathogens as a point of entry?
A. M cells
B. Goblet cells
C. Paneth cells
D. Macrophages
E. None of the above

Q3: What is the term used to describe the community of bacteria that live in your digestive tract?
A. Immunoglobulin
B. Intestinal colony
C. GI tract microbial colony
D. Human gut microbiome
E. Mammalian gut microbiome

Q4: The M (microfold) cells are characterized by all the following EXCEPT:
A. It is specialized epithelial cells located at the surface of the ileum and in Peyer's patches
B. Its cytoplasm contains lymphocytes and macrophage cells
C. They release and store the lysozyme enzyme
D. Their main function is to engulf foreign bodies at the surface
E. Its basement membrane is discontinuous

Q5: Which of the following diseases affects the normal microbiota of the gut?
A. Obesity
B. Type I diabetes
C. Atopic diseases
D. A+B
E. All the above

Q6: What are prebiotics?
A. Live bacteria
B. Dead bacteria
C. Diary pathogens
D. Bacterial nutrition
E. Microbes

Q7: Primary feces inhabitants shortly after birth are:
A) Clostridium botulinum
B) Clostridium tetani
C) Bifidobacterium
D) Clostridium perfringens
E) Clostridium difficile

Q8: Which bacteria are most associated with an increased proportion in obesity?
A. Proteobacteria
B. Firmicutes
C. Actinobacteria
D. Bacteroidetes
E. Cyanobacteria

Q9: Which of the following is true about Gut-associated lymphoid tissue (GALT)?
A. It is mainly composed of neutrophils
B. Peyer's patches are covered by goblet cells
C. Mesenteric lymph nodes drain lymph from the gut
D. It has very few lymphocytes compared to other organs
E. It is in the subcutaneous tissue

Q10: Which of the following best describes microbial antagonism?
A. Microbes that directly infect epithelial cells
B. Microbes that help pathogens attach to mucosal surfaces
C. Microbes competing with pathogens for nutrients and space
D. Microbes that produce toxins only under immune suppression
E. Microbes that prevent immune tolerance development

Q11: Which mucosal structure is primarily responsible for trapping microbes and holding secretory IgA?
A. Mucus layer
B. Peyer's patch
C. Paneth cells
D. Goblet cells
E. Enterocytes

Q12: Which of the following statements about microbiota colonization after birth is correct?
A. The intestines are heavily colonized before birth
B. Vaginally delivered babies acquire mainly skin flora
C. C-section babies acquire mainly vaginal flora

D. Colonization starts immediately after birth and stabilizes later
E. Microbiota in infants are dominated by Bacteroidetes initially

Q13: The majority of intraepithelial lymphocytes (IELs) in the small intestines are?
A. B cells expressing IgA
B. CD4+ T helper cells
C. CD8+ T cells
D. NK cells
E. M cells

Q14: What is the function of the glycan chains in mucins secreted by goblet cells?
A. Decrease the viscosity of mucus
B. Allow epithelial penetration
C. Trap microbes and hold immune molecules
D. Digest pathogens directly
E. Facilitate IgG secretion

Q15: What is the process called by which M cells transport antigens across the epithelium?
A. Phagocytosis
B. Exocytosis
C. Endocytosis
D. Transcytosis
E. Pinocytosis

Q16: Which bacterial protein binds to GP2 on M cells to facilitate bacterial entry?
A. Flagellin
B. Exotoxin
C. LPS
D. FimH
E. Pilin

Q17: The immunoglobulin class most frequently responsible for inhibition of bacteria on mucosal surfaces is?
A. IgG
B. IgM
C. IgA
D. IgE
E. IgD

Answers:
Q1: A
Q2: A
Q3: D
Q4: C
Q5: E
Q6: D
Q7: C
Q8: B
Q9: C
Q10: C
Q11: A
Q12: D
Q13: C
Q14: C
Q15: D
Q16: D
Q17: C

The University of Jordan
Gastroenterology Interest Group (UJ-GIG)
Booklet

Microbiology

Gram-positive & spore-forming bacterial infection of the gastrointestinal tract

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[Overview]

➔ **What are the spore-forming bacteria?**

Some **Gram-positive bacteria** can form **spores** when nutrients are limited. Spores **lack metabolic activity and are highly resistant to heat and chemicals**.
The core contains dipicolinic acid, which is responsible for heat resistance. Must **autoclave** to kill spores (as is done to surgical equipment) by steaming at 121°C for 15 minutes.

Examples: B. anthracis (anthrax), B. cereus (food poisoning), C. botulinum (botulism), C. difficile (pseudomembranous colitis), C. perfringens (gas gangrene), C. tetani (tetanus).
Autoclave to kill **B**acillus and **C**lostridium (ABC).

[Bacillus Species]

➔ **General Features**
- Large **gram-positive rods**
- **Spore**-forming
- **Aerobic** or **facultatively anaerobic**
- Widely distributed in soil, water, and air
- Many are **saprophytes** (*An organism that lives on and obtains nutrients from dead or decaying organic matter*).

➔ **Medically Important Species**
1) **Bacillus anthracis** → Anthrax
2) **Bacillus cereus** → Food poisoning & opportunistic infections
3) **Bacillus thuringiensis** → Insect pathogen found in insecticides

Bacillus Cereus

➔ **Key Characteristics**
- **Gram-positive**, **motile**, **spore**-forming rod
- **Aerobic** or **facultative anaerobe**
- Widely present in the environment
- Common cause of **food poisoning**

➔ **Other Infections**
May cause serious infections, especially with:
- IV drug use
- Medical devices
- Immunocompromised states

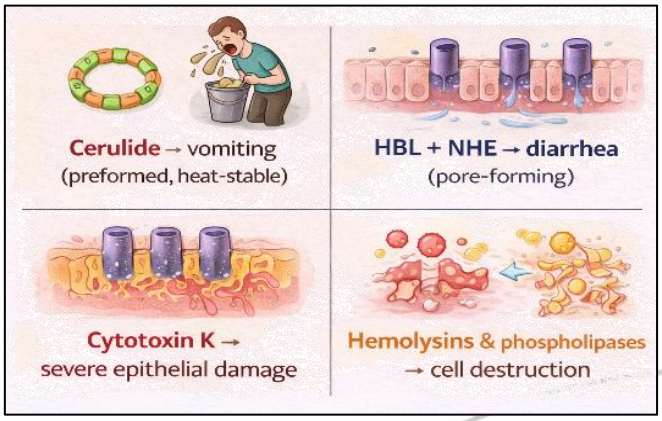
Examples:
- Endocarditis
- Meningitis (especially transplant patients)
- Osteomyelitis
- Pneumonia

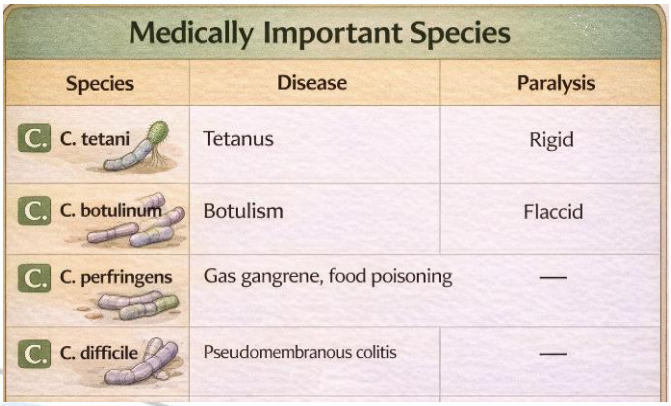
➔ **Morphology & Identification**
- Size: 3–4 µm, arranged in **long chains**
- **Spores** located in the center of the **motile** bacilli.
- Differentiated from *B. anthracis* by:
  1) **Colony morphology**:
     i. B. cereus → **large spreading feathery colonies** (blood/MYP agar)
     ii. B. anthracis → **dry waxy "Medusa head" colonies**.
  2) **Motility**:
     i. B. cereus → **motile**
     ii. B. anthracis → **non-motile**.
  3) **Hemolysis**:
     i. B. cereus → **β-hemolytic**
     ii. B. anthracis → **non-hemolytic**.
  4) **Lecithinase**:
     i. B. cereus → **positive**
     ii. B. anthracis → **negative**.
  5) **Antibiotics**:
     i. B. cereus → **resistant to penicillins/cephalosporins**
     ii. B. anthracis → **sensitive**.

Clinically, B. cereus causes **food poisoning** → supportive management (no antibiotics).

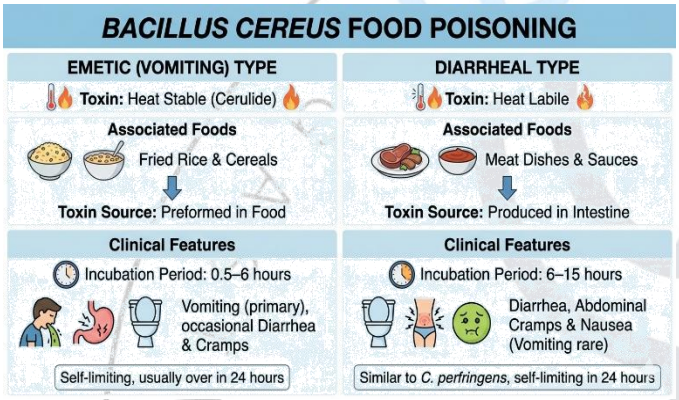
➔ **Epidemiology**
- Heat-resistant spores contaminate: **Rice and Cereals**
- Spores **germinate** at room temperature.
- Produces a **heat-stable toxin that survives frying**.
- Reservoirs include: **Soil, Water, Vegetables, Fomites, and Invertebrate intestines**
- Spores germinate when they come into contact with organic matter (rice & soil) or within an insect or animal host.

➔ **Pathogenesis (Toxins)**
B. cereus produces:
- Phospholipases
- Hemolysins
- Emetic toxin (**cerulide/ heat-stable**)
- Diarrheal Enterotoxins (**heat-labile**):
  1) Hemolysin BL (HBL)
  2) Non-hemolytic enterotoxin (NHE)
  3) Cytotoxin K





➔ Food Poisoning (Two Types)



Note: The enterotoxin may be formed in the food or produced in the intestine

➔ Diagnosis:

- Clinical diagnosis
- Culture from: food, stool, and vomitus
- Gram stain

➔ Treatment

- **<u>Fluid and electrolytes replacement</u>**
- Usually **<u>no antibiotics</u>** required
- Resistant to: Penicillins and Cephalosporins

[Clostridium species]

➔ General Characteristics

- **Gram-positive**, **spore**-forming **rods**.
- Strict **anaerobes**.
- Spores **wider than the diameter of the rods** in which they are formed
- Most are **motile** with peritrichous flagella *except for C.perfringenes.*
- Grow well on **blood-enriched anaerobic** media.

Clostridium Botulinum

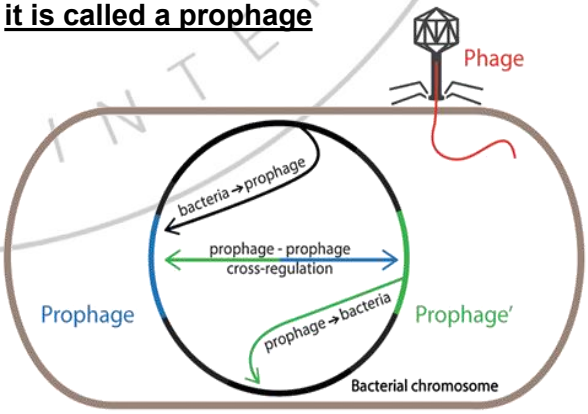
➔ Key Features

- **Anaerobic**, **spore**-forming **gram-positive** bacillus
- Produces **botulinum toxin** (neurotoxin) characterized by <u>symmetrical, **descending**, flaccid paralysis of motor and autonomic nerves</u> usually beginning with cranial nerves.
- Found in **soil, vegetables, fish, mammals**.

➔ Botulinum Toxin

- One of the **most potent toxins known**
- Encoded by a **prophage**
- Seven serotypes (A–G)

What is a prophage?
A **bacteriophage** = virus that infects bacteria
When the phage's DNA enters a bacterium, it integrates into the bacterial genome and remains inactive (latent).
→ **<u>it is called a prophage</u>**



➔ Mechanism of Action

Source of the Toxin (How exposure happens), the most common sources are:

- Spiced
- Smoked
- Vacuum-packed
- Canned alkaline foods

These foods **are often not heated properly**.
Why is this important?

1) C. botulinum spores survive in harsh conditions.
2) In anaerobic environments they produce:
   - Spores
   - Vegetative bacteria
   - Botulinum toxin

The toxin is **preformed** in the food, not produced inside the body.
After ingestion:

- Botulinum toxin is absorbed from the gut
- Enters the bloodstream
- Is carried to peripheral nerve synapses

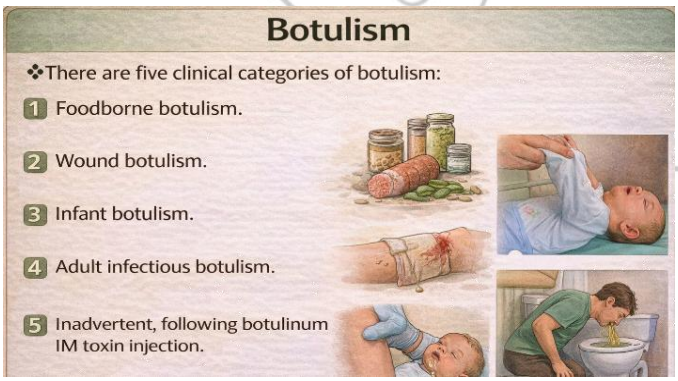
Site of Action

- The toxin acts at the **neuromuscular junction**
- Specifically at the **presynaptic nerve terminal**

Molecular Mechanism

- Botulinum toxin **blocks the release of acetylcholine (ACh)**
- It does this by: **Cleaving SNARE proteins required for vesicle fusion**

Without ACh: Muscle fibers cannot contract
Result: **Flaccid Paralysis**



➔ Clinical Findings of Botulism

Botulism is caused by botulinum toxin, which **reversibly blocks acetylcholine release at neuromuscular junctions**.
As a result, symptoms progress from gastrointestinal → neurological → respiratory**.**

1. **Early Gastrointestinal Symptoms**:

- Symptoms usually begin **18–36 hours after ingestion** of contaminated food.
- Early GI features include:
  1) Nausea
  2) Vomiting
  3) Abdominal cramps
  4) Diarrhea

2. **Early Neurological Symptoms**:

The earliest neurological symptoms involve cranial nerves:

1) Dry mouth (due to autonomic dysfunction)
2) Blurred vision
3) Diplopia (double vision)

These are followed by:

1) Dysphagia (difficulty swallowing)
2) Dysarthria (difficulty speaking)

This pattern is typical of botulism and reflects **descending paralysis**.

3. **Progressive Neuromuscular Paralysis**:

Paralysis progresses from:

1) Cranial nerves
   1) To neck and upper limbs
   2) Then to the lower limbs

Paralysis is **flaccid, symmetric, descending**.

4. **Respiratory Failure (Life-Threatening):**

In severe cases:

- Paralysis **involves the respiratory muscles**
- Leads to ventilatory failure

This is **the most common cause of death in botulism** if untreated.

5. **Infant Botulism**:

Occurs in infants during the first months of life.
Ingested spores germinate within the intestinal tract, producing toxin.
Honey is the most common vehicle. In adults, spores do not germinate because of the presence of a mature microbiome.

Clinical features:

- Poor feeding
- Weak cry
- Hypotonia
- “Floppy baby” syndrome

Infant botulism may be a **cause of sudden infant death syndrome (SIDS).**
SIDS is the sudden and unexplained death of an apparently healthy baby aged up to 12 months old, usually during sleep.

➔ **Diagnosis**

- Toxin detection (ELISA, PCR)
- Mouse bioassay → **gold standard**

➔ **Treatment**

Management focuses on **supportive care first**, then neutralizing circulating toxins, and addressing the source.

1. **Supportive Treatment**

The most critical part of management includes:

- Continuous heart rate and respiratory rate monitoring.
- Airway protection.
- Adequate mechanical ventilation.

Needed because:

- Botulinum toxin causes **respiratory muscle paralysis**.
- Death usually occurs from **respiratory failure**.

2. **Surgical Debridement (Wound Botulism)**

Indicated in wound botulism

- **Remove necrotic tissue**
- **Eliminate anaerobic conditions**
- **Stop further toxin production**

3. **Antitoxin Therapy**

- Trivalent antitoxin (A, B, E) is given intravenously
- Must be administered as early as possible

How antitoxin works:

- **Neutralizes circulating (free) toxin**
- Does **NOT** reverse paralysis already established
- **Prevents progression** ONLY

Earlier administration = better outcome

4. **Infant Botulism**

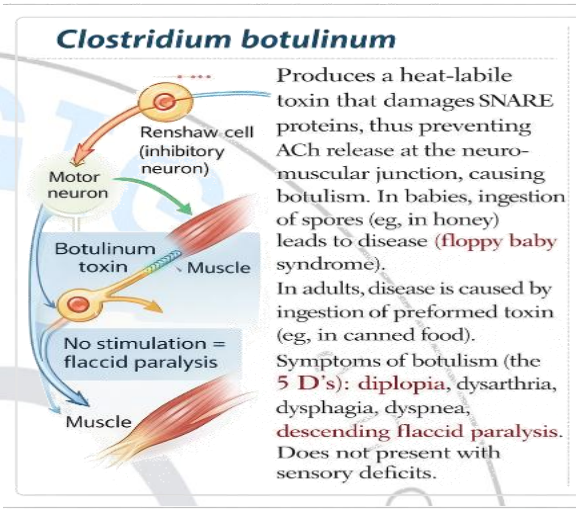
Many infants recover with supportive care alone, however:

- Antitoxin therapy is recommended
- Prevents progression and complications

➔ **Prevention**

- Proper Heating of Canned Foods
- No Honey for Infants (<1 year)

➔ **Summary of C. Botulinum**



Clostridium Perfringens

➔ **Key Concept**

- Many toxin-producing Clostridium species can cause invasive infections
- These infections occur when bacteria are:
  1) Introduced into **damaged or devitalized** tissue
  2) Exposed to **anaerobic** (low oxygen) conditions
     - About 30 Clostridium species can cause invasive disease
     - **Clostridium perfringens** is responsible for ~90% of cases

➔ **Clinical manifestations**

1) Myonecrosis (gas gangrene) → muscle death
2) Severe soft tissue infections
3) Food poisoning (via enterotoxin, not invasion)

Important distinction:

- Invasive disease → **toxin-mediated tissue destruction** (myonecrosis & soft tissue infection)
- Food poisoning → **toxin acting in the intestine** (non-invasive)

➔ **Distinguishing Features of Clostridium perfringens**

- **Large gram-positive rods**
- **Spore**-forming
- **Non-motile**
- Strict **anaerobe** (Grows best in low-oxygen environments such as devitalized tissue and deep wounds)
- **Stormy Fermentation** (Classic Lab Feature), seen in **milk** media**.**
  Rapid fermentation causes **gas production and clot disruption**.
- **Double Zone of Hemolysis** (Blood Agar)
- Reservoir: **soil and human colon** (normal flora)
- Transmission: **Foodborne** → food poisoning
- **Traumatic implantation** → gas gangrene: open wounds, crush injuries, and surgical wounds

➔ **Epidemiology**

C. perfringens is widely distributed in:

- The intestines of humans
- The intestines of domestic animals
- The environment (soil)

How does food poisoning occur?

- During meat preparation, meat can become contaminated with:
  - Soil
  - Intestinal contents

-Spores of *C. perfringens* are:

- **Heat-resistant**
- **Able to survive cooking, especially in large pieces of meat**

After cooking:

-If food is **slowly cooled** or **improperly stored**:

- Spores germinate
- Bacteria multiply rapidly

Ingestion of large numbers of bacteria → food poisoning

This explains why outbreaks often occur with buffets, stews, and large meat dishes.

A rare but serious illness called:

Necrotizing enteritis, also known as **pigbel disease**.

- Caused by: **Type C strains of *C. perfringens***

-Occurs after ingestion of contaminated food.

-Characterized by:

- Severe intestinal necrosis
- High mortality if untreated

➔ **Pathogenesis**

Pathogenesis differs depending on whether the infection is invasive or intestinal**.**

1) ***Entry of Spores*** (Invasive Infections)
Spores enter tissues through:
   1) **Traumatized or devitalized tissue**
   2) **Soil-contaminated wounds**
   3) **Crush injuries**
   4) **Endogenous source** → from the intestinal tract.

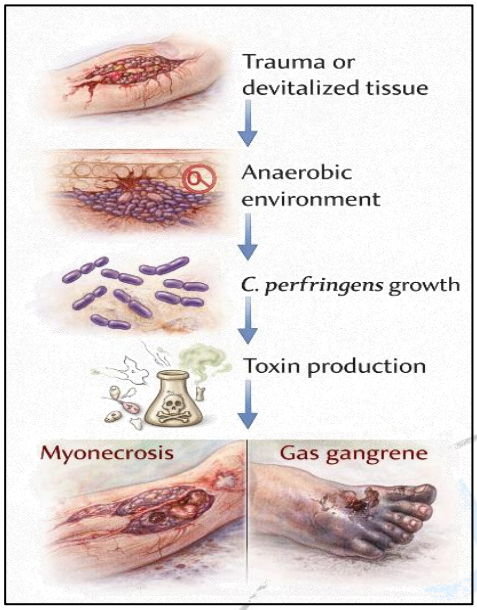
2) ***Germination & Growth***
Spores germinate in tissues with:
   - **Low oxidation–reduction potential**, associated with lower oxygen levels.
   - **Low oxygen** (anaerobic environment)

   Spores germinate into vegetative cells which:
   - Multiply rapidly
   - Ferment carbohydrates in tissues
   - Produce gas
     Gas production causes **crepitus**, which is a crunching, popping sound caused by air bubbles. A classic sign of gas gangrene.

3) ***Toxin Production***
C. *perfringens* produces multiple toxins:
   1) **α-toxin** (Most important)
   2)
   3) **Lecithinase** (phospholipase C), causes **tissue necrosis, hemolysis, and cell membrane destruction**.
   Responsible for:
   - **Gas gangrene**
   - **Myonecrosis**

   4) **Theta-toxin**, which contributes to:
   - **Tissue damage**
   - **Hemolysis**
   - **Shock**

   5) **Enterotoxin** (Some strains)
   - Produced in the intestine
   - Causes:
     - **Food poisoning**
     - **Watery diarrhea and cramps**



➔ **Clinical Manifestations**

1. **Invasive Infection (Gas Gangrene / Myonecrosis)**

Occurs after contaminated wounds, such as:

- **Compound fractures** (bone fractures that pierces the skin)
- **Postpartum uterine infections**
- **Traumatic injuries** contaminated with soil or feces

Timeline

Infection spreads rapidly within **1–3 days**

Clinical features

1) **Crepitation (gas in tissues)**
2) **Severe pain**
3) **Muscle swelling and discoloration**
4) **Rapidly progressing necrosis**
5) **Systemic signs**: fever, hemolysis, toxemia
6) **Shock, and death if untreated**

2. **Food Poisoning (Non-Invasive)**

Occurs after ingestion of large numbers of bacteria commonly from:

- Improperly stored food
- Warmed meat dishes
-

Mechanism: Bacteria sporulate **in the gut** (not the food) and release **enterotoxin**.

Clinical features

1) **Watery diarrhea.**
2) **Abdominal cramps**, without vomiting or fever.

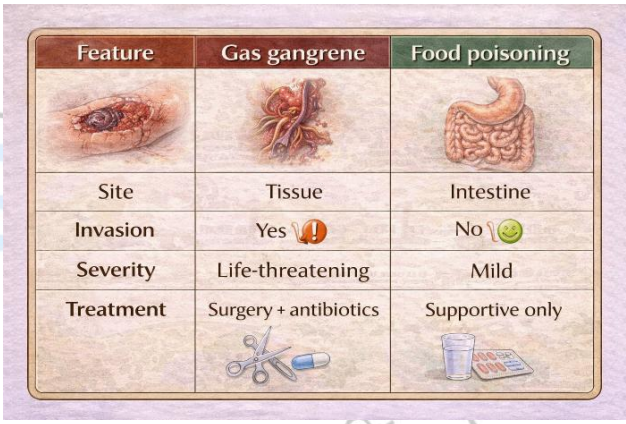
Onset

7–30 hours after ingestion

Illness duration

1–2 days

Usually **self-limiting**



➔ **Diagnostic Laboratory Tests**

Diagnosis depends on clinical suspicion + laboratory confirmation**.**

1) **Microscopy**: Gram-stained smears from: **Wounds, Pus, and Tissue**
   Show**: Large gram-positive rods** and few leukocytes (due to toxin-mediated destruction)
2) **Culture**: Specimens cultured in:
   - **Anaerobic** blood agar
   - **Thioglycolate** medium

   Laboratory features: rapid growth, gas production, rarely produces spores.

3) **Toxin Detection**: Diagnosis confirmed by**:**
   - Neutralization by specific antitoxin
   - Toxin production

   Example: **Nagler test.**
   This is the **definitive test** for detecting toxin-producing *Clostridium perfringens*.

   The agar plate is divided into two halves; antitoxin (against lecithinase) is applied to one side. After inoculation with *C. perfringens*, **a zone of opalescence appears only on the antitoxin side**, indicating a lecithinase-producing strain.

➔ **Treatment and Prevention**

Treatment of Invasive Disease (Gas Gangrene)

1) ***Surgical management***
   - Immediate and extensive **surgical debridement**
   - Removal of devitalized tissue & necrotic muscle
   - Prevents further bacterial growth and toxin production

2) ***Antibiotic therapy***
   - Started immediately
   - Commonly: **Penicillin**
   - Adjunct therapies: Hyperbaric oxygen
     - Inhibits anaerobic growth
     - Helps tissue oxygenation
     - Reduces toxin production

3) ***Antitoxins***
   - Antitoxins exist (immune globulins)
   - Limited role
   - Should not be relied upon alone

Treatment of Food Poisoning
- Supportive care only
- Fluids and symptomatic treatment
- Antibiotics are not required

Clostridium Difficile

➔ **Epidemiology**

Where is *C. difficile* found?
- Ubiquitous in the environment
- Colonizes:
  - 50% of healthy neonates
  - 4% of healthy adults

Disease occurs only **when conditions allow overgrowth and toxin production**.

One of the most common **healthcare-associated infections**, especially affects:
- **Hospitalized patients**
- **Elderly**
- **Immunocompromised individuals**

Antibiotics **disrupt** normal bowel flora; this allows *C. difficile* to overgrow.
Common high-risk antibiotics:
- **Clindamycin**
- **Cephalosporins**
- **Fluoroquinolones**

This leads to **antibiotic-associated diarrhea**.

Source of infection can be:
- **Endogenous** (from patient's own gut)
- **Exogenous** (ingestion of spores from hospital environment)

Spores are:
- **Resistant to alcohol**
- **Persist on surfaces**
- **Easily transmitted in hospitals**

➔ **Pathogenesis**

Disease is caused by **toxin production**, not invasion.
C. difficile produces two major toxins and other virulent factors:

1. **Toxin A (Enterotoxin)**, which causes:
   - Cytokine release
   - Inflammation
   - Hypersecretion of fluid

   Toxin A leads to watery diarrhea
2. **Toxin B (Cytotoxin)**, which causes:
   - Depolymerization of actin
   - Loss of cytoskeleton
   - Cell death

   Results in:
   - **Mucosal damage**
   - **Pseudomembrane formation**

   Toxin B is essential for the disease.
3. Additional Virulence Factors
   - **Adhesion factors** → allow attachment to intestinal epithelium.
   - **Hyaluronidase** → tissue spread.

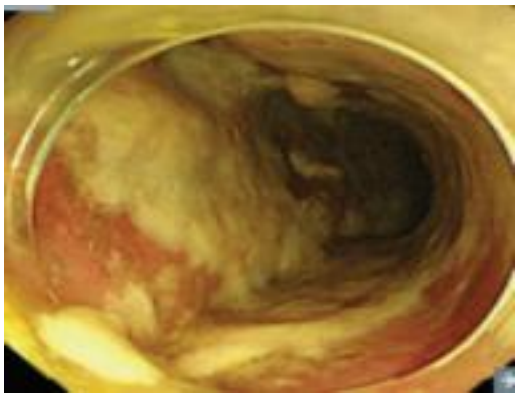
4. Hypervirulent Strains (Exam-important)
- New strains **produce higher amounts of toxins**.
- Examples:
  - **Ribotype 027**
  - **Ribotype 078**
  - Associated with:
    - **More severe disease**
    - **Higher relapse rates**
    - **Increased mortality**

➔ **Disease**

1. **Antibiotic-Associated Diarrhea**
   - Most common presentation
   - Mild to moderate
   - Due to **disruption of normal gut flora and overgrowth of *C. difficile***.
   - Symptoms:
     - **Watery diarrhea**
     - **Mild abdominal discomfort**

This is the early and less severe form of CDI.

2. **Pseudomembranous Colitis (PMC)**
- Severe form of CDI
- Characterized by:
    - **Formation of pseudomembranes on colonic mucosa**, which is composed of fibrin, mucus, necrotic epithelial cells, and inflammatory cells
- Can progress to:
    - **Fulminant colitis**
    - **Toxic megacolon**
    - **Perforation**
    - **Sepsis**



Pseudomembranes in pseudomembranous coliti.

Fulminant colitis is a life-threatening, rapidly-progressive, severe inflammation of the colon.
Toxic megacolon is a condition in which there is diffuse, severe dilation of the colon due to inflammation.
Sepsis is an exaggerated, systemic reaction to infection.

➔ Diagnosis

Diagnosis of CDI requires **clinical suspicion PLUS laboratory confirmation.**

1) ***Clinical Criteria***
    - **Diarrhea** (≥ 3 unformed stools per 24 hours For ≥ 2 days)
    - **No other obvious cause of diarrhea**
2) ***Laboratory Confirmation*** by at least **one** of the following:

a. Stool toxin detection
- Detect Toxin A or Toxin B
- Methods:
    - ELISA
    - Latex agglutination
    - PCR (most sensitive) commonly used today

b. Stool culture
- Culture of *C. difficile* on selective media
- Mainly used for epidemiology, not routine diagnosis

c. Endoscopy (for severe cases)
- Shows: **Yellow-white pseudomembranous**
- Not required in all patients
- Seen in: 50% of patients with positive toxin tests and severe diarrhea.

➔ Treatment

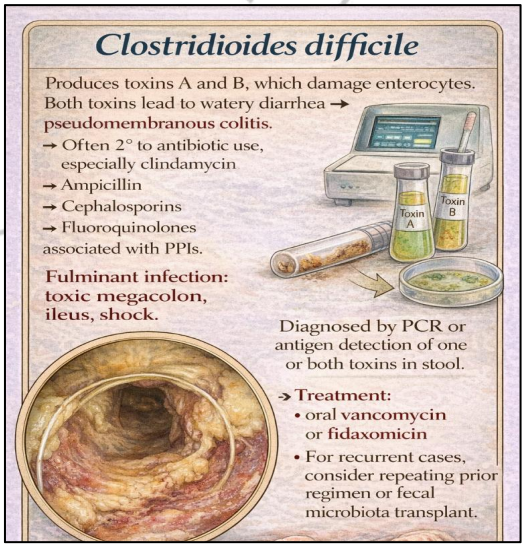
- Stop the offending antibiotic
- Antibiotic therapy for CDI: **Oral vancomycin or fidaxomicin** → drug of choice and **Metronidazole** → alternative (mild cases or limited resources)
- For recurrent cases, consider repeating prior regimen or fecal microbiota transplant.

➔ Infection Control & Prevention

1. Antibiotic stewardship
- Avoid unnecessary broad-spectrum antibiotics
- Prefer narrow-spectrum agents when possible

2. Isolation
- Symptomatic patients should be isolated
- Especially important in:
    - Hospitals
    - Nursing homes

3. Environmental control
- *C. difficile* spores are resistant
- Autoclaving bedpans and equipment is required
- Alcohol hand rubs do not kill spores → soap and water preferred



[Questions]

Q1: Which food item is most frequently associated with emetic type food poisoning by Bacillus cereus?
A) Rice and cereals
B) Honey
C) Lettuce and spinach
D) Meat dishes and sauces
E) Canned alkaline foods

Q2: A woman eats farm eggs, and 12 hours later has nausea, vomiting, then descending flaccid paralysis needing ventilation. Diagnosis?
A) Viral gastroenteritis
B) Botulism
C) Salmonella infection
D) Shigellosis
E) Campylobacter infection

Q3: Which of the following causes pseudomembranous colitis?

A) Clostridium difficile
B) Shigella
C) Salmonella
D) Bacillus cereus
E) All of the above

Q4: Which is the most frequent vehicle for infant botulism?
A) Rice and cereals
B) Honey
C) Lettuce and spinach
D) Meat dishes and sauces
E) Canned foods

Q5: The test of choice for confirmation of botulism is:
A) ELISA
B) PCR
C) Mouse lethality bioassay
D) Gram stain
E) Nagler reaction

Q6: Food poisoning from Bacillus cereus produces two syndromes. Emetic toxin is:
A) Heat-labile toxin
B) Hemolysin
C) Enterotoxin A
D) Heat-stable toxin
E) Neurotoxin

Q7: Which Clostridium species is associated with gas gangrene?
A) Clostridium difficile
B) Clostridium tetani
C) Clostridium perfringens
D) Clostridium botulinum
E) Clostridium septicum

Q8: A patient develops diarrhea after taking antibiotics, which of the following is the causative agent?

A) Salmonella typhi
B) Clostridium difficile
C) Escherichia coli O157:H7
D) Shigella sonnei
E) Vibrio cholera

Q9: Which organism's food poisoning is associated with consumption of improperly stored fried rice?

A) Salmonella typhi
B) Clostridium botulinum
C) E. coli O157:H7
D) Bacillus cereus
E) Vibrio cholera

Q10: Which organism produces a potent neurotoxin that causes flaccid paralysis?

A) Clostridium perfringens
B) Clostridium tetani
C) Clostridium botulinum
D) Escherichia coli
E) Bacillus cereus

Q11: Clostridium perfringens causes all the following EXCEPT:
A) Food poisoning
B) Gas gangrene
C) Pseudomembranous colitis
D) Stormy fermentation in milk media
E) Alpha-toxin mediated hemolysis

Q12: A 70-year-old man hospitalized for infection treated with clindamycin later develops mucoid green stools with yellow plaques seen on colonoscopy. Most likely cause?
A) C. difficile infection
B) Bacillus cereus infection
C) Clostridium perfringens infection
D) Salmonella typhi infection
E) Shigella infection

Q13: Which of the following is most associated with pseudomembranous colitis after antibiotic use?
A. Escherichia coli
B. Clostridium difficile
C. Bacteroides fragilis
D. Lactobacillus species
E. Staphylococcus aureus

Q14: Antimicrobial therapy can decrease the amount of susceptible bowel flora and allow proliferation of relatively resistant colonic bacteria. Which one of the following species can proliferate and produce a toxin that causes diarrhea?

A. Enterococcus species
B. S epidermidis
C. Pseudomonas aeruginosa
D. Clostridium difficile
E. B fragilis

Answers:
Q1: A
Q2: B
Q3: A
Q4: B
Q5: C
Q6: D
Q7: C
Q8: B
Q9: D
Q10: C
Q11: C
Q12: A
Q13: B
Q14: D

The University of Jordan
Gastroenterology Interest Group (UJ-GIG)
Booklet

Microbiology

Enteric Gram-Negative Rods (Enterobacteriaceae) 1&2

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[Introduction to Enteric Gram-Negative Bacteria]

Enteric gram-negative bacteria are a large group of organisms that primarily inhabit the gastrointestinal tract of humans and animals. Many are part of the normal gut flora, while others are important pathogens causing gastrointestinal and systemic infections.

➔ General Characteristics

- Gram-negative **rods**
- Grow **aerobically** and **anaerobically** (are facultative anaerobes)
- Grow well on **MacConkey** agar, **peptone** or **meat extract** media
- **Ferment glucose**
- **Reduce nitrates to nitrites**
- **Oxidase negative**
- **Catalase positive**
- Have a 39–59% **G + C** DNA content.

Clinical importance: They are a major cause of **diarrhea**, **food poisoning**, **urinary tract infections**, **septicemia**, and **enteric fever**.

➔ Classification

Based on Lactose Fermentation (MacConkey Agar)

Group	Examples	Colony appearance
Lactose fermenters	*E. coli, Klebsiella, Enterobacter*	Pink colonies
Non-lactose fermenters	*Salmonella, Shigella*	Pale/colorless colonies

Note: MacConkey agar s**howing lactose vs non-lactose fermenters**.

➔ Antigenic Structure of Enteric Gram-Negative Bacteria

Enteric gram-negative bacteria (like *E. coli, Salmonella, Shigella*) have important surface antigens that are used for identification, serotyping, and understanding virulence**.**

1) ***O-antigen*** (Somatic Antigen):
- Part of the **lipopolysaccharide (LPS)** on the cell wall of all gram negative bacteria.
- **Heat-stable**.
- Detected by bacterial **agglutination tests**.
- Antibodies produced are mainly **IgM**.
- Important for:
  - **Serotyping**
  - **Immune response**
  - **Endotoxin-related effects** (fever, inflammation)

2) ***K-Antigen*** (Capsular Antigen):
- **Heat-labile**
- The K antigens make up a **large polysaccharide capsule** that cover the O (or H) antigen
- Identified by **capsular swelling (Quellung) test**.
- Contributes to:
  - **Virulence**
  - **Resistance to phagocytosis**

3) ***H-Antigen*** (Flagellar Antigen):
- Found on flagella.
- **Heat-labile.**
- Detected by **agglutination with anti-H antibodies**.
- Antibodies are mainly **IgG**.
- Important for:
  - **Motility**
  - **Serotyping (especially in *Salmonella*)**

4) ***Vi Antigen*** (Special Capsule)
- Found in Salmonella Typhi
- A type of capsular antigen
- Helps bacteria:
  - **Evade immune response**
  - **Survive inside host**

5) ***Colicins*** (Bacteriocins)
- Protein toxins produced by some gram-negative bacteria
- Kill or inhibit closely related bacteria
- Provide a competitive advantage in the gut

[*E. coli*–Associated Diarrheal Diseases]

➔Normal Habitat of *E. coli*

Normal resident of:

1) Intestine
2) Upper respiratory tract (small numbers)
3) Genital tract

- Most strains are **harmless**
- Disease occurs only with pathogenic strains

➔ **Why are some *E. coli* pathogenic?**

Pathogenic *E. coli* strains differ by:
- **Virulence factors**
- **Mechanism of disease**
- **Site of action (small vs large intestine)**

Each group causes diarrhea by a different mechanism.

➔ **Genetic Basis of Virulence**

- Virulence genes are often:
  - **Plasmid-encoded**
  - **Phage-mediated**
- These genes encode:
  1) **Adhesins** (attachment to gut epithelium)
  2) **Toxins**

Horizontal gene transfer is key.

➔ **Adhesion to Intestinal Epithelium**

Some strains adhere to:
- Small intestine → **watery diarrhea**
- Large intestine → **inflammatory or bloody diarrhea**

Adherence is essential for disease production

➔ **Laboratory Identification**

E. coli is: **Oxidase negative** and a **Lactose fermenter**.
On EMB (Eosin Methylene Blue agar) → Produces **green metallic sheen**.

Enteropathogenic *E. coli* (EPEC)

EPEC is a **non-toxin–mediated** cause of diarrhea, especially important in infants and young children.

➔ **Epidemiology & Importance**

- Major cause of infantile diarrhea
- Common in:
  - **Nurseries**
  - **Developing countries**
- Causes outbreaks, especially where hygiene is poor.

➔ **Pathogenesis (How EPEC Causes Disease)**

Unlike other *E. coli* strains, EPEC does **NOT** produce classic enterotoxins.
Instead, disease is caused by **attachment and effacement**.

Key Virulence Factors

EPEC requires two important genetic elements:
1. **EAF** (EPEC adherence factor) : **plasmid-encoded**
   - Encodes bundle-forming pili (BFP)
   - **Allows initial attachment to intestinal epithelial cells**
2. **LEE** (Locus of Enterocyte Effacement) : **chromosomal**
   - Encodes protein that injects bacterial proteins into host cells which result in **rearrangement of the host cell cytoskeleton**.

Effacement

After attachment:
- Microvilli are destroyed (**effacement**)
- **Loss of absorptive surface**
- Leads to:
  - **↓ absorption**
  - **↑ fluid loss**

Key mechanism: Structural damage → diarrhea.

➔ **Clinical Picture of EPEC Infection**

Typical Presentation (Infants)
- **Severe watery diarrhea**
- **Vomiting**
- **Fever**
- **Stool: non-bloody, contains mucus**.

This distinguishes **EPEC from EHEC and Shigella** (which are **invasive** and cause **bloody diarrhea**)

Course of Illness: usually **self-limited**
- Can become:
  - Prolonged
  - Chronic (especially in malnourished infants)

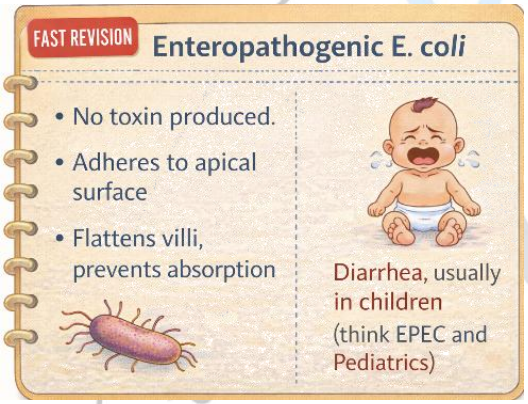
➔ **Serotyping & Identification**

EPEC strains are identified by:
- O antigen typing
- Sometimes H antigen typing

Helps in outbreak investigation.

➔ **Treatment**
- Supportive care is mainstay.
- Antibiotics:
  - May shorten duration
  - Useful in chronic or severe cases

Oral rehydration is always essential - *remember that patients end up losing a large amount of fluids that need to be replaced*.



Enterotoxigenic *E. coli* (ETEC)

ETEC is a **toxin-mediated** cause of watery diarrhea and **traveler's diarrhea** and is one of the most important diarrheal pathogens worldwide**.**

➔ **Epidemiology & Importance**

- Most common cause of "traveler's diarrhea".
- Major cause of diarrhea in: Infants in developing countries.
- Transmitted by **contaminated food and water**.

➔ **Colonization of the Intestine**

- ETEC infects **the small intestine**.
- Uses **Colonization Factor Antigens** (CFAs):
  - Surface adhesins
  - Allow firm **attachment** to intestinal epithelial cells.

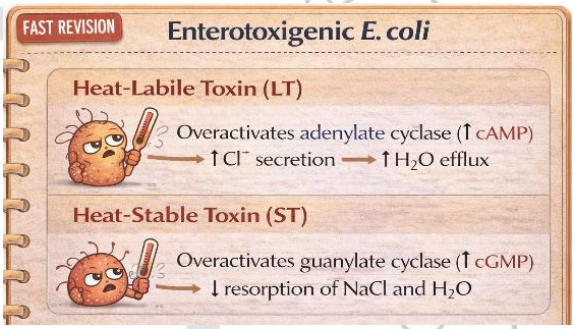
  CFAs are human-specific.

Adhesion is essential before toxin action.

➔ **Toxins Produced by ETEC**

ETEC produces two enterotoxins, both causing secretory diarrhea:

1) **Heat-Stable Toxin (ST)**
   - Small peptide toxin
   - Activates guanylate cyclase
   - ↑ cGMP
   - Leads to:
     - **↓ sodium absorption**
     - **↑ water secretion**
2) **Heat-Labile Toxin (LT)**
   - Large protein toxin
   - Similar to cholera toxin
   - Activates adenylate cyclase
   - ↑ cAMP
   - Causes:
     - **Increased chloride secretion**
     - **Water follows → diarrhea**



➔ **Clinical Picture of ETEC Infection**

Typical Features
- **Profuse watery diarrhea**
- **No blood or pus**
- **Abdominal cramps**
- **Dehydration if severe**

Physiological Effects
- **Hypersecretion** of:
  - Water
  - Chloride
- **Inhibition of sodium reabsorption**
- Gut becomes:
  - Distended
  - Hyper-motile

Diarrhea may last several days

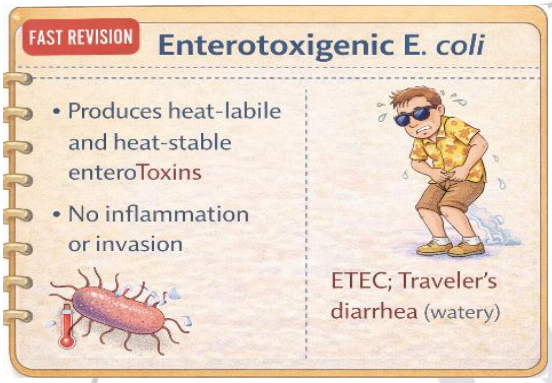
Remember

Secretion refers to the process of moving "stuff" from the body and systemic circulation to the lumen of the GI tract. **Absorption** means moving "stuff" back into the systemic circulation.

So, when we say water secretion → **water is being lost outside the body**.

➔ **Immunity & Cross-Reactivity**

- LT is antigenically **similar to cholera toxin**.
- Infection stimulates the production of **neutralizing antibodies** in the serum of persons previously infected with enterotoxigenic E coli.
- People in endemic areas often **develop partial immunity and less severe disease** on re-exposure.



Shiga toxin–producing *E. coli* (STEC / EHEC)

A strain of *E. coli* that causes disease mainly by producing a toxin called **Shiga toxin**.

Classically linked to:

- **Undercooked ground beef** (hamburgers)
- **Fresh produce** (lettuce, spinach, sprouts)
- **Unpasteurized products** (e.g., apple cider, milk)

➔ **Key toxins + mechanism**

- Produces **Shiga-like toxin 1 (Stx1) and/or Shiga-like toxin 2 (Stx2)**.
- These toxins **inactivate the 60S ribosomal subunit → stop protein synthesis → cell death** (especially intestinal epithelium + kidney endothelium).

This explains:

- **Bloody diarrhea** (hemorrhagic colitis)
- Can cause **Hemolytic uremic syndrome** (HUS): Occurs more often in children but can occur in adults too.

Hemolytic Uremic Syndrome (HUS) → is a serious complication caused by the shiga toxin where the blood vessels become damage leading to **microangiopathic hemolytic anemia** (rupture of red blood cells) + **thrombocytopenia** (low platelets) + **acute kidney injury**.

Clinical clues

- **Decreased urine output**, edema (acute kidney injury)
- **Pallor/fatigue** (anemia from RBC's getting shredded and dying)
- **Bruising/petechiae** (low platelets → bleeding)

Important serotype: **O157:H7** is the most common classic STEC and easiest to identify in many labs.

- (Classic lab pearl: often **does not ferment sorbitol** on sorbitol MacConkey agar)

➔ **STEC clinical picture**

Typical illness pattern:

1) **Starts** with **watery**/ secretory (non-bloody) diarrhea.
2) **Progresses** to grossly **bloody** diarrhea (hemorrhagic colitis).

Clinical picture you should expect:

- **Crampy abdominal pain** is common and can be severe.
- **Fecal leukocytes** (white blood cells) may be present (in many cases).
- **Fever is often absent** (**very testable point**).
  - If fever is absent but pain + bloody diarrhea are strong, don't jump too quickly to "noninfectious" causes like intussusception or ischemic bowel disease; STEC can do this.

Course:

- Often self-limited: symptoms commonly last ~ 5–10 days.
- 80–90% of cases spontaneously resolve without HUS.
- 10–20% develop HUS.
- Among those who do develop HUS:30% → chronic kidney failure and Up to 10% → mortality.

➔ **Diagnosis and treatment**

Diagnosis (stool testing):

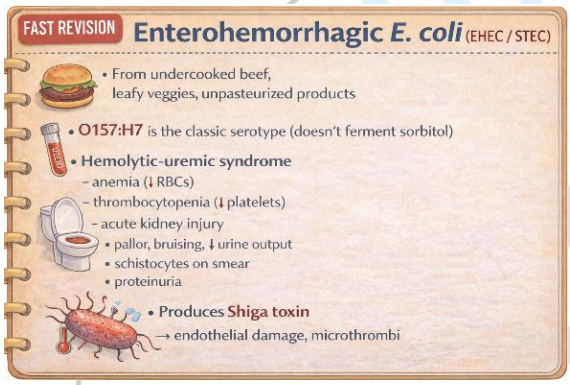
- Many labs do direct toxin detection:
  - **EIA (enzyme immunoassay)** for Shiga toxins.
- More sensitive options:
  - **PCR** for toxin genes (stx1/stx2).
  - Some settings use cell culture cytotoxicity tests (e.g., Vero cells).

Treatment:

- Supportive care is the mainstay
  - Oral/IV fluids, electrolytes
- **Avoid antibiotics** in suspected/confirmed STEC because it can increase toxin release → **higher HUS risk**

Prevention:

- Cook ground beef thoroughly
- Avoid unpasteurized products (like unpasteurized apple cider)
- Wash produce carefully
- Reduce cross-contamination in kitchens by hand-washing



Enteroinvasive *E. coli* (EIEC)

EIEC causes an illness very similar to Shigella. Seen mainly in:

- Children in developing countries
- Travelers to endemic areas

➔ Key microbiology

- **Non-lactose** or **late lactose** fermenters are **non-motile**.
- Requires a **large inoculum** (10^8–10^{10} CFU) → unlike Shigella, which needs only a small dose.

Inoculum refers to the number of bacteria needed to initiate an immune response.

➔ Pathogenesis

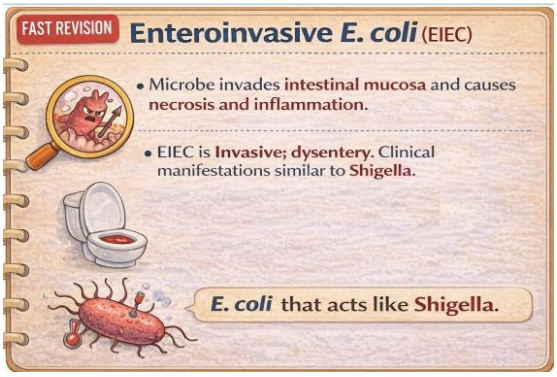
- **Invades** intestinal mucosal epithelial cells
- Spreads cell-to-cell → **inflammation and tissue destruction**.

➔ Clinical features

Inflammatory diarrhea

- **Bloody or mucoid stools**
- **Abdominal pain**
- **Fever is common**

Inflammatory diarrhea is characterized by blood or mucus in stool.



Enteroaggregative *E. coli* (EAEC)

- Causes **acute** and **chronic diarrhea (>14 days)**
- Important causes of: foodborne illness in developed countries, **traveler's diarrhea**, and persistent diarrhea in **HIV patients**.

➔ Key adherence pattern

- Bacteria attach tightly to intestinal cells in a: Diffuse or "**Stacked-brick**" pattern
- Leads to **persistent colonization** of the gut.

➔ Pathogenesis

- Does **not** invade intestinal cells
- Produces toxins: **EAST-1 toxin** (enteroaggregative ST-like toxin) and Other **plasmid-encoded enterotoxins**
- Results in: Mucosal damage and Prolonged **watery** diarrhea.

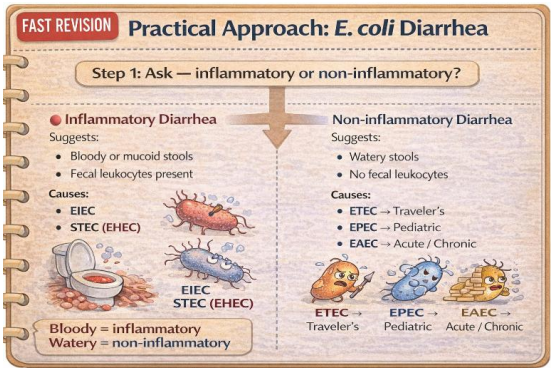
➔ Clinical features

Non-inflammatory diarrhea

- Watery stools
- Minimal or no blood
- Can be long-lasting

➔ Diagnosis

- Often suspected clinically
- Confirmation requires specialized adherence assays (not routinely available)



Treatment of *E. coli* infections

➔ General principles

- Severe *E. coli* infections (especially gram-negative sepsis and bacteremia) require:
  - **Rapid fluid and electrolyte replacement**
  - Antimicrobial therapy
  - Support of cardiovascular function (if septic shock develops)

➔ Antibiotic therapy

- There is no single antibiotic that works for all *E. coli* strains.
- Commonly used antibiotic classes include:
  - Sulfonamides
  - Ampicillin
  - Cephalosporins
  - Fluoroquinolones
  - Aminoglycosides

Note:

- Antibiotic susceptibility varies widely among *E. coli* strains.
- Therefore, culture and sensitivity testing are essential before choosing definitive therapy.

➔Antibiotic resistance

- *E. coli* frequently shows multiple drug resistance**.**
- Resistance genes are often carried on **plasmids**, which can be transmitted between bacteria**.**

Prevention of *E. coli* diarrhea (especially traveler's diarrhea)

➔ Preventive measures

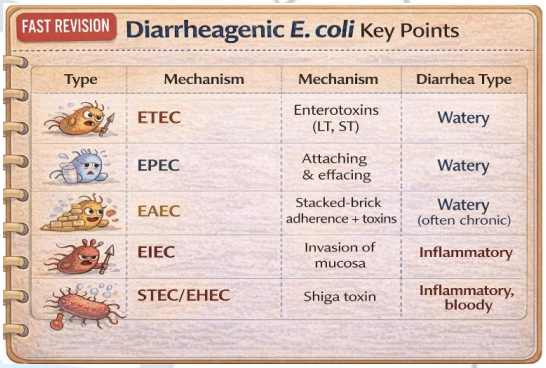
- Prevention is particularly important in traveler's diarrhea.
- Options include:
  - **Bismuth subsalicylate**: Taken daily has protective antimicrobial effects.
  - Short-term prophylactic antibiotics
  - e.g., **tetracyclines** or other antimicrobials (limited use)

➔ Caution with prevention

- These methods can cause side effects, so:
  - Use cautiously, especially in areas with poor sanitation and food hygiene.

➔ High-risk travelers

- In travelers at high risk or with early symptoms:
  - Early treatment may be given with:
    - **Trimethoprim–sulfamethoxazole**
    - **Ciprofloxacin**



Control of *E. coli* spread

➔ Normal flora concept

- *E. coli* normally colonizes the gut within days after birth.
- It becomes a major component of normal intestinal flora.
- Because it is part of normal flora: complete eradication is **neither possible nor desirable**.

➔ Environmental control

- Presence of *E. coli* in water or milk indicates fecal contamination.
- However, control measures cannot eliminate *E. coli* because it is **endogenous**.

➔ **Opportunistic infections**

- Some *E. coli* strains act as opportunistic pathogens:
  - Causes disease when introduced into:
    1) Immunocompromised hosts
    2) Hospitals
- Transmission occurs via:
  - **Medical personnel**
  - **Instruments**
  - **Parenteral medications**

➔ **Hospital infection control**

Key control strategies include:
- Strict handwashing
- Rigorous aseptic technique
- Proper sterilization of equipment
- Controlled use of IV lines
- Closed drainage systems for urinary catheters

[Shigellosis (Bacillary Dysentery)]

- **Shigellosis** is an acute inflammatory diarrheal disease caused by Shigella species.
- Humans (and some primates) are the only natural host.
- The infection causes **bacillary dysentery**, characterized by bloody, mucoid diarrhea.

➔**Microbiology**

- Gram-negative **rods**
- Slender, sometimes **coccobacillary** in young cultures.
- **Non-motile**
- **Facultative anaerobes** but grow best aerobically.

➔ **Culture characteristics**

- ***On agar plates***
  Colonies are small, circular, transparent
  Smooth edges
  Reach ~2 mm diameter in 24 hours

➔ **Sugar fermentation**

- All Shigella **ferment <u>glucose</u>**
- Do **NOT** ferment lactose
  Exception: **Shigella sonnei** (late lactose fermenter)
- On EMB agar: **Colorless colonies**
- Do **NOT** produce H_2S

Note: Non-motile, non-lactose fermenter, no H_2S → think **Shigella**.

➔ **Epidemiology of Shigellosis**

Host
- Humans and certain primates only.
- **No animal reservoir**.

Age group
- Can occur at any age
- Most common in children under 5 years

Seasonal pattern
- More common in:
  - Temperate climates
  - Rainy seasons in tropical regions
  - Warm months

Geographic distribution
- Endemic areas: Asymptomatic infections are common.
- Industrialized countries:
  - Shigella *sonnei* → **most common**.
  - Shigella *flexneri* → **second most common**.

Transmission
- Fecal–oral route
- Spread via:
  - Toilets, door handles
  - Contaminated food and water
  - Flies (mechanical vectors)
  - Person-to-person contact

➔ **Etiology (Classification of Shigella)**

General features
- Genus Shigella is divided into 4 species (Groups A–D) based on:
  - Antigenic structure
  - Biochemical reactions
- Very low inoculum needed to cause disease: 10–100 organisms (extremely infectious)

1. **Group A – *Shigella dysenteriae***
- 12 serotypes: the most important serotype is "**type 1**" which produces **shiga toxin**.
- Causes the most severe disease
- Associated with:
  - **Complications (e.g. HUS)**
  - **Severe dysentery**

2. **Group B – *Shigella flexneri***
- 8 serotypes
- Causes milder disease
- <u>Common in **developing** countries</u>

3. **Group C – *Shigella boydii***
- 18 serotypes
- Less common globally

4. **Group D – *Shigella sonnei***
- Single serotype
- Causes intermediate severity disease
- Most common in **industrialized** countries

➔ Pathogenesis of Shigellosis

Site of infection

- Shigella infection is almost always **limited to the gastrointestinal tract**, specifically the **colon**.
- Shigella is highly communicable.
- Very **low infectious dose**: As few as 10–100 organisms can cause disease. (much lower than Salmonella or Vibrio)

Mechanism of invasion

- Shigella **invades** intestinal epithelial cells **through M cells** overlying Peyer's patches.
- Steps:
  1) Induced phagocytosis by M cells
  2) Escape from the phagocytic vacuole
  3) Multiplication in the epithelial cytoplasm
  4) Cell-to-cell spread

Note: Shigella is an invasive pathogen, not just toxin-mediated.

Tissue damage

Infection of the large intestine causes:
1) Necrosis of the mucosal membrane
2) Superficial ulceration
3) Bleeding
4) Formation of microabscesses

Pseudomembrane formation on ulcers consists of:
- Fibrin
- Leukocytes
- Cell debris
- When inflammation subsides:
  - Granulation tissue fills ulcers
  - Scar tissue may form

➔ Toxins of Shigella

A. Endotoxin (LPS)
- On bacterial autolysis, all Shigella release endotoxin (LPS).
- LPS contributes to:
  - **Irritation of the bowel wall**
  - **Inflammatory response**
  - **Fever**

B. Shiga Exotoxin (Group A – *Shigella dysenteriae*)

- Shigella dysenteriae type 1 produces Shiga toxin.
- Properties:
  - **Heat-labile**
  - Neurotoxic → *harmful to neural tissue*
  - Cytotoxic → *harmful to cells*
  - Enterotoxic → *harmful to the GI tract*

Mechanism of action

- Similar to E. coli Shiga-like toxin
- **Inhibits protein synthesis**
- Causes:
  - Cell death
  - Severe intestinal damage

Systemic effects

- In severe cases, Shiga toxin may cause:
  1) Neurologic symptoms (e.g., meningismus, coma)
  2) Vascular injury

Sequence of disease

1) Early phase:
   - **Watery diarrhea (toxin-mediated)**
2) Later phase:
   - **Invasion** of the colon
   - **Bloody, purulent diarrhea (dysentery)**

➔ Clinical Findings of Shigellosis

Incubation period

- Short incubation: 1–2 days

Early symptoms

- Sudden onset of:
  - Abdominal pain
  - Fever
  - Watery diarrhea

Progression

- As disease advances:
  - Stool volume decreases
  - Stool frequency increases
  - Blood and mucus appear in stool

Bowel symptoms

- Each bowel movement is associated with:
  - Straining
  - Tenesmus (*rectal spasms which cause a feeling of incomplete defecation*)
  - Lower abdominal pain

Disease course

- In more than half of cases:
  - Fever and diarrhea resolve within 2–5 days
- However, in children and elderly, **severe dehydration and electrolyte imbalance** may lead to death if untreated.

Immunity and recurrence

- Infection produces **strain-specific** immunity
- Immunity is:
  - **Incomplete**
  - **Short-lived**
- Patients may experience recurrent infections.

➔ Diagnostic Laboratory Tests for Shigellosis

A. ***Specimens***

- Common specimens include:
  1) Fresh stool
  2) Mucus flecks
  3) Rectal swabs

  Microscopy often shows:
  - **Many fecal leukocytes**
  - **Red blood cells**

  These findings support **inflammatory** diarrhea.

B. ***Culture (most important diagnostic test)***

- Stool samples are cultured on:
  - Differential media:
    - MacConkey agar
    - EMB agar
- Selective media (suppress other Enterobacteriaceae and gram-positive organisms):
  1) Salmonella–Shigella (SS) agar
  2) Hektoen enteric agar
- These media:
  - **Suppress normal gut flora**
  - **Allow growth of Shigella (non-lactose fermenter)**

C. ***Serology***

- Normal individuals may already have agglutinating antibodies to Shigella.
- Serial antibody titers may rise during infection, but:
  - **Serology is not used to diagnose Shigella infections**
- Diagnosis relies mainly on **stool culture**.

➔ Treatment of Shigellosis

Antibiotic therapy

- Most commonly used antibiotics:
  - **Ciprofloxacin**
  - **Ampicillin**
  - **Doxycycline**
  - **Trimethoprim–sulfamethoxazole (TMP-SMX)**

Benefits of antibiotics

1) **Suppress** acute infection
2) **Shorten** duration of symptoms
3) **Reduce** transmission

Important considerations

- Multidrug resistance is common
  - Resistance is often **plasmid-mediated**.
- Many infections are self-limited
- Avoid opioids in Shigella dysentery
  1) Opioids slow gut motility
  2) Can worsen illness and prolong infection

➔ Prevention and Control of Shigellosis

Immune protection

- IgA antibodies in the gut (not serum) help limit reinfection.
- Serum antibodies against Shigella somatic antigens are mainly IgM.
- Immunity is **partial and temporary**

Transmission routes

- Shigella spreads via:
  1) Food
  2) Feces
  3) Fingers
  4) Flies
  5) Person-to-person contact

Key idea: Humans are **the only** reservoir.

Control measures

1. Improved sanitation
2. Safe water supply
3. Early diagnosis
4. Isolation of infected patients
5. Disinfection of excreta
6. Detection of subclinical carriers, especially food handlers
7. Antibiotic treatment of infected individuals

FAST REVISION

Shigella

Reservoirs	Humans only
Spread	Cell to cell; no hematogenous spread
H_2S Production	No
Flagella	No
Virulence Factors	Endotoxin; Shiga toxin (enterotoxin)
Infectious Dose (ID_{50})	Low—very small inoculum required; acid stable (resistant to gastric acids)
Immune Response	Primarily PMN infiltration
GI Manifestations	Crampy abdominal pain → tenesmus, *bloody mucoid* stools (bacillary dysentery)
Unique Properties	**4 F's:** fingers, fliys, food, feces. In order of decreasing severity **D > F > B > S** (most → least toxic *produced Dysenteriae*)

[The *Salmonella* Group]

➔ General characteristics

- Salmonella are **gram-negative bacilli** that commonly cause disease in **humans and animals**.
- Infection is usually acquired by the oral route.
- Transmitted from animals and animal products (eggs, poultry, meat)

➔ Motility & metabolism

- **Motile** organisms with peritrichous flagella
- Do **NOT** ferment lactose or sucrose
- Produce:
  - Acid
  - Gas
  - Usually H_2S

➔ Pathogenesis

- After ingestion, Salmonella:
  - Invades intestinal mucosa
  - Causes enteritis
- Some strains can:
  - Enter the bloodstream
  - Cause systemic infection

➔ Laboratory identification

- Salmonella are identified using selective media containing chemicals that suppress normal gut flora:
  - Brilliant green
  - Sodium thiosulfate
  - Sodium deoxycholate
- These allow isolation of Salmonella from feces

➔ Classification

- Salmonella are named by:
  - Genus: *Salmonella*
  - Species: *enterica*
  - Subspecies: e.g., *Typhi*, *Enteritidis*

➔ Subspecies of Medical Importance

All belong to Salmonella enterica, but differ in disease patterns:

- S. enterica subsp. Typhi
  - Causes **typhoid fever**
- S. enterica subsp. Enteritidis
  - Common cause of **gastroenteritis**
- S. enterica subsp. Typhimurium
  - Common cause of **gastroenteritis**
- S. enterica subsp. Choleraesuis
  - More likely to cause **bacteremia**
- S. enterica subsp. Paratyphi
  - Causes **paratyphoid fever**
- S. enterica subsp. Dublin
  - Often associated with **systemic infection**

Note: Enteritidis & Typhimurium → **diarrhea**
Typhi & Paratyphi → **enteric (typhoid) fever**

"Enteric Fevers" (Typhoid Fever)

Definition:

- Enteric fever is a systemic illness caused by certain Salmonella serotypes
- Diagnosed by:
  - Biochemical tests
  - Serologic tests

FAST REVISION

Major Salmonella Serotypes Causing Enteric Fever

Four serotypes are clinically important and should be **routinely identified:**

Serotype	Serogroup
Salmonella Typhi	Group D
Salmonella Paratyphi A	Group A
Salmonella Paratyphi B	Group B
Salmonella Choleraesuis	Group C1

➔ **Epidemiology**

- In developed countries:
  - S. Enteritidis
  - S. Typhimurium

  → most commonly reported (cause gastroenteritis, not typhoid)

➔ **Incidence**

- Marked difference between regions:
  - Developing countries: very high incidence (up to ~500 cases / 100,000 population)
  - Developed countries: low incidence (0.2–4 / 100,000)

➔ **Reservoir & transmission**

- Humans are **the only natural reservoir**.
- Major sources of spread:
  - **Feces** of asymptomatic carriers
  - **Food handlers** who are chronic carriers (important exam point)
- Routes of transmission:
  - **Food and water** contaminated with human feces
  - **Many animals**, including cattle, rodents, and fowl, are naturally infected with a variety of salmonellae and have the bacteria in their tissues (meat), excreta, or eggs.
  - **Vertical transmission** (through the placenta) – rare but possible

Note: Chronic carriers (especially food handlers) are **<u>the most important</u>** source of infection.

➔ **Pathogenesis of Typhoid Fever**

Entry & survival

- Infection occurs after oral ingestion.
- Disease severity depends on:
  - **Gastric acid** (low acidity increases risk)
  - **Normal intestinal flora**

Intestinal invasion

- Bacteria invade:
  - Peyer's patches in the ileum
- Transported to:
  - Mesenteric lymph nodes
- Then enter the bloodstream via:
  - Thoracic duct
- Cause primary bacteremia

Systemic spread

- Organisms multiply in:
  - **Liver**
  - **Spleen**
  - **Bone marrow**
- Results in prolonged **bacteremia** (*the presence of bacteria in the blood stream*) and **toxemia** (*the presence of toxins in the blood stream*)
- Infection of lymphoid tissue causes:
  - Necrosis and sloughing
  - Formation of intestinal ulcers
  - Ulcers usually heal without scarring

Immunity

- **Cell-mediated** immunity plays a major role in recovery.

➔ **Clinical Manifestations of Typhoid Fever**

Incubation period

- 7–14 days
- Onset is insidious (gradual)

1st week

- **Stepwise rising fever** (unremitting and high (**a high plateau**)
- **Malaise**
- **Anorexia**
- **Myalgia**
- **Headache**
- **Abdominal pain**
- **Diarrhea early**, may progress to constipation

2nd week

- **High, sustained fever**
- **Fatigue**
- **Cough**
- **Epistaxis (nosebleed)**
- **Worsening abdominal symptoms**
- Appearance of:
  - **<u>Rose spots</u>** (maculopapular rash on trunk)

3rd–4th weeks

- If untreated:
  - Symptoms may gradually improve
- In the pre-antibiotic era, major complications included:
  - **Intestinal hemorrhage**
  - **Intestinal perforation**
- Mortality rate historically: 10–15%

Enterocolitis (Non-typhoidal Salmonella)

➔ **Definition**
- Enterocolitis is the most common manifestation of Salmonella infection.
- In the United States, the most common causes are:
  - Salmonella Typhimurium
  - Salmonella Enteritidis
- However, >1,400 Salmonella serotypes can cause enterocolitis.

➔ **Clinical features**
- Symptoms begin 8–48 hours after ingestion.
- Typical symptoms include:
  - **Nausea**
  - **Headache**
  - **Vomiting**
  - **Profuse diarrhea**
- Stool findings: Fecal **leukocytes** present
- Fever: Usually **low-grade**
- Intestinal involvement: Lesions occur in both **the small and large intestine**
- Disease course:
  - **Self-limited**
  - Symptoms usually resolve in 2–3 days
- Blood cultures: Usually **negative**
- Stool cultures:
  - **Positive**
  - May remain positive for weeks after clinical recovery

➔ **Bacteremia**
- Occurs in 2–4% of cases
- Much more likely in: Immunodeficient patients

Bacteremia with Focal Lesions

➔ **Definition**
- A less common but more severe form of Salmonella infection.
- Associated commonly with S ***choleraesuis*** but may be caused by any Salmonella serotype**.**

➔ **Pathogenesis**
- After initial infection: Salmonella invades the bloodstream.
- Leads to **focal** infections in:
  - Lungs
  - Bones (osteomyelitis)
  - Meninges
  - Other organs

Gastrointestinal symptoms are often **absent** in this form.

➔ **Laboratory findings**
- **Blood cultures are positive**
- Stool cultures may be **negative**

Diagnostic Laboratory Tests for Salmonella

A. Specimens

Cultures may be taken from:
- Blood
- Bone marrow
- Stool
- Urine

Culture timing

1. Enterocolitis
- Stool culture: Positive **early** in disease
- Blood culture: Usually negative

2. Enteric (Typhoid) fever
- Blood culture: Positive in the first week
- Stool culture: Positive in the second or third week
- Bone marrow culture: **Highly sensitive**

Note: in simple words, a "**sensitive test**", is a test that is able to detect a disease effectively. When a sensitive test is negative, that means the patient is **unlikely** to have the disease.

In chronic carriers
- **Duodenal drainage culture**:
  - Confirms presence of Salmonella in the biliary tract
  - Important in identifying carriers

B. Bacteriologic Culturing for Isolation of *Salmonella*

Step 1: **Enrichment** culture
- Stool specimens are first placed in enrichment broths, such as:
  - Selenite F broth
  - Tetrathionate broth
- Purpose:
  - **Inhibit normal intestinal flora**
  - **Allow Salmonella to multiply**
- This step **increases the chance of detecting Salmonella** when present in small numbers.

Step 2: **Differential & selective** media
- After enrichment, specimens are plated onto:
  - EMB agar
  - MacConkey agar
  - Deoxycholate agar
  - Salmonella–Shigella (SS) agar
  - Hektoen enteric agar
  - Xylose-lysine-deoxycholate (XLD) agar

Key lab features
- Salmonella:
  - **Non-lactose** fermenter
  - **Produces H_2S** (black colonies on some media)

Step 3: **Final identification**
- Suspect colonies are confirmed by:
  - Biochemical reaction patterns
  - Slide agglutination tests using specific antisera

C. Serologic Methods:

1. Slide Agglutination

Process
- This is a rapid screening test
- Known and unknown bacterial cultures are mixed on a slide.
- Clumping (agglutination) occurs within minutes **if antigens match antibodies**.

Uses
- Preliminary identification (there are commercial kits) of:
  - Salmonella
  - Serogrouping based on O antigens
    - Groups: A, B, C1, C2, D, E

2. Tube Agglutination (Widal Test)

Process
- A tube dilution agglutination test
- Detects antibodies against:
  - O antigen (somatic)
  - H antigen (flagellar)
- Used mainly for **Typhoid** fever

Antibody response
- Antibody titers rise during: 2nd and 3rd weeks of infection
- Because baseline antibodies may exist:
  - A single test is unreliable
  - Paired sera are required

Diagnostic criteria
At least two serum samples, taken 7–10 days apart, must show:
A rising antibody titer (Titer is the **highest serum dilution that still Produces agglutination**; higher titers reflect higher antibody concentrations)
- Positive titer:
  - O antigen > 1:320
  - H antigen > 1:640
  - A high titer of antibody to the Vi antigen occurs in some carriers.

Limitations of the Widal test
- False-positive and false-negative results are common.
- Interpretation varies by geographic region.
- Cannot reliably confirm diagnosis alone. (Alternatives to the Widal test include rapid colorimetric and EIA methods.)

Clinical use
- Most useful in:
  - **Resource-limited** settings where blood cultures are not available.

Immunity in Salmonella (Typhoid Fever)

Nature of immunity
- Infection with Salmonella Typhi or Paratyphi usually confers **partial immunity**.
- Protection is not complete and not long-lasting.
- Reinfection can occur, but is often **milder and shorter** in duration

Antibodies
- Circulating antibodies (**IgG**):
  - Directed **against O and Vi antigens**
  - Appear **2–3 weeks** after recovery
- Secretory **IgA**:
  - Helps prevent attachment of Salmonella to intestinal epithelium
  - Important for **mucosal defense**

Special risk groups
- Patients with **hemoglobin disorders** are more susceptible:
  - Sickle cell disease S/S trait (S/S means homozygous hemoglobin S (HbS), which causes sickle cell disease)
  - Hemoglobin A/S trait (Sickle cell trait (A/S) is heterozygous HbS, and the other Hb is the normal HbA)

- Increased risk of:
  - **Salmonella bacteremia**
  - **Osteomyelitis**

Treatment of Salmonella Infections

When antibiotics are required

- Always treat in:
  - **Enteric (typhoid) fever**
  - **Bacteremia** with focal lesions
  - **Salmonella enteritis in neonates**

- Usually NOT required in:
  - **Enterocolitis** is mild and uncomplicated: clinical symptoms and excretion of the salmonellae may be prolonged by antimicrobial therapy
  - In **severe diarrhea**, **fluid and electrolyte replacement** is essential to prevent dehydration and shock

Antibiotic options

- Commonly used antibiotics include:
  - **Fluoroquinolones**
  - **Ampicillin**
  - **Trimethoprim–sulfamethoxazole**
  - **Third-generation cephalosporins**

Antibiotic resistance

- Multidrug resistance is common
- Resistance genes are often plasmid-mediated
- Antibiotic susceptibility testing is important before definitive therapy

3% of survivors of typhoid become healthy permanent carriers:

- Salmonella may persist in:
  - **Gallbladder** (especially with gallstones)
  - **Biliary tract**
  - Rarely in the intestines or the urinary tract
- Treatment:
  - Some chronic carriers have been cured by **ampicillin** alone
  - Often requires **cholecystectomy (removal of the gallbladder) + antibiotics**

Prevention and Control of Salmonella

Public health measures

1) **Sanitation**
   - Safe food and water supplies
   - Prevention of contamination by:
     - Humans
     - Rodents
     - Domestic animals

2) Food safety

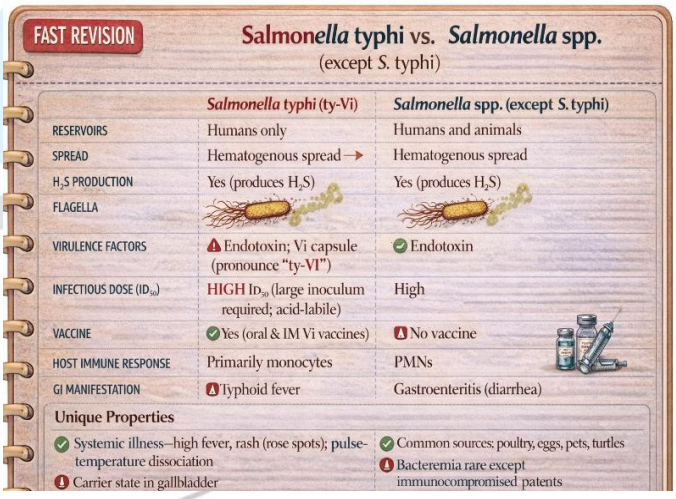
- Meat and eggs must be thoroughly cooked

3) Carrier control

- Must not work as food handlers
- Must follow strict hygiene practices

4) **Vaccination**

- Two typhoid vaccines available:
  1. Oral live attenuated vaccine
  2. IM Vi capsular polysaccharide vaccine
- Vaccination is recommended for:
  - **Travelers to endemic areas**
  - **Regions with poor sanitation**
- Note:
  - Vaccine efficacy is limited
  - Does **not** provide lifelong immunity



[Questions]

Q1: Heat-labile toxin of ETEC acts by which of the following mechanisms?
A) Attachment and effacement
B) Ribosomal dysfunction
C) None of the above
D) Activation of adenylyl cyclase
E) Aggregative adherence

Q2: Intestinal infection with which of the following organisms should NOT be treated with antibiotics?
A) Salmonella typhi
B) Clostridium difficile
C) Escherichia coli O157:H7
D) Shigella sonnei
E) Vibrio cholera

Q3: Which of the following bacterial agents has the lowest infective dose for producing gastrointestinal disease in the human host?
A) Enteropathogenic Escherichia coli
B) Enterotoxigenic Escherichia coli
C) Vibrio cholerae
D) Salmonella (nontyphoid serotypes)
E) Shigella flexneri

Q4: Heat-stable toxin of ETEC acts by which of the following mechanisms?

A) Activates adenylyl cyclase
B) Ribosomal dysfunction
C) Activates guanylyl cyclase
D) Attachment and effacement
E) Aggregative adherence

Q5: What is the identity of the organism forming black colonies (H2S production) on S-S agar?

A) Staphylococcus species
B) Escherichia coli
C) Shigella species
D) Streptococcus pyogenes
E) Salmonella species

Q6: An HIV-positive man recently traveled to the Caribbean. Three weeks later, he has persistent watery diarrhea and weight loss. Most likely cause?

A) Enteroinvasive Escherichia coli
B) Salmonella typhi
C) Enteropathogenic Escherichia coli
D) Shigella flexneri
E) Enteroaggregative Escherichia coli

Q7: Regarding Enterobacteriaceae, which is most accurate?
A) All are part of the normal microbiota
B) All have endotoxin
C) All ferment lactose
D) All are strict anaerobes
E) All produce enterotoxin

Q8: About typhoid fever (Enteric fever), which one is wrong?

A) Highly contagious
B) Vi antigen presence
C) Carriers are more important than cases
D) Bacteremia is characteristic
E) Zoonotic disease

Q9: Which condition is associated with Shiga toxin-producing E. coli (STEC/EHEC)?
A) Hemolytic uremic syndrome (HUS)
B) Non-bloody diarrhea only
C) Necrotizing fasciitis
D) Botulism
E) Pseudomembranous colitis

Q10: Which bacteria produce heat-stable and heat-labile toxins?

A) EPEC
B) EHEC
C) ETEC
D) Shigella
E) Klebsiella

ANSWERS:
Q1: D
Q2: C
Q3: E
Q4: C
Q5: E
Q6: E
Q7: B
Q8: E
Q9: A
Q10: C

The University of Jordan
Gastroenterology Interest Group (UJ-GIG)
Booklet

Microbiology

Vibrio, Campylobacters, Helicobacter, and Associated Bacteria

Written by: Ammar Alirani

Edited by: Sireen Basel

Reviewed by: Amr Abdallah

[Overview: Vibrio, Campylobacters, Helicobacter & Related Bacteria]

General features

- This group includes gram-negative **rods**.
- They are widely distributed in nature, especially in:
  - **Water**
  - **Food**
  - **Animals**
- Most cause gastrointestinal disease in humans.

➔ Vibrio cholerae

- Produces a potent **enterotoxin**.
- Disease severity is due to toxin production, **not** invasion
- Causes cholera:
  - Profuse watery ("**rice-water**") diarrhea
  - Rapid loss of fluids and electrolytes
- Can lead to:
  - Severe dehydration
  - Shock (decreased tissue perfusion)
  - Death if untreated

➔Campylobacter jejuni

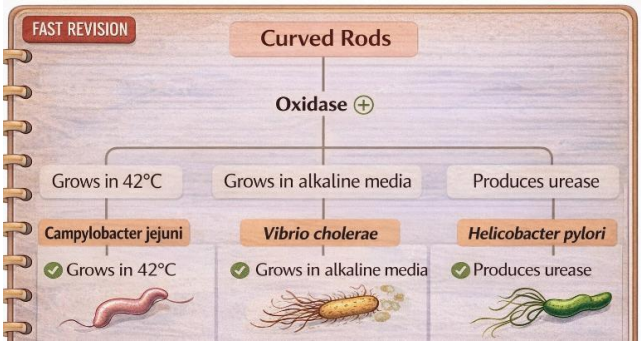
- One of the most common causes of bacterial enteritis worldwide.
- Causes:
  - **Inflammatory** diarrhea
  - Often **bloody**
  - Abdominal pain and fever

➔Aeromonas & Plesiomonas

- Less common causes of diarrhea.
- Usually associated with:
  - Water exposure
  - Contaminated food
- Causes acute gastroenteritis.

➔ Helicobacter pylori

- **Colonizes** the stomach.
- Associated with:
  - Chronic gastritis
  - Duodenal ulcers
  - Some gastric cancers and MALT lymphoma (later detail)



[The Vibrios – General Overview]

- Vibrios are gram-negative, **curved** (**comma-shaped**) rods.
- They are widely distributed in surface waters worldwide, especially:
  - Coastal waters
  - Brackish water

➔ Diseases caused by Vibrios

- Vibrios cause a spectrum of human diseases, including:
  - **Severe diarrheal illness** (e.g. cholera, which is responsible for 7 global pandemics in the past two centuries. Still a major public health problem today.)
  - **Mild gastroenteritis**
  - **Soft tissue and wound infections**

Other important Vibrio species:

- ***V. parahaemolyticus***
  - Causes gastroenteritis
  - Often associated with **raw or undercooked seafood**
- ***V. alginolyticus***
  - Causes **eye, ear, and wound** infections
- ***V. vulnificus***
  - Causes severe **sepsis** and wound infections
  - Seen in patients with **liver disease** (cirrhosis)
  - Associated with **raw oysters**
  - Known as the "wound maker."

Cirrhosis is a term used to describe chronic, irreversible scarring (fibrosis) of the liver.

Vibrio Cholrea

➔ **Epidemiology**
- Cholera outbreaks are closely linked to:
  - **Contaminated water**
  - **Poor sanitation**
- Control of cholera parallels:
  - **Development of safe water systems**
  - **Improved hygiene and sanitation**

➔ **Morphology**
- **Comma-shaped**, curved rod
- Size: 2–4 µm long
- Actively **motile** via polar flagellum
- serogroups O1 and O139
- After prolonged cultivation:
  - May lose curvature
  - Can resemble straight gram-negative rods

➔ **Growth conditions**
- Grows best in **alkaline** environments
  - Optimal pH: 8.5–9.5
- Rapidly **killed by acid**
  - Explains why a **high infectious dose** is needed (to overcome stomach acid)
  - Antacids **increase** susceptibility

➔ **Colony morphology**
- On standard media:
  - Colonies are convex, smooth, round
  - Appear opaque and granular under transmitted light

➔ **Selective media**
- **Thiosulfate-citrate-bile salts-sucrose** (TCBS) agar is selective for Vibrio species
- **Sucrose** fermentation: *Vibrio cholerae* and *V. alginolyticus*.
  - Produces **yellow** colonies
- Easily distinguished from **non-sucrose-fermenting** vibrios like V. vulnificus and V. parahaemolyticus
- **Dark green** background enhances visibility

➔ **Identification & General Properties of Vibrio**

1) Oxidase test
- Vibrios are **oxidase-positive**
- A positive oxidase test is a **key first step** in identifying *Vibrio cholerae* and other Vibrio species.

2) O/129 susceptibility
- Vibrio are **susceptible to O/129** (2,4-diamino-6,7-diisopropylpteridine phosphate).
- This helps differentiate:
  - Vibrio → susceptible
  - Aeromonas → resistant

3) Salt tolerance
- Most vibrios are **halotolerant**: growth is stimulated by NaCl
- Some species are halophilic: require NaCl to grow

Term	Needs salt?	Exam clue
Halotolerant	✗ Not required	Salt enhances growth
Halophilic	✓ Required	Cannot grow without NaCl

➔**Antigenic Structure & Biologic Classification**

1. Flagellar antigen
- Many vibrios share a **single heat-labile H** (flagellar) antigen
- Antibodies to the H antigen:
  - Are **not** strongly protective

2. O (somatic) antigens
- *Vibrio **cholerae*** has **lipopolysaccharide (LPS) O** antigens
- There are ≥206 O antigen groups
- Only two groups cause classic cholera:
  - O1
  - O139

Clinical significance of O groups
- O1 and O139 cause classic cholera
- Non-O1 / non-O139 strains usually cause milder diarrheal illness
- Note that these strains **belong to Vibrio cholera**, not all Vibrio species.

Cholera biotypes (A biotype is a subclassification within the same bacterial species based on biochemical, phenotypic, or biological behavior, not on major genetic differences)
- *V. cholera* O1 has two biotypes: (discussed further on the next page)
  - Classical
  - El Tor
- Each biotype is subdivided into two serotypes:
  - Inaba
  - Ogawa

➔ **Vibrio cholera Enterotoxin (Cholera Toxin)**

General features

- Cholera toxin is a potent protein **enterotoxin**
- Produced in **the small intestine**
- Molecular weight: ~84,000
- Structure → AB toxin: 1A subunit (MW,28,000) and 5B subunits.

Genetics: Genes encoding cholera toxin are located on the bacterial **chromosome**

Mechanism of action: B subunits bind to **GM1 ganglioside receptors** on intestinal epithelial cells.

1. This allows entry of the A subunit
2. A subunit:
   - Activates adenylate cyclase
   - ↑ **intracellular cAMP**
3. Results in:
   - Massive secretion of water and electrolytes
   - Profuse watery diarrhea

Note: Disease is due to toxin, not mucosal invasion

➔ **Pathogenesis of Vibrio cholerae**

Host & infectious dose

- Under natural conditions, *V. cholerae* is pathogenic only to humans.
- The infectious dose is **high** because vibrios are **acid-sensitive**:
  - Normal gastric acidity kills most organisms.
  - With water, infection usually requires ingestion of ~10^8–10^{10} organisms.

When gastric acidity is reduced (e.g., food buffering, antacids, malnutrition), as few as 10^3–10^4 organisms can cause infection.

Note: ↓ stomach acid → ↓ infectious dose

Survival & colonization.

- Toxin-coregulated pilus (TCP), whose expression is co-regulated with cholera toxin:
  - **Adherence**
  - **Colonization of the small intestine**
- Note: Vibrio do **NOT** invade the bloodstream; they remain in the intestinal lumen

Mechanism of disease

- Organisms attach to microvilli of the brush border of intestinal epithelial cells
- They:
  - **Multiply locally**
  - **Produce cholera toxin**
- May also release:
  - Mucinases
  - Endotoxin
- Resulting in massive secretion of water and electrolytes into the gut lumen

➔ **Clinical Findings: Epidemiology & Onset**

Epidemiology

- Disease burden peaks during "cholera seasons":
  - **High temperatures**
  - **Heavy rainfall**
  - **Flooding**
- However, cholera can occur year-round.

Asymptomatic infection

- Classic biotype: ~50% of infections are asymptomatic
- El Tor biotype: ~75% of infections are asymptomatic
- El Tor infections are generally milder.

Incubation period

- Ranges from 12 hours to 3 days
- Depends on the size of the inoculum

Diarrhea

- Sudden onset of:
  - **Nausea**
  - **Vomiting**
  - **Profuse watery diarrhea**
- Stool appearance:
  - "**Rice-water**" stools
  - Contain:
    - Mucus
    - Epithelial cells
    - Large numbers of vibrios
- Usually no blood or pus.
- Rapid loss of:
  - Water
  - Sodium
  - Potassium
  - Bicarbonate
- Leads to:
  - **Severe dehydration**
  - **Circulatory collapse**
  - **Anuria** (absence or near-absence of urine production)

Without treatment: Mortality rate: 25–50%

Diagnosis

- During epidemics, diagnosis is clinical.
- Sporadic or mild cases may be difficult to distinguish from other diarrheal illnesses.

➔ **Diagnostic Laboratory Tests for Vibrio cholerae**

1. **Specimens**
   - Best specimen for culture: **Mucus flecks from stool** because they are concentrated in the mucus, not the watery portion.

2. **Smears**
   - **Dark-field or phase-contrast** microscopy may show rapidly **motile** Vibrio
   - This gives a quick presumptive diagnosis, especially during outbreaks.

3. **Culture**
   - *V. cholerae* grows rapidly on:
     - **Peptone** agar
     - **Blood agar** at alkaline pH (~9.0)
     - **TCBS** agar → **yellow** colonies (sucrose fermenter)
   - Typical colonies can be identified in ~18 hours.

4. Specific identification

- Confirmed by slide agglutination tests with:
  - Anti-O1 antiserum
  - Anti-O139 antiserum
- And confirmed by biochemical tests

➔ **Treatment of Cholera**

- **Water and electrolyte replacement is the cornerstone of therapy**.
- Corrects severe dehydration and salt depletion
- Oral rehydration therapy (ORT) saves lives.

Note: Fluids first — antibiotics are secondary.

Antibiotic therapy

- Antibiotics:
  - **Reduce stool output**
  - **Shorten duration of illness**
  - **Decrease duration of bacterial shedding**
- Common options:
  - **Tetracycline**
  - **Doxycycline**

Antibiotic resistance

- In some endemic areas:
  - Tetracycline resistance has emerged.
  - Resistance genes are **plasmid-mediated**.

Special populations

- Avoid tetracyclines in **children and pregnant women**:
- Use alternatives such as:
  - **Erythromycin**
  - **Furazolidone**

Note: in children and pregnant women, tetracyclines are avoided due to *risk of permanent teeth discoloration and possible effects on bone development*.

➔ **Prevention of Cholera**

- Safe water supply
- Sanitary disposal of feces
- Improved nutrition
- Safe food preparation and storage
- These measures dramatically reduce cholera incidence.

Vaccines

Two oral killed cholera vaccines are available internationally and prequalified by WHO:

1. **WC-rBS (Dukoral)** contains:
- Killed V. cholerae O1
- Cholera toxin B subunit
2. **BivWC (Shanchol**)
- Contains killed *V. cholerae* O1 and O139
- Does **not** contain cholera toxin B subunit.

[Campylobacter]

Campylobacters are:

- Gram-negative
- **Curved** (comma or S-shaped) rods
- **Motile**
- **Non-spore**-forming

Reservoirs

- Found in the gastrointestinal tract of many animals, especially:
  - **Poultry** (most important)
  - Cattle, sheep, pigs
  - Household pets (dogs and cats)
- Humans become infected through contaminated food, especially undercooked poultry.

Diseases caused

- Campylobacter causes:
  - Diarrheal disease (most common)
  - Systemic and extraintestinal infections (less common)

- It is among the most widespread causes of bacterial enteritis worldwide.

The classification of Campylobacter has changed. Some organisms previously classified as Campylobacter are now:
  - Helicobacter
  - Arcobacter
- Human pathogens fall into two major groups:
  - **Enteric** pathogens → cause diarrhea.
  - **Extraintestinal** pathogens → cause systemic disease.

➔ Important Campylobacter Species

1) ***Campylobacter jejuni***
- Prototype organism of the group
- One of the most common causes of bacterial diarrhea in humans
- Leading cause of gastroenteritis in developed countries

2) ***Campylobacter fetus***
- Has two subspecies:
  - C. fetus subsp. fetus
  - C. fetus subsp. venerealis
- Acts as an **opportunistic** pathogen
- Causes:
  - Systemic infections
  - Mainly in immunocompromised patients
- May occasionally cause diarrhea

3) ***Other species (less common)***
- Campylobacter coli
- Campylobacter lari
- Campylobacter upsaliensis
- Campylobacter hyointestinalis
- Campylobacter fetus
- Related genera:
  - Arcobacter butzleri
  - Helicobacter cinaedi
  - Helicobacter fennelliae

➔ Campylobacter jejuni and Campylobacter coli

Clinical importance
- C. jejuni and C. coli are now recognized as:
  - Common human pathogens
  - Cause mainly enteritis
  - Occasionally causes systemic infection

Similarity between species
- Infections caused by C. jejuni and C. coli are clinically indistinguishable.
- Most laboratories do not differentiate between the two species

Epidemiology
- C. jejuni causes ~95% of cases
- C. coli accounts for ~5–10% of infections
- Together, Campylobacter species are:
  - At least as common as Salmonella and Shigella
  - Especially important causes of diarrhea in **developed** countries.

Campylobacter Jejuni

Basic characteristics
- Gram-negative **rods**
- Shape:
  - **Comma**-shaped
  - S-shaped
  - "Gull-wing" appearance (classic exam clue)
- **Motile** with a single polar flagellum
- Non-spore-forming

Growth requirements
- Requires selective media
- Requires a microaerophilic environment:
  - Reduced oxygen (~5% O_2)
  - Increased CO_2 (~10% CO_2)

Temperature preference
- Incubation at 42°C
- Grows well at 36–37°C
- Growth at **42°C**:
  - Inhibits most normal fecal flora
  - Helps identify C. jejuni
- Reflects adaptation to avian hosts (poultry)

➔ Pathogenesis of Campylobacter jejuni

Transmission
- Acquired by the oral route from:
  - Contaminated food or water
  - Undercooked poultry (most common)
  - Infected animals or animal products

Infectious dose
- Low infectious dose
- About 10^4 organisms are sufficient
- Organism is susceptible to gastric acid

Mechanism of disease

- Pathogenesis involves:
  - **Adherence** to the intestinal mucosa
  - **Production** of:
    1) Enterotoxins
    2) Cytotoxins
    3) Cytolethal distending toxin (CDT)
  - **Multiply** in the small intestine
  - **Invade** the intestinal epithelium

Note: Campylobacter causes disease mainly by invading the intestinal lining and triggering inflammation, rather than by producing toxins.

- Results in:
  - **Inflammation**
  - **Ulceration**
  - **Bleeding**
- **Blood and leukocytes** may appear in stool

➔ Clinical Findings of Campylobacter jejuni Infection

Onset

- Incubation period: 1–4 days
- Prodrome:
  - **Fever**
  - **Headache**
  - **Myalgia**
  - **Malaise**
- Followed by:
  - **Diarrhea** (may be watery or bloody)

Course of illness

- Usually self-limited
- Duration:
  - 5–8 days
  - Occasionally longer
- Most patients recover without antibiotics
- However:
  - ~5–10% may relapse

Complications

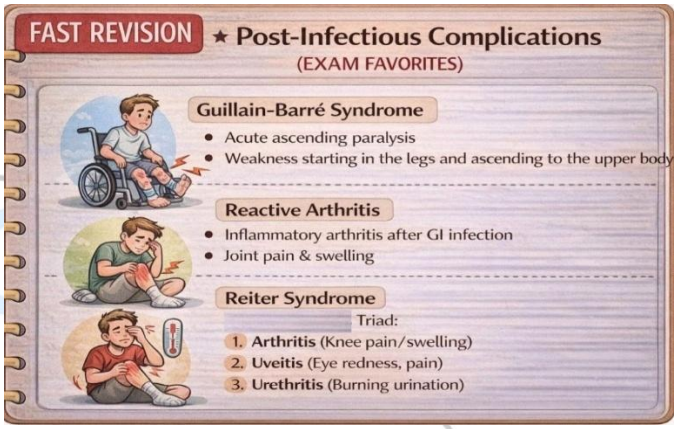
1) **Local complications (rare)**

- Cholecystitis
- Pancreatitis
- Cystitis

2) **Systemic complications** (rare, more common in immunocompromised patients)

- Meningitis
- Endocarditis
- Arthritis
- Peritonitis
- Cellulitis
- Septic abortion
- Hepatitis
- interstitial nephritis
- hemolytic-uremic syndrome

3) **Post-Infectious Complications:**



➔ Diagnostic Laboratory Tests for Campylobacter

A. **Specimens**

- Stool is the usual specimen for diagnosis.
- Blood cultures: occasionally positive, more likely in:
  - Immunocompromised
  - Elderly patients

B. **Smears**

- Gram-stained stool smear may show curved "**gull-wing**" shaped gram-negative rods
- Dark-field or phase-contrast microscopy may show **rapid darting** motility (classic feature)

C. **Culture** (Definitive diagnosis)

- Requires selective media, such as:
  - Skirrow's
  - Butzler's
  - Blaser's
  - Campy-BAP
  - Preston media

Note: If non-jejuni Campylobacter is suspected, use media **without cephalosporins**, which may inhibit growth.

➔ Treatment of Campylobacter Infection

Supportive care (MOST IMPORTANT)

- Fluid and electrolyte replacement is the cornerstone of therapy.
- Many cases are:
  - Mild
  - Self-limited

Antibiotics are NOT routinely required, but are indicated if:

- High fever
- Bloody diarrhea
- Severe or prolonged illness
- Symptoms > 1 week
- Worsening clinical course

Antibiotic therapy

- First-line treatment:
  - **Erythromycin** (5–7 days)
- Alternative (adults):
  - **Ciprofloxacin or other fluoroquinolone** (5–7 days)

Systemic infection

- For severe or invasive disease:
  - Start empiric therapy with:
    - **Gentamicin**
    - **Imipenem**
    - **Chloramphenicol**
- Adjust treatment after susceptibility testing

[Helicobacter pylori]

➔ General Features

Morphology & physiology

- **Spiral**-shaped, gram-negative rod
- Actively **motile** with multiple flagella at one pole
- **Microaerophilic** (requires low levels of oxygen)
- **Oxidase-positive** and **catalase-positive**.

Acid resistance

- Survives gastric acidity via:
  - **Urease** (key enzyme)
    - **Converts urea → ammonia**
    - Raises local pH
- This allows survival in the acidic stomach environment

Note: Urease positivity = H. pylori

Diseases associated with H. pylori

- Chronic gastritis
- Duodenal ulcers
- Gastric ulcers
- Gastric adenocarcinoma
- MALT lymphoma
- May trigger:
  - Pernicious anemia
  - Iron deficiency anemia (↓ iron absorption, occult blood loss)

➔ Epidemiology of H. pylori

Prevalence

- Colonizes the stomach in ~50% of the world's population
- Infection is often lifelong unless treated

Reservoir & transmission

- Humans are **the only important reservoir**
- Transmission:
  - Usually person-to-person
  - Often from parents to children

Clinical outcomes

- Most colonized individuals are asymptomatic
- Disease develops in some patients due to:
  1) **Bacterial virulence factors**
     - CagA
     - VacA
     - type IV secretion system
  2) **Host susceptibility**
     - Immune response
     - IL-1 gene polymorphisms
  3) **Environmental factors**
     - Smoking

➔ Pathogenesis of H. pylori

Site of colonization

- Lives **deep in the mucus layer** near the gastric epithelium
- Does **not** invade epithelial cells
- Colonizes gastric-type epithelium only, not intestinal-type epithelium

Motility & penetration

- Highly motile, even in thick mucus
- Flagella allow movement toward the epithelial surface, where pH is higher

Mechanisms of mucosal injury

1) **Urease**
   - Produces ammonia → **buffers acid**
   - Ammonia damages epithelial cells
2) **Protease production**
   - **Degrades gastric mucus**
   - Allows acid to reach the epithelium
3) **Inflammation**
   - Leads to gastritis and ulcer formation

➔ Pathologic Effects of Helicobacter pylori

Mechanism of mucosal damage

- The exact mechanism is multifactorial, involving:
  - Bacterial factors
  - Host inflammatory response
- *H. pylori*:
  - Invades the epithelial surface to a limited degree.
  - Does not deeply invade tissue.

1) **Direct bacterial injury**

- Damage occurs due to:
  - **Toxins and lipopolysaccharide** (LPS)
  - Ammonia produced by urease
    - Directly toxic to epithelial cells
    - Disrupts mucosal integrity

2) **Inflammatory response**

- Gastric mucosa shows:
  - **Polymorphonuclear (PMN) infiltration**
  - **Mononuclear cell infiltration**
- Changes seen:
  - **Vacuolization** of epithelial cells
  - Epithelial **destruction**
  - Glandular **atrophy**

Chronic gastritis

- *H. pylori* induces:
  - Chronic superficial gastritis
- Characterized by:
  - **Persistent** inflammatory infiltrate
  - Both **PMNs** and **mononuclear** cells
- This chronic process explains long-term complications.

➔ Clinical Findings of H. pylori Infection

Acute infection

- May present as acute upper GI illness, with:
  - **Nausea**
  - **Epigastric pain**
  - **Vomiting**
  - **Fever** (occasionally)
- Duration:
  - Usually < 1 week
  - May last up to 2 weeks

Chronic infection

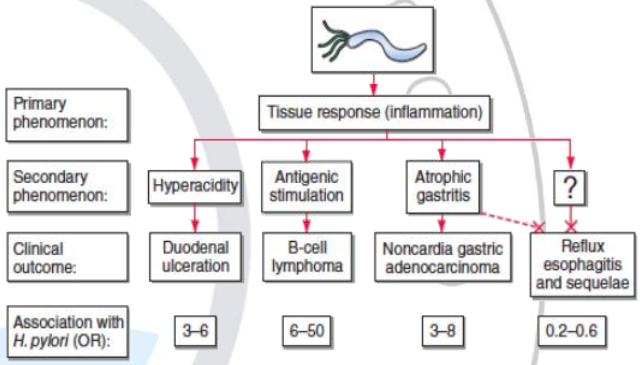
- After colonization:
  - Infection often persists for years or decades
  - May last a lifetime if untreated

Association with ulcers

- Strongly associated with peptic ulcer disease:
  - ~90% of duodenal ulcers
  - 50–80% of gastric ulcers
- Eradication of *H. pylori*:
  - Dramatically reduces ulcer recurrence

Malignancy risk

- Chronic *H. pylori* infection is a major risk factor for:
  - **Gastric adenocarcinoma**
  - **Gastric MALT lymphoma**



Core idea: H. pylori **colonization** → chronic gastric **inflammation** → different diseases depending on the gastric response.

1. Primary Phenomenon

Tissue response = Chronic inflammation

- *H. pylori* colonizes the gastric mucosa
- This triggers a chronic inflammatory response
- Inflammation is the starting point for all outcomes

2. Secondary Phenomena (What happens next?)

Depending on how the stomach responds, four different pathways can occur:

A. **Hyperacidity**

- **Increased acid production** (especially in the duodenum)
- Leads to **duodenal ulceration**

Association with H. pylori: Odds ratio (OR): 3–6.

B. **Antigenic stimulation**
- Chronic infection **stimulates B-cell proliferation**
- Can lead to **Gastric MALT (B-cell) lymphoma**

Association with H. pylori: OR: 6–50 (VERY STRONG)

C. **Atrophic gastritis**
- Long-standing inflammation causes:
  - **Loss of gastric glands**
  - Reduced acid secretion
- Leads to:
  - Noncardia **gastric adenocarcinoma**

Association with H. pylori: OR: 3–8

D. **Protective effect against reflux disease** (IMPORTANT & CONFUSING)
- H. pylori may **reduce acid secretion**
- This can **lower the risk of reflux esophagitis**

Association with H. pylori: OR: 0.2–0.6 (protective)

Note: Odds Ratio (OR) tells you how strongly an exposure is associated with a disease.
In this context:
- Exposure = *H. pylori* infection
- Outcome = disease (ulcer, cancer, lymphoma, etc.)
- **OR = 1** → No association
- **OR > 1** → Increased risk
- **OR < 1** → Protective effect

➔ **Diagnostic Laboratory Tests for Helicobacter pylori**

A. **Smears / Histology**
- *H. pylori* infection can be diagnosed by gastric biopsy during endoscopy.
- Routine stains may show gastritis.
- Special stains (e.g. Giemsa, silver stains):
  - Reveal **curved or spiral-shaped** organisms on the gastric epithelium.

B. **Culture**
- Culture is not routine.
- Performed when:
  - Patient fails standard therapy
  - Antibiotic susceptibility testing is needed

C. **Special Tests (MOST IMPORTANT)**

Urease tests
- Based on strong **urease activity** of *H. pylori*.
- Two main types:

a) **Rapid urease test** (biopsy-based)
- Gastric biopsy placed in urea-containing medium
- Color change → **positive**

b) **Urea breath test** (NON-INVASIVE, VERY HIGH-YIELD)
- Patient **ingests urea** labeled with ^{13}C or ^{14}C
- If *H. pylori* is present:
  - Urease → CO_2 released
  - Labeled CO_2 detected in exhaled breath

Best test to **confirm eradication** is **stool antigen test.**
- Detects H. pylori antigen in stool
- Used to:
  - Diagnose infection
  - Confirm cure after treatment

➔ **Treatment of Helicobacter pylori**
- H. pylori must be eradicated to:
  - **Heal ulcers**
  - **Prevent recurrence**
  - **Reduce cancer risk**

A. ***Triple Therapy*** (Classic) for 7–14 days:
- **PPI (proton pump inhibitor)**
- **Amoxicillin**
- **Clarithromycin**
- **(Metronidazole used if penicillin allergy)**

Eradicates infection in ~70–95% of patients.

B. ***Quadruple Therapy*** (Preferred in resistance or failure) for 10–14 days:
- **PPI**
- **Bismuth**
- **Metronidazole**
- **Tetracycline**

Used when:
- Clarithromycin **resistance** suspected
- Previous treatment **failed**

Role of PPIs:
- Suppress gastric acid
- Enhance antibiotic effectiveness
- Directly inhibit H. pylori urease activity

[Plesiomonas & Aeromonas]

Plesiomonas

➔ **General features**
- Uncommon cause of diarrheal disease
- Gram-negative **rod**
- **Oxidase positive**
- Has a polar flagellum
- Found mainly in:
  - **Freshwater**
  - **Soil**
  - **Fish and aquatic animals**
- More common in **tropical and subtropical** regions

➔ **Clinical relevance**
- Human infection is usually associated with:
  - **Diarrhea**
- Organism is most often isolated from stool cultures of patients with diarrhea

➔ **Laboratory identification**
- Can grow on Salmonella–Shigella selective media
- Shares antigens with Shigella sonnei → may **cross-react**
- Key differentiating test:
  - Plesiomonas → oxidase **POSITIVE**
  - Shigella → oxidase NEGATIVE
- **DNase positive**
- Biochemical tests help distinguish it from Aeromonas

Aeromonas

➔ **General features**
- Gram-negative **rods**
- **Oxidase positive**
- Often confused with enteric gram-negative bacteria

➔ **Laboratory clues**
- Can be distinguished from Enterobacteriaceae by:
  - Positive oxidase test
  - **Resistance to O/129** (Vibrio is sensitive → Aeromonas is resistant)
  - **lack of on media containing 6% NaCl**

➔ **Virulence factors**
- Produces:
  - Hemolysins
  - Cytotoxins
- Some strains can:
  - **Invade** intestinal cells
  - Cause **tissue culture damage**

➔ **Clinical manifestations**
- Gastroenteritis:
  - Ranges from **acute watery diarrhea to bloody dysentery-like** illness
- Extraintestinal infections:
  - **Bacteremia**
  - **Wound infections**
- Often associated with:
  - **Trauma** in **aquatic** environments
- Most commonly caused by:
  - Aeromonas hydrophila

Treatment of Aeromonas & Plesiomonas

Antibiotic susceptibility
- Generally susceptible to:
  - **Fluoroquinolones** (e.g. ciprofloxacin)
  - **Third- and fourth-generation cephalosporins**
  - **Carbapenems**
  - **Aminoglycosides**

Resistance considerations
- Resistance has been reported
- Aeromonas can produce:
  - **β-lactamases**
  - Including carbapenemases

VERY IMPORTANT
- Antibiotic **susceptibility testing is required**
- Therapy must be **guided** by culture and sensitivity.

[Questions]

Q1: A 47-year-old male is diagnosed with peptic ulcer disease. Treatment includes antibiotics for infection of the stomach with Helicobacter pylori. Which enzyme is secreted by this organism and enables it to survive in the acidic environment of the stomach?

A. Transpeptidase
B. Oxidase
C. Catalase
D. Urease
E. Protease

Q2: A 34-year-old woman presents with fever, nausea, severe abdominal cramping, and bloody diarrhea that was fecal leukocyte-positive. A few days previously, she had eaten undercooked chicken. Of the following, which is the most likely etiologic agent?

A. Salmonella typhi
B. Shigella dysenteriae
C. Clostridium perfringens
D. Campylobacter jejuni
E. Yersinia enterocolitica

Q3: The primary reservoir for Helicobacter pylori is?

A. Dog
B. Aquatic water
C. Cat
D. Rat
E. Human

Q4: Vibrio cholera, one is WRONG?

A. Cholera toxin causes an increase in intracellular cGMP
B. Non-O1/O139 Vibrio causes acute gastroenteritis
C. Has an enterotoxin
D. Motile curved bacilli
E. Are halotolerant

Q5: The characteristic 'rice water stool' diarrhea is associated with infection by?

A. Campylobacter jejuni
B. Vibrio cholera
C. Salmonella typhimurium
D. Shigella sonni
E. Coxiella burnetii

Q6: Which of the following is false regarding Campylobacter Jejuni?

A. It is a microaerophile
B. It is associated with Guillain-Barré syndrome
C. It is a small, gram-negative, curved rod
D. Pathogenesis is mainly through toxins like enterotoxins
E. All the above are true

Q7: Which of the following bacteria grow well at 42°C in a microaerophilic environment?

A. Vibrio cholerae
B. Yersinia Pseudotuberculosis
C. Campylobacter coli
D. Helicobacter pylori
E. None of the above

Q8: Which isn't associated with H. Pylori infection?

A. Gastritis
B. Duodenal ulcer
C. Urease
D. Chronic meningitis

Q9: Vibrio Parahaemolyticus?

A. Food poisoning
B. Septicemia
C. Wound infection
D. A +B
E. A + B+ C

Q10: A 52-year-old woman presented with indigestion and heartburn occurring shortly after meals, which she treated with over-the-counter antacids. Physical examination revealed mild epigastric tenderness. A radiolabeled urea breath test is positive. What is the most probable etiologic agent:

A. Campylobacter jejuni
B. Helicobacter pylori
C. Yersinia enterocolitica
D. Shigella dysenteriae
E. Clostridium difficile

Q11: The most effective non-invasive test for the diagnosis of Helicobacter pylori-associated gastric ulcers is?

A. Detection of H. pylori antigen in stool
B. Growth of H. pylori from a stomach biopsy
C. Growth of H. pylori in the stool
D. IgM antibodies to H. pylori

E. Culture of stomach contents for H. pylori

Q12: Which of the following statements best describes Vibrio cholera?

A. Motile by multiple polar flagella
B. Oxidase negative
C. Give yellow colonies on TCBS
D. Give green colonies on TCBS
E. Gram-positive curved bacilli

Q13: A female who had sushi at a party is presented with gastroenteritis. Which of the following agents may have caused the gastroenteritis?

A. H. Pylori
B. C. Jejuni
C. V. parahaemolyticus
D. Campylobacter lari
E. V. Cholera

Q14:Clinical case/ people who ate chicken from an all-you-can-eat fried chicken buffet are presented with abdominal pain, vomiting, diarrhea, and there are leukocytes in the stool. Which of the following is the causative agent?

A. H. Pylori
B. C. Jejuni
C. V. parahaemolyticus
D. Campylobacter lari
E. V. Cholera

Q15: T.C.B.S media is selective for?

A. H. Pylori
B. C. Jejuni
C. S. Aureus
D. Campylobacter lari
E. V. Cholera

Q16: We cultured the blood of a person with bloody diarrhea, and bacteria grew at 42 °C and at 5% oxygen. What are these species?

A. H. Pylori
B. C. Jejuni
C. S. Aureus
D. Salmonella
E. V. Cholera

Answers:
Q1: D
Q2: D
Q3: E
Q4: A → Cholera toxin ↑ **cAMP**, not cGMP
Q5: B
Q6: D Disease mainly due to **invasion and inflammation**, not toxins alone.
Q7: C
Q8: D
Q9: A
Q10: B
Q11: A
Q12: C Due to **sucrose fermentation**
Q13: C
Q14: B
Q15: E TCBS is selective for **Vibrio species**
Q16: B

The University of Jordan
Gastroenterology Interest Group (UJ-GIG)
Booklet

Microbiology

The Brucellae, Leptospira and Mycobacterium of the GIT

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Reviewed by: Amr Abdallah

[Brucella]

➔ General Characteristics

- **Gram-negative** bacteria
- **Obligate intracellular** organisms
- Primarily pathogens of animals, but infect humans
- Relatively **inactive** metabolically

Species & Hosts

- Brucella melitensis → goats, sheep (most common & most virulent in humans)
- Brucella abortus → cattle
- Brucella suis → swine
- Brucella canis → dogs
- Humans are **incidental** hosts

Disease Pattern

- Causes brucellosis (**undulant** fever, Malta fever)
- Characterized by:
  - Acute bacteremic phase
  - Followed by chronic infection
- Chronic disease may:
  - Last for years
  - Involve multiple organs

➔ Morphology and Identification

- Shape: Short **rods to coccobacilli**
- Size:1.2 µm in length
- Staining:
  - **Poorly stained** on Gram stain
  - Appear **faint** and **irregular**
- **Non-motile**
- **Non-spore** forming

Growth Characteristics

- Adapted for **intracellular** survival
- Nutritional requirements are complex
- Oxygen requirements:
  - *B.* ***abortus*** → needs 5–10% CO_2
  - Other species → grow in air

Biochemical Features

- **Catalase positive**
- **Oxidase positive**

Resistance

- Killed by:
  - **Boiling**
  - **Pasteurization**
- Resistant to:
  - **Freezing**
  - **Drying**

➔ Epidemiology

- Zoonotic infection (animal → human)
- Transmission occurs through:
  - **Direct contact** with infected animals
  - **Exposure to animal tissues, blood, urine**
  - Ingestion of **unpasteurized milk or cheese** (most common)
- High-risk groups:
  - Farmers
  - Veterinarians
  - Slaughterhouse workers
  - Laboratory personnel

Modes of Transmission

- Ingestion (most common)
- Inhalation
- Mucocutaneous exposure
- Percutaneous exposure

Special Notes

Accidental injection of live vaccines (*B. abortus* (S19, RB51) and *B. melitensis* (Rev-1)) can cause disease.
B. *melitensis* and *B. suis*: **highly infectious** and considered potential **biological weapons**.

➔ Pathogenesis

- Each Brucella species has a preferred animal host
- All species can infect humans (accidental hosts)

Spread in the Body

- Organisms enter through:
  - Skin
  - Mucosa
  - Gastrointestinal tract
- Spread via:
  - **Lymphatics** → regional lymph nodes then to Thoracic duct → bloodstream
- Hematogenous spread to:
  - **Liver**
  - **Spleen**
  - **Bone marrow**
- Can form abscesses within the reticuloendothelial system

Tissue Damage

- Osteomyelitis
- Meningitis
- Cholecystitis (occasionally)

- Histologic features:
  - Proliferation of **mononuclear** cells
  - Fibrin exudation
  - Coagulation necrosis
  - Fibrosis

Granuloma Formation

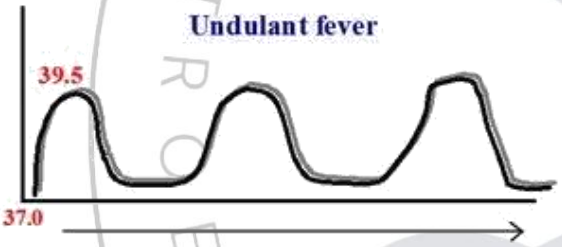
- Granulomas composed of:
  - Epithelioid cells
  - Giant cells
- Central necrosis with peripheral fibrosis

➔ **Clinical Findings**

- Incubation period:1–4 weeks
- Onset: **Gradual** (insidious)
- Common Symptoms :**fever, malaise, fatigue, weakness, headache, and sweats**

Fever Pattern - Important

- Fever rises in the afternoon
- Falls at night
- Associated with drenching sweats
- Known as **undulant** fever



Organ Involvement

- Lymphadenopathy
- Splenomegaly
- Hepatomegaly
- Hepatitis

Complications

- Osteomyelitis
- Endocarditis (most serious complication)
- Deep organ abscesses

Disease Course

- Generalized infection:
  - Symptoms may improve within **weeks or months**.
- Localized disease:
  - Symptoms may persist **longer**.

Chronic Brucellosis

- Fatigue
- Aches and pains
- Low-grade fever
- Nervousness
- Depression
- Nonspecific neuropsychiatric symptoms

➔ **Diagnostic Laboratory Tests**

A. **Specimens**

- Blood cultures
- Biopsy material:
  - Lymph nodes
  - Bone
- Serologic tests

B. **Culture**

- Use Brucella agar
  - Enriched medium designed for *Brucella*
- Some species require:
  - **5–10% CO_2**
- Growth is **slow**

Other Media

- Sheep blood agar
- Brain–heart infusion medium
- Thayer–Martin medium
- Chocolate agar

Colony Morphology

- Typical virulent strains form:
  - **Smooth**
  - **Transparent** colonies

C. **Serology**

- Antibody response pattern:
  - **IgM**:
    - Appears in **the first week** of acute illness
    - Peaks at ~3 months
  - **IgG and IgA**:
    - Rise about **3 weeks after onset**
    - Peak at 6–8 weeks
- High antibody titers are typical during acute infection

Agglutination Tests: IgG agglutinin **titers above 1:80 indicate active infection**

- Agglutination tests may show false-positive reactions due to **cross-reactivity** from individuals vaccinated with the cholera vaccine may develop agglutinins to Brucella

ELISA

- Detects:
  - IgG
  - IgA
  - IgM
- Uses cytoplasmic antigens

- Advantages:
  - More sensitive
  - More specific than agglutination tests
- Especially useful in:
  - Chronic brucellosis

➔ **Treatment & Immunity**

Antibiotic susceptibility

- **Tetracyclines**
- **Rifampin**
- **Trimethoprim–sulfamethoxazole**
- **Aminoglycosides**
- **Some quinolones**

Treatment Principles

- Symptoms may improve within days
- Complete eradication is difficult due to:
  - Intracellular location
- High relapse rate if treated inadequately

Recommended Therapy

- **Combination** therapy is required
- Standard regimen: **Doxycycline + rifampin for 6 weeks**
- Alternative regimen: Doxycycline + streptomycin for 2–3 weeks

➔ **Prevention and Control**

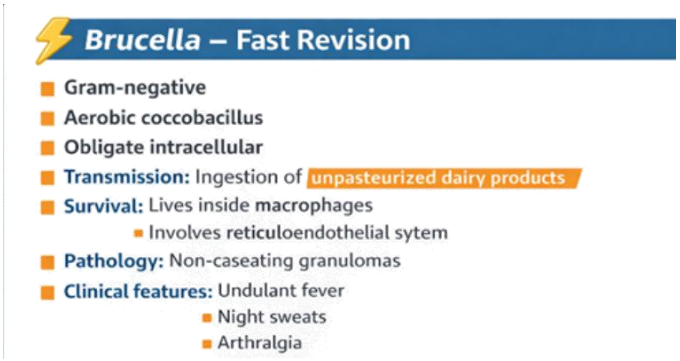
- Control in animals:
  - Test-and-slaughter programs
  - Active immunization of cattle
  - Vaccination of heifers (young female cattle that have not yet given birth to a calf) with attenuated live strain 19
- Diagnosis in cattle:
  - Agglutination tests

Human Prevention

- No widely used human vaccine
- Active immunization in humans is experimental

Public Health Measures

- **Pasteurization** of milk and dairy products
- Control and elimination of animal infection
- Reduction of occupational exposure:
  - Farmers
  - Veterinarians
  - Slaughterhouse workers



[Leptospira]

➔ **General Features**

- Genus *Leptospira* includes:
  - Pathogenic: *Leptospira interrogans*
  - Free-living (nonpathogenic): *Leptospira biflexa*
- Disease caused by pathogenic Leptospira species

Clinical Spectrum

Wide range of disease severity:

- **Asymptomatic infection**
- **Mild, self-limited illness**
- **Severe, fatal disease** (Weil syndrome)

Reservoir & Shedding

- Chronic kidney infection in many animals
- Animals shed large numbers of leptospires in urine
- Animal urine contaminates:
  - Water
  - Soil
- Major source of human infection

Human **Urine** may contain spirochetes during the 2nd and 3rd weeks of disease

Leptospira Interrogans

➔ **Morphology**

- **Spirochete**
- Tightly **coiled**
- **Thin** and flexible
- Size:
  - Length: 5–15 µm
  - Width: 0.1–0.2 µm
- Ends often **bent** into a hook shape
- Actively **motile**
- Best visualized using **dark-field** microscopy

Metabolism

- Energy source:
  - **Oxidation of long-chain fatty acids**

- Cannot use:
  - Amino acids
  - Carbohydrates
- Nitrogen source:
  - Ammonium salts

Environmental Survival
- Can survive for weeks in water
- Survival **enhanced at alkaline** pH

➔ **Epidemiology**
- Worldwide distribution
- Most common in:
  - **Tropical and subtropical** regions
- Favored by:
  - Warm climate
  - Poor hygiene
  - Environmental exposure to contaminated water

Disease Severity
- Most infections:
  - Mild or asymptomatic
- Small proportion (~1%):
  - Severe disease (approximately 1 million severe cases occur per year, with a mean case–fatality rate of nearly 10%)
  - Potentially fatal complications

➔ **Pathogenesis (Leptospirosis)**
- Mode of transmission through:
  - Cuts or abraded skin
  - Mucous membranes
  - Conjunctiva
  - Oral mucosa
- Incubation period: 1–2 weeks

After entry
- Organisms multiply
- Cross tissue barriers
- Disseminate hematogenously (leptospiremic phase)
- Organ involvement preferentially localize in:
  - Liver
  - Kidneys
- Tissue damage: **Hemorrhage and Necrosis**
- Resulting organ dysfunction:
  1) **Jaundice**
  2) **Hemorrhage**
  3) **Nitrogen retention (renal failure**)

➔ **Clinical Findings**
- Disease course:
  - Biphasic illness
- Phase 1 (Leptospiremic phase):
  - Initial symptoms
  - Temporary improvement
- Phase 2 (Immune phase):
  - Occurs when **IgM titers rise**
  - Often presents as aseptic meningitis (severe headache, stiff neck, and pleocytosis of CSF)

Pleocytosis means an increase in white blood cells in a bodily fluid (e.g., cerebrospinal fluid)

Organ Involvement
- Nephritis
- Hepatitis
- Skin lesions
- Muscle involvement
- Eye lesions
- Severity and organ distribution vary by species

Disease Severity
- Many infections are:
  - Mild
  - Subclinical
- Hepatitis is common in leptospirosis

➔ **Diagnostic Laboratory Tests**

A. Specimens
- Blood
- CSF
- Urine
- Tissue samples

B. Microscopy
- Dark-field examination
- Thick smears stained with Giemsa

C. Culture
- Culture media: EMJH medium (Ellinghausen-McCulloughJohnson- Harris)
- Growth characteristics:
  - Very slow
  - Cultures examined for up to 8 weeks

D. Serology
- **Main method of diagnosis**
- Tests:
  - Microscopic agglutination test (MAT)
  - ELISA
- Most cases confirmed serologically

➔ **Treatment**
- Mild leptospirosis:
  - **Oral doxycycline**
  - **Or ampicillin**
  - **Or amoxicillin**
- Severe leptospirosis:
  - **IV penicillin**
  - Start as soon as diagnosis is suspected
- Early treatment reduces:
  - Severity
  - Complications

➔ **Immunity**
- Infection induces serovar-specific immunity (Immunity develops **only** against the exact serovar of the organism that caused the infection, so **protection is not broad across all strains of the same species**)
- **Reinfection** can occur with different serovars

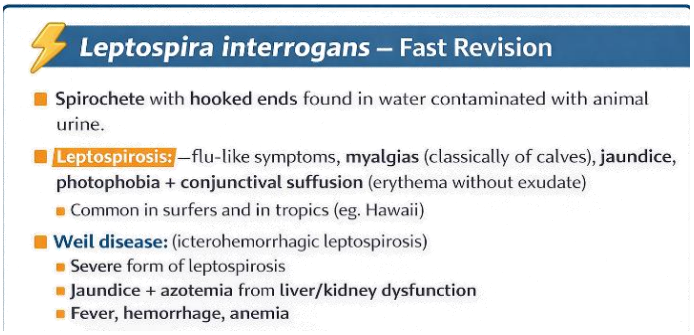
➔ **Prevention and Control**

Routes of Human Exposure
- Drinking contaminated water
- Swimming or bathing in contaminated water
- Food contamination

High-Risk Groups
- Farmers
- Fishermen
- Sewer workers
- Miners
- Individuals exposed to rat-contaminated water

Preventive Measures
- Avoid contact with:
  - Animal urine
  - Animal tissues
- Use protective equipment:
  - Gloves
  - Boots
  - Protective eyewear
- Rodent control strategies are important

Vaccination
- Vaccines available for:
  - Agricultural animals
  - Companion animals
- Use of animal vaccines should be encouraged



[Mycobacterium tuberculosis (Mtb)]

➔ **General Features**

TB historically called:
- Consumption (consume patients, weight loss)
- White plaque (extreme pallor seen among patients)

Mycobacterium tuberculosis Complex (MTC)
- Causes TB in humans and animals
- Includes:
  - Mycobacterium tuberculosis (most important in humans)
  - Mycobacterium africanum
  - Mycobacterium bovis
  - Mycobacterium microti
  - Mycobacterium caprae
  - Mycobacterium pinnipedii
  - Mycobacterium suricattae
  - Mycobacterium mungi
  - Mycobacterium dassie
  - Mycobacterium orygis
  - Mycobacterium canettii

Basic Characteristics
- **Slow-growing** organism
- Obligate **aerobe**
- **Facultative intracellular**
- **Acid-fast** bacillus
- **Non-motile**
- **Non-spore** forming
- doesn't gram stain well (due to mycolic acids in cell wall and fatty acids and complex lipids)

➔ **Epidemiology**
- Two TB-related conditions:
  - Latent TB infection (LTBI)
    - **No symptoms**
    - Not contagious
    - About one-third of the world's population
  - Active TB disease

- Symptomatic
- Can be fatal if untreated

Transmission

- Primarily **airborne**
- Other routes (less common):
  - Unpasteurized milk (M. bovis)
  - Direct inoculation

➔ **General Spread**

- Primary site of TB: Lung
- From the lungs, TB may:
  - Disseminate **hematogenously**
  - Spread to other organs
- Other routes of spread:
  - Contiguous spread to adjacent tissues
  - Primary involvement of extrapulmonary organs.

Extrapulmonary TB Sites

- Pleura
- Lymph nodes
- Pericardium
- Kidney
- Spine
- Brain
- Abdomen (abdominal TB)

Abdominal TB

- Less common than pulmonary TB
- Can cause significant morbidity and mortality
- Often diagnosed late due to:
  - Nonspecific symptoms

Forms of Abdominal TB:

- Tuberculous **lymphadenopathy**
- **Peritoneal** TB
- **Gastrointestinal** (GI) TB
- **Visceral** TB (solid organs)

Gastrointestinal (GI) TB

➔ **Pathogenesis**

Mechanisms of Infection

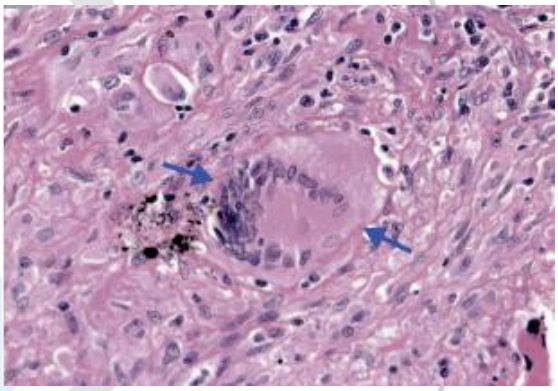
- Reactivation of latent TB
- Ingestion of *Mycobacterium tuberculosis*:
  - **Unpasteurized** milk (*M. bovis*)
  - Contaminated **sputum**

Spread to GI Tract

- From active pulmonary or miliary TB
- Hematogenous spread
- Lymphatic spread
- Retrograde spread via fallopian tubes (females)

Pathologic Process

- Bacilli penetrate
  - **Intestinal mucosa**
- Involve:
  - Submucosal **lymphoid** tissue
- Leads to:
  - **Granuloma formation**
  - **Caseating necrosis**, after 2-4 weeks: with **central necrosis** and **Langhans giant cells** are characteristic of 2° tuberculosis.
- Results in:
  - Ulceration of the mucosa
  - Possible spread to deeper layers and peritoneum
- Rarely:
  - Portal circulation or hepatic artery →Spread to liver, pancreas, and spleen



Granuloma with central necrosis as seen in TB infection.

➔ **Clinical Findings**

- Presentation: Often **nonspecific**
- Common symptoms:
  - **Abdominal pain** (at times similar to that associated with appendicitis)
  - **General malaise**

Commonly Affected Sites

- Terminal ileum
- Cecum

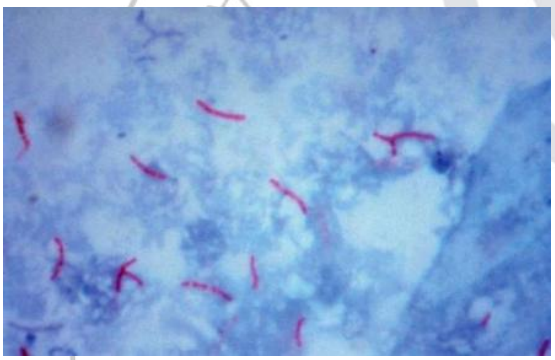
Local Symptoms

- **Abdominal pain**
- **Diarrhea**
- **Constipation**
- **Weight loss**
- **Obstruction**
- **Hematochezia**
- **Palpable abdominal mass**

Systemic Symptoms
- **Fever**
- **Weight loss**
- **Anorexia**
- **Night sweats**

➔ **Laboratory Diagnostic Methods (TB)**

A. Smear Microscopy
- All suspected TB patients:
  - Examine **three** fresh specimens
- Methods:
  - **Acid-fast bacilli (AFB)** staining
    - Ziehl–Neelsen stain appears as: **Red, slender rods**.
    - Acid-fast due to: **High mycolic acid content** in cell wall, Does not decolorize with acid–alcohol
  - Fluorescent staining
    - Auramine stain



Mycobacterium as it appears after AFB staining.

B. Culture
- Perform both liquid and solid cultures
- Incubation:
  - Up to 8 weeks (slow growing)
- Culture media:
  - **Lowenstein–Jensen**
  - **Middlebrook 7H10 / 7H11**
- Automated systems:
  - BACTEC
  - MGIT (Mycobacterial Growth Indicator Tube)
- Culture is:
  - **Most specific test**
  - Allows:
    - Species identification
    - Drug susceptibility testing

C. Additional Tests
- **NAAT** (nucleic acid amplification tests)
- **TST** (tuberculin skin test) (**type 4 hypersensitivity reaction**, false positive in vaccinated individuals)
- **IGRA** (interferon-gamma release assays, no false positive in vaccinated individuals)

➔ **Treatment**
- Treatment depends on:
  - Latent TB infection (LTBI)
  - Active TB disease
  - Risk of progression

1) **Active TB Treatment**
- Requires multi-drug therapy
- Two phases:
  - Intensive phase (2 months)
  - Continuation phase (4–6 months)

First-Line Anti-TB Drugs
- **Isoniazid (INH)**
- **Rifampin (RIF)**
- **Pyrazinamide (PZA)**
- **Ethambutol (EMB)**
- **± Streptomycin**

2) **Latent TB**
- **Isoniazid** preventive therapy (IPT)
- Limitation:
  - Long treatment duration

➔ **Prevention**
- Most effective prevention:
  - Early diagnosis
  - Isolation of infectious patients
  - Appropriate treatment until non-infectious
- Patients usually become non-infectious:
  - After 2–4 weeks of effective therapy

Additional Measures
- **Treatment of latent TB** infection in high-risk individuals
- **Vaccination**:
  - Bacillus Calmette–Guérin (BCG) vaccine
    - Live attenuated vaccine
    - Derived from Mycobacterium bovis
    - Only licensed TB vaccine

[Questions]

Q1: Leptospirosis, one is wrong?

A. Varying from asymptomatic to fatal disease
B. Feco-oral transmission
C. Caused by Leptospira interrogans
D. Zoonosis
E. Hepatitis is frequent

Q2: Human transmission of Brucella can occur by any of the following EXCEPT?

A. Ingestion of infected milk
B. Ingestion of contaminated meat
C. Person to person
D. Inhalation
E. Direct contact with animal tissues

Q3: A 36-year-old butcher presents with undulating fever, night sweats with a musty odor, and hepatosplenomegaly. He often handles raw animal tissue. Which pathogen is most likely responsible?

A. Brucella melitensis
B. Leptospira interrogans
C. Mycobacterium bovis
D. Salmonella typhi
E. EHEC

Q4: Which of the following Brucella species is most associated with chronic brucellosis in individuals working with pigs?

A. Brucella abortus
B. Brucella canis
C. Brucella suis
D. Brucella melitensis
E. Brucella ovis

Q5: A veterinarian accidentally pricks his finger with a live vaccine for Brucella. Which form of transmission best describes this exposure?

A. Ingestion
B. Inhalation
C. Percutaneous
D. Feco-oral
E. Sexual

Q6: All of the following are true about Brucella melitensis EXCEPT?

A. Most severe form of brucellosis
B. Associated with unpasteurized goat milk
C. Facultative intracellular organism
D. Gram-positive coccobacillus
E. Causes undulant fever

Q7: Which of the following best explains why Brucella abortus causes abortion in cattle but not in humans?

A. Human immunity is stronger
B. Brucella can't infect humans
C. Human placenta lacks erythritol
D. Humans are not exposed to cattle
E. Cattle produce antibodies that fail to protect

Q8: A lab worker accidentally inhales aerosols while processing a suspected brucellosis sample. Which phase of the disease would be optimal for isolating Brucella via blood culture?

A. Chronic stage
B. Immune phase
C. Latent stage
D. Acute bacteremic phase
E. Recovery phase

Q9: Which species of Brucella is catalase and oxidase-positive but cannot be detected serologically?

A. Brucella melitensis
B. Brucella abortus
C. Brucella suis
D. Brucella canis
E. Brucella ovis

Q10: In chronic brucellosis, bone marrow samples are preferred over blood for culturing because?

A. Bone marrow is easier to obtain
B. Brucella is extracellular in the chronic phase
C. ELISA is not available
D. Brucella prefers muscle tissue
E. The bacteremic phase has ended

Q11: Weil's syndrome, a severe complication of leptospirosis, includes all of the following EXCEPT?

A. Pneumonia
B. Hepatitis
C. Hemorrhage
D. Nephritis
E. Jaundice

Answers:

Q1: B Leptospirosis is transmitted via contact with urine-contaminated water/soil through skin or mucosa, not feco-oral.
Q2: C
Q3: A
Q4: C Brucella suis → swine
Q5: C
Q6: D
Q7: C
Q8: D
Q9: D
Q10: E
Q11: A

The University of Jordan
Gastroenterology Interest Group (UJ-GIG)
Booklet

Microbiology

Viral gastroenteritis

Written by: Ammar Alirani

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Reviewed by: Amr Abdallah

[Gastroenteritis – Overview]

➔ General Characteristics

Gastroenteritis is an **inflammatory** condition of the gastrointestinal tract, mainly involving:

- Stomach
- Small intestine

It is usually **infectious**, rather than non-infectious
Infectious causes include:

- **Viruses (most common)**
- Bacteria
- Parasites
- Fungi (less common)

Non-infectious causes are less frequent and include:

- Drugs (e.g., antibiotics, NSAIDs)
- Certain foods or toxins

The inflammation leads to:

- **Vomiting** (due to gastric irritation)
- **Diarrhea** (due to **impaired intestinal absorption**)

◆ Causes of Gastroenteritis (Summary Table)

Category	Examples	Frequency
Viral	Norovirus, Rotavirus	Most common
Bacterial	Salmonella, E. coli	Common
Parasitic	Giardia	Less common
Fungal	Candida (rare)	Rare
Non-infectious	Drugs, food intolerance	Less common
	Drugs, food intolerance	Less common

➔ Viral Gastroenteritis Etiology

Viral gastroenteritis is caused by viruses that infect the intestinal epithelium.

These viruses lead to:

- **Impaired absorption**
- **Increased secretion** → watery diarrhea

Severity depends on:

- Age
- Immune status
- Type of virus

Diagnosis is usually **clinical**, but lab tests are used in outbreaks or severe cases.

◆ Common Viral Causes of Gastroenteritis (Summary Table)

Virus	Family	Genome	Primary Age Group at Risk	Clinical Severity	Detection Methods
Rotavirus	Reoviridae	Double-stranded segmented RNA	Children < 5 years	+++ (severe)	ELISA (EIA), RT-PCR, PAGE, EM
Norovirus	Caliciviridae	Positive-sense single-stranded RNA	All ages	++ (moderate)	RT-PCR, EM

Abbreviation: EIA: enzyme immunoassays, EM: electron microscopy, PAGE: polyacrylamide gel electrophoresis, PCR: polymerase chain reaction, RT-PCR: reverse transcriptase

➔ Viral Gastroenteritis Epidemiology

- Acute infectious gastroenteritis is one of the most common illnesses globally.
- It is a major cause of morbidity and mortality in children, especially in developing countries, causing ~0.7 million deaths per year in children.
- In industrialized countries, it is responsible for 10–12% of all pediatric hospitalizations.

High-risk groups for severe disease and death include:

- **Children**, especially under 5 years
- **Elderly** patients
- **Patients with chronic or debilitating illnesses**

In contrast:

- Healthy young adults usually have mild disease
- Fatal outcomes are rare

◆ Risk Groups and Outcomes (Summary Table)

Population Group	Risk Level	Typical Outcome
Children (<5 yrs)	High	Severe dehydration, hospitalization, death (esp. developing countries)
Elderly	High	Severe complications, increased mortality
Chronically ill	High	Poor outcomes
Healthy young adults	Low	Mild, self-limited illness

[Norovirus]

Fast revision: related to Caliciviruses family: N**on-enveloped**, **(+)** ssRNA, **linear**, **icosahedral**.

➔ Norovirus Epidemiology

- Norovirus causes illness in ~20 million people each year
- It is the leading cause of foodborne outbreaks, especially in **restaurants, catering settings, and closed communities**.
- Infection is worldwide

Because exposure is common:
- Most adults have antibodies
- Reinfection can still occur (immunity is not long-lasting)

Food handlers play a major role in outbreaks.

➔ Norovirus Transmission

- Norovirus is extremely contagious.

Transmission occurs via:
- **Fecal–oral route (most important)**
- Contaminated food or water
- Person-to-person contact
- Contaminated surfaces (fomites)
- Aerosolization from vomitus

A very **small inoculum is sufficient**: as few as 10–100 viral particles.

◆ Transmission Routes (Summary Table)

Route	Example
Fecal–oral	Poor hand hygiene
Foodborne	Contaminated meals
Person-to-person	Close contact
Fomites	Door handles, surfaces
Aerosolized vomit	Vomiting episodes

➔ Norovirus Pathogenesis

- Norovirus binds to carbohydrate receptors on the gastroduodenal epithelium.
- Primary site of injury: **Upper jejunum**.

Pathologic changes include:
- **Blunting and broadening** of villi
- **Shortening of microvilli**
- **Vacuolization** of epithelial cells
- **Crypt hyperplasia**
- **Inflammatory infiltration** of the lamina propria (neutrophils & lymphocytes)

These changes cause:
- ↓ **Absorptive surface area**
- ↑ **Fluid loss → watery diarrhea**

The stomach and colon are usually not affected

➔ Norovirus Clinical Manifestation

- Incubation period: 24 hours
- Onset: **Sudden**
- Duration: 12–60 hours

Common symptoms
- **Nausea**
- **Vomiting**
- **Abdominal cramps**
- **Watery diarrhea**

Age-related differences
- Children → more vomiting
- Adults → more diarrhea

Constitutional symptoms are common
- **Headache**
- **Fever**
- **Chills**
- **Myalgias**

Stool characteristics
- Loose, watery
- No blood, mucus, or leukocytes

➔ Norovirus Diagnosis

- Advances in genome cloning and sequencing have allowed the development of molecular assays.
- **RT-PCR** is the **most sensitive and specific** test: detects **viral RNA in stool or vomitus**.
- **Enzyme immunoassays (EIAs)**: detect **viral antigens in stool** and are less sensitive than PCR.
- Due to high genetic and antigenic diversity, no single assay detects all human caliciviruses.

Testing is mainly used in
- Outbreak investigations
- Severe or atypical cases

◆ Diagnostic Methods Summary Table

Method	Detects	Use
RT-PCR	Viral RNA	Gold standard
EIA (ELISA)	Viral antigen	Screening
Clinical diagnosis	Symptoms + history	Most cases

➔ **Norovirus Treatment**

- Norovirus infection usually resolves **without** specific treatment.
- **Oral rehydration therapy (ORT)** is adequate in most cases.
- IV fluids are required if: **severe dehydration** or **persistent vomiting**.
- No specific antiviral drugs are available.

Supportive care includes
- Antipyretics
- Antiemetics (if needed)

➔ **Norovirus Prevention**

Prevention of Norovirus outbreaks relies on situation-specific epidemic control.

Key preventive strategies include
- Control of food and water contamination.
- Exclusion of ill food handlers from work.
- Reduction of person-to-person transmission through:
  - Proper hand hygiene
  - Use of gloves
- Disinfection of contaminated fomites (surfaces, utensils)

Strict hygiene measures are essential, especially in:
- Restaurants
- Schools
- Hospitals
- Long-term care facilities

[Rotavirus]

Fast revision: related to reovirus family: **Non-enveloped**, **dsRNA**, **icosahedral**, **segmented**, **linear** viruses

➔ **Rotavirus Epidemiology**

- Rotavirus infection is nearly universal in childhood: most children are infected by 3–5 years.

Neonatal infections
- Common
- Usually asymptomatic or mild
- Likely due to:
  - **Maternal antibodies**
  - **Breast milk protection**

First symptomatic infections
- Occur after 3 months of age
- **Peak incidence** between 4–23 months

In developing countries
- Limited access to oral rehydration therapy
- Rotavirus remains a leading cause of diarrheal mortality

Geographic pattern
- Tropical regions:
  - Rotavirus infection occurs year-round
  - Seasonal variation is less pronounced
- Temperate regions:
  - Infections occur mainly during **cooler and winter** months

Vaccination impact
- Routine rotavirus vaccination of U.S. infants began in 2006
- This led to:
  - Marked **decline** in rotavirus detection.

➔ **Rotavirus Pathogenesis**

- Rotavirus **infects mature enterocytes** on the villus epithelium of the proximal **small intestine**.
- Infected enterocytes are destroyed.

Pathologic consequences:
- **Loss of absorptive villi**
- **Villous atrophy**
- **Shortening of microvilli**

Compensatory response: Proliferation of secretory crypt cells
Net effect:
- ↓ Absorption
- ↑ Secretion

→ **Secretory diarrhea**

Decreased brush border enzymes lead to:
- **Poor digestion of carbohydrates**
- Osmotic effect → worsened diarrhea

Rotavirus Diagnosis
- Clinically, rotavirus gastroenteritis is difficult to distinguish from other enteric viral infections.
- Diagnosis is aided by the fact that **large amounts of virus are shed** in stool.

Confirmatory tests include:

- **Enzyme immunoassays** (EIAs / ELISA) to detect **viral antigen in feces**.
- Molecular techniques such as:
  - PCR
  - Nucleic acid probe hybridization

Testing is especially useful in:

- Severe cases
- Epidemiologic surveillance
- Hospitalized children

◆ Diagnostic Methods Summary Table

Method	Detects	Use
ELISA (EIA)	Viral antigen	Routine diagnosis
PCR	Viral RNA	High sensitivity
Clinical diagnosis	Symptoms	Initial assessment

➔ Rotavirus Treatment

- Rotavirus infection can cause **severe dehydration**, especially in infants, so early treatment is critical.
- **Oral rehydration therapy (ORT)**: effective for most children who can drink

IV fluid replacement is indicated if

- **Severe dehydration**
- **Frequent vomiting**
- **Failure of oral therapy**

Antibiotics

- Not effective
- Should be avoided

Antimotility agents

- Should be **avoided** in children

◆ Treatment Summary Table

Aspect	Management
Primary concern	Dehydration
First-line therapy	Oral rehydration
Severe cases	IV fluids
Antibiotics	Avoid
Antimotility drugs	Avoid

➔ Rotavirus Prevention

Rotavirus causes disease at similar rates in both developing and industrialized countries
This showed that **improvements in hygiene and sanitation** alone were **insufficient** to reduce disease incidence.

Therefore, **vaccine** development was prioritized
In 2006:

- Two rotavirus vaccines showed good safety and efficacy
- They were recommended for routine infant immunization.

Widespread vaccination resulted in: >70–80% reduction in:

- Rotavirus-related hospitalizations
- Emergency department visits

◆ Characteristics of Viral vs Bacterial Gastroenteritis (Summary Table)

Feature	Viral Gastroenteritis	Bacterial Gastroenteritis
Setting	Similar incidence in developing and developed countries	More common with poor hygiene and sanitation
Infectious dose	Low (10–100 viral particles)	High (10^5 bacteria for E. coli, Salmonella, Vibrio; lower for Shigella)
Seasonality	Winter peak in temperate climates; year-round in tropic-	More common in summer or rainy months
Incubation period	1–3 days (shorter for norovi- rus)	1–7 days (hours for toxin-mediated disease)
Reservoir	Primarily humans	Humans, animals, water (species-dependent)

CHARACTERISTICS OF GASTROENTERITIS CAUSED BY VIRAL AND BACTERIAL AGENTS

FEATURE	VIRAL GASTROENTERITIS	BACTERIAL GASTROENTERITIS
Fever	Common with rotavirus and norovirus; uncommon with other agents	Common with agents causing inflammatory diarrhea (e.g., Salmonella, Shigella)
Vomiting	Prominent and can be the only presenting feature, especially in children	Common with bacteria producing preformed toxins; less prominent in diarrhea due to other agents
Diarrhea	Common; nonbloody in almost all cases	Prominent and occasionally bloody with agents causing inflammatory diarrhea
Duration	1–3 days for norovirus and sapovirus; 2–8 days for other viruses	1–2 days for bacteria producing preformed toxins; 2–8 days for most other bacteria
Diagnosis	This is often a diagnosis of exclusion in clinical practice. Commercial enzyme immunoassays are available for detection of rotavirus and adenovirus, but identification of other agents is limited to research and public health laboratories.	Fecal examination for leukocytes and blood is helpful in dif erential diagnosis. Culture of stool specimens, sometimes on special media, can identify several pathogens. Molecular techniques are useful epidemiologic tools but are not routinely used in most laboratories.
Treatment	Supportive therapy to maintain adequate hydration and nutrition should be given. Antibiotics and antimotility agents are contraindicated.	Supportive hydration therapy is adequate for most patients. Antibiotics are recommended for patients with dysentery caused by Shigella or diarrhea caused by Vibrio cholerae and for some patients with Clostridium dif cile colitis.

Questions:

Q1: Which of the following is true of rotavirus gastroenteritis?
A. Has a high mortality rate even with hydration therapy.
B. Presence of antibodies against rotaviruses is rare worldwide.
C. Most infections occur in the elderly.
D. Causes a distinct set of clinical symptoms that is different from other viral gastroenteritides.
E. Infections in neonates often cause very few or no symptoms at all.

Q2: The following histological changes commonly take place following viral Gastroenteritis except:
A. Shortening of microvilli.
B. Death of enterocytes in the villous epithelium.
C. Immune cell infiltration of lamina propria.
D. Blunting of villi.
E. Atrophy and shortening of crypts.

Q3: A clinical case; it was stated that the patient has non-bloody diarrhea, and he is vomiting, he is unable to intake hard food, but he can keep soft food and water. The best management is:
A. Stay at home & rehydration.
B. Antibiotics
C. NSAIDs as typical treatment
D. Corticosteroids
E. All the above

Q4: Most common cause of gastroenteritis in children:
A. Norovirus
B. Rotavirus
C. Cytomegaly virus
D. Adenovirus
E. Hepatitis B virus

Q5: What's the typical incubation period of Norovirus?
A. 1-3 Days
B. 3-7 hours
C. 24 hours
D. 5-10 days
E. 2 weeks

Q6: Which of the following is a key preventive measure specifically associated with controlling Norovirus outbreaks?
A. Mass vaccination of school outbreaks
B. Eradication of mosquitoes
C. Exclusion of ill food handlers and disinfection of fomites
D. Prophylactic antibiotic use in travelers
E. National vaccination program

Q7: What is the most appropriate treatment approach for both Norovirus and Rotavirus?
A. Broad-spectrum antibiotics
B. Oral rehydration, IV fluids if severe
C. Antiviral drugs
D. High-dose steroids
E. None of the following

Q8: What type of stool is typically seen in both Norovirus and Rotavirus infections?
A. Bloody and mucus-filled
B. Watery, with no blood or leukocytes
C. Greasy and foul-smelling
D. Constipated pellets
E. Normal

Q9: Which diagnostic technique is commonly used for detecting both Norovirus and Rotavirus in stool samples?
A. Gram stain
B. Enzyme immunoassays (EIA)
C. Acid-fast stain
D. Blood culture
E. Chocolate agar

Q10: Which of the following is not a typical symptom of Norovirus gastroenteritis?
A. Nausea
B. Vomiting
C. Bloody diarrhea
D. Abdominal cramps
E. All are symptoms of norovirus gastroenteritis

Answers:
Q1: E
Q2: E
Q3: A
Q4: B
Q5: C
Q6: C
Q7: B
Q8: B
Q9: B
Q10: C

The University of Jordan
Gastroenterology Interest Group (UJ-GIG)
Booklet

Microbiology

Protozoal infections of the GI tract

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[Introduction to Protozoa]

The parasitic kingdom includes **three** major groups of human pathogens:

1) Protozoa
2) Helminths
3) Arthropods

Among these, **protozoa** are **unicellular eukaryotic** organisms that cause a wide range of clinically significant infections.

Protozoa are classified based on their mechanism of locomotion and whether sexual reproduction occurs in their life cycle. This classification aids in predicting pathogenesis, transmission, and intracellular behavior.

The four major classes of protozoa are:

- **Rhizopoda (Amoebae):**
  - Move using **pseudopodia** (crawling motion).
  - Reproduce asexually via **binary fission**.
- **Flagellates**:
  - Move using **flagella**.
  - Reproduce **asexually**.
- **Ciliates:**
  - Move using **cilia**.
  - Reproduce primarily **asexually**.
- **Sporozoa (Apicomplexa):**
  - Do not possess traditional locomotor organelles and move via **gliding motility**.
  - They are the only class that undergoes **sexual reproduction** (although they also have asexual stages).
  - They are **obligate intracellular** parasites.

[Protozoa of the GI tract]

The major protozoa infecting the gastrointestinal tract include:

Organism	Class	Reproduction	Disease Caused	Transmission
Entamoeba histolytica	Rhizopoda (Amoebae)	Asexual (binary fission)	Amoebiasis	Fecal–oral route (ingestion of infective stage in contaminated food or water)
Giardia lamblia	Flagellates	Asexual (binary fission)	Giardiasis	
Cryptosporidium spp.	Sporozoa (Coccidia subclass)	Both asexual and sexual	Cryptosporidiosis	

[Entamoeba histolytica]

- Entamoeba histolytica is a protozoan parasite (not a bacterium or virus).
- It infects the **large intestine** and can **invade** tissues → causing amoebiasis.

➔ Geographical Distribution

Worldwide, especially in **temperate** zones and areas with poor sanitary conditions.

- The parasite spreads through the **fecal–oral route**.
- Therefore, it is common where: drinking water is contaminated, sewage disposal is poor, and food hygiene is inadequate.

➔ Habitat

- The organism mainly lives in the large intestine, especially: (caecum, colonic flexures, and sigmoidorectal region).
- These areas have slow stool movement, which allows the parasite to attach and invade.

➔ Host

- **Definitive host** (where sexual or main life cycle occurs)**: Humans** only
- **Reservoir host** (who can carry the parasite)**:** Humans (main reservoir), also dogs, pigs, rats, monkeys, but transmission to humans is mostly from other humans, not animals.
  - Humans are the principal source of transmission.
  - Approximately 90% of infected individuals are asymptomatic carriers (cyst passers).

➔ Disease Caused

Amoebiasis (Amoebic dysentery)

➔ Morphology of Entamoeba histolytica

The parasite exists in two main forms:

1. **Trophozoite** (vegetative / tissue form)
2. **Cyst** (**infective**/luminal form)

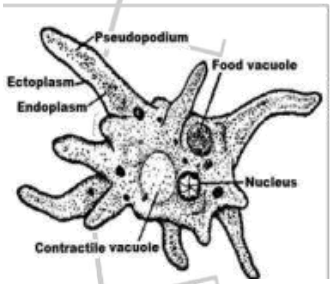
1) **Trophozoite Stage** – “Disease-causing form.”

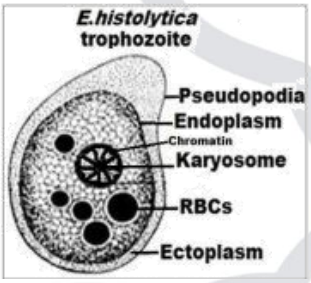
Characteristics

- The **active**, **motile** feeding form of the parasite.
- Lives in the intestinal wall and tissues.
- Responsible for:
  - Tissue invasion
  - Ulcers
  - Dysentery
  - Liver abscess

Key Morphological Features

- Size: 15–20 µm
- Contains:
  1) Clear ectoplasm
  2) Granular endoplasm
  3) Single nucleus with:
     - Central karyosome
     - Fine peripheral chromatin (“cartwheel” appearance)
  4) Pseudopodia → used for movement





Diagnostic Feature

- **Ingested red blood cells** (RBCs) in cytoplasm.
  → Pathognomonic for E. *histolytica*
  → Distinguishes it from: *Entamoeba coli, Entamoeba dispar, Entamoeba moshkovskii*
  These non-pathogenic species do not ingest RBCs.

2) **Cyst Stage** – “Infective Form.”

Characteristics

- The **dormant, resistant form**
- Survives outside the body and **survives gastric acidity**
- Responsible for transmission

Humans get infected by **swallowing mature cysts in contaminated food/water**.

Types of Cysts

A) Immature cyst
May contain:

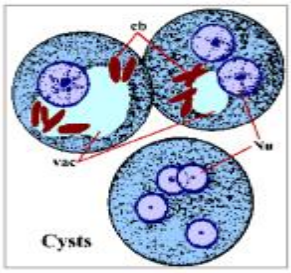
- 1 nucleus → uninucleate cyst
- 2 nuclei → binucleate cyst

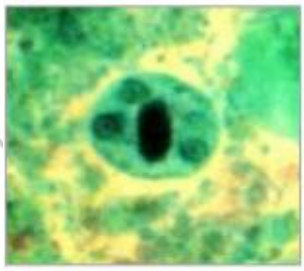
B) Mature cyst (Most Important)

- Contains 4 nuclei = Quadrinucleate cyst
- This is **the infective stage** for humans

Additional Features

- Chromatoid bodies (**cigar-shaped**)
- Central karyosome





Exposure to immature cysts does not cause infection.

➔ **Life cycle**

1. Ingestion of mature Quadrinucleate cyst
2. Survival through stomach acid
3. Excystation in the small intestine
4. Each cyst releases 8 trophozoites
5. Trophozoites migrate to the large intestine
6. Either:
   - Remain luminal (asymptomatic carrier)
   - Invade mucosa → disease
7. Re-encystation → **cysts passed in stool**

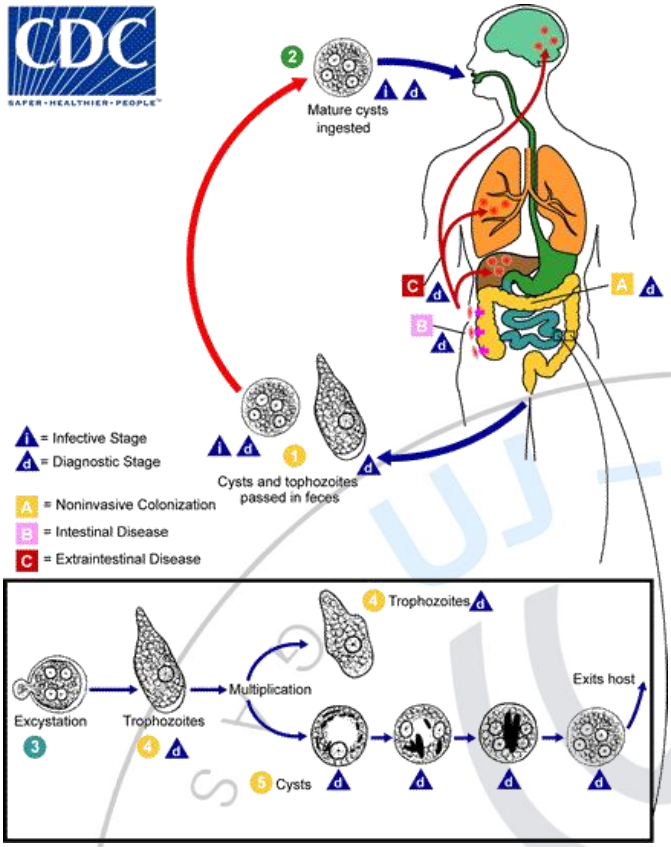
Infective Stage: Mature Quadrinucleate cyst

Diagnostic Stage

- Cysts (formed stool)
- Trophozoites (loose stool)

Comparison of Trophozoite vs. Cyst Forms – *Entamoeba histolytica*

Feature	Trophozoite	Cyst
Stage type	• Invasive / pathogenic	Infective
Motility	• Motile with pseudopodia	Non-motile
Nuclei	• (1) nucleus	• (1)(2)(3)(4) 1–4 nuclei
RBC ingestion	Present (diagnostic)	Absent
Found in	• Tissues, diarrheal stool	Formed stool, environment
Role	• Causes disease	Transmission



➔ Mode of Infection

1) Contaminated food and water (Most common)
- Drinking water contaminated with human feces
- Raw vegetables washed with unsafe water
- Uncooked food exposed to cysts

This is why the disease is common in areas with poor sanitation.

2) Infected food handlers
- Cooks or waiters who carry cysts on their hands
- Poor hand hygiene after using the toilet
- Direct contamination of meals

3) Mechanical vectors
- Flies and cockroaches can carry cysts
- Transfer them from feces → exposed food

4) Autoinfection
- Person re-infects himself by:
  - Contaminated hands
  - Poor personal hygiene
  - Hand-to-mouth contact

5) Homosexual Transmission

➔ Clinical Pictures of Intestinal Amoebiasis

Intestinal infection with *E. histolytica* can present in three major ways:
i. **Asymptomatic infection**
ii. **Symptomatic intestinal disease**
iii. **Complications**

1) **Asymptomatic Infection** (Most Common)
What happens?
- Trophozoites live in the intestinal lumen
- They feed on bacteria and nutrients
- **No tissue invasion**

Patient features
- Asymptomatic patients no diarrhea or pain
- Person acts as:
  - healthy carrier
  - **cyst passer** → important source of spread.

2) **Symptomatic Intestinal Amoebiasis**
A) ***Acute Amoebic Dysentery***
Clinical features
- **Fever**
- **Abdominal pain & tenderness**
- **Tenesmus (painful urge to defecate)**
- **Frequent small motions**
- **Stool contains**:
  - mucus
  - blood
  - Trophozoites

Pathology
- Trophozoites invade colonic mucosa
- Cause ulcer formation → **bleeding**

B) ***Chronic Amoebic Colitis***
- Low-grade dysentery for weeks–months
- Recurrent episodes of:
  - **Abdominal discomfort**
  - **Episodic diarrhea alternating with constipation**
- Stools usually show **cysts** only (not trophozoites)
- Often confused with inflammatory bowel disease.

3) **Complications**
- **Intestinal hemorrhage** → from erosion of blood vessels
- **Intestinal perforation** → peritonitis (life-threatening)
- **Appendicitis-like** presentation

- **Ameboma** (Amoebic granuloma):
  - Granulomatous mass around ulcer
  - May mimic colon cancer
  - Can cause intestinal obstruction/stricture

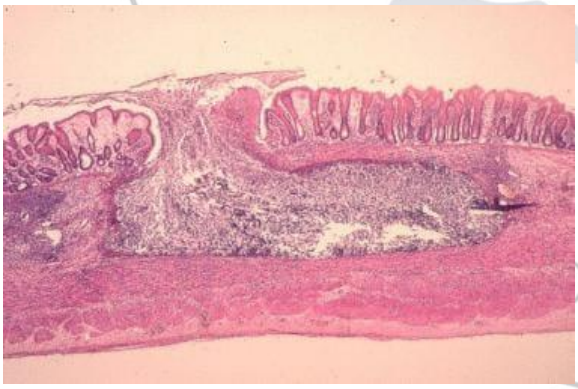
Pathogenesis – How Damage Occurs.

Step 1 – **Invasion**
- With heavy infection or ↓ immunity
- Trophozoites invade:
  - Mucosa
  - Submucosa of the large intestine
- They secrete lytic enzymes → tissue destruction

Step 2 – **Ulcer Formation**
- Characteristic **flask-shaped ulcer** (shown below)
  - **Narrow neck**
  - **Wide base**
- Edges contain:
  - Mucus
  - Cytolyzed (dead) cells
  - Trophozoites

Step 3 – Common sites of ulcer formation
Most common locations:
- Caecum
- Colonic flexures
- Sigmoid & rectum

Because t**hese areas have slow peristalsis** and colonic flow, allowing invasion.



➔ **Extra - Intestinal Amoebiasis**

- Trophozoites from the intestine can invade blood vessels.
- They spread via the portal circulation → mainly to the liver, then to other organs.

Clinical Manifestations of Intestinal Amoebiasis – *Entamoeba histolytica*

Type	Key Features	Stool Findings
Asymptomatic	• No invasion, carrier state	Cysts only
Acute dysentery	• Fever, abdominal pain, bloody mucus stool	Trophozoites + RBCs
Chronic colitis	• Recurrent mild symptoms	Mostly cysts
Amoeboma	• Mass lesion, mimics cancer	Variable
Amoeboma	• Mass lesion, mimics cancer	

A) Liver (Most Common)

Amoebic **liver abscess** or **diffuse amoebic hepatitis**.
- Trophozoites reach the liver through the **portal vein or extension from a perforating ulcer in right colonic flexure**.
- Cause necrosis of liver tissue → abscess formation.

Clinical Picture
- **Fever**
- **Right upper quadrant pain**
- **Tender hepatomegaly**
- **Sometimes jaundice**

Important Facts
- Affects **the right lobe** commonly either due to spread via the portal vein or extension from a perforating ulcer in the right colonic flexure.
- Abscess content:
  - Thick
  - Golden-brown
  - "**Anchovy paste**."
  - Usually sterile
- Complications
  - **Rupture** into:
    - Pleura
    - Lung
    - Peritoneum
    - Pericardium (rare but fatal)

B) ***Lung***

Amoebic lung abscess (Pulmonary Amoebiasis)

Mechanisms
1. **Direct spread** from liver abscess through the diaphragm (most common)
2. Rarely via the **bloodstream**

Features
- **Chest pain**
- **Cough**

- **Fever**
- **Lung abscess or pneumonitis**

Usually affects the **lower part** of the right lung

C) ***Brain***

Cerebral Amoebiasis

- Brain abscess → Meningoencephalitis
- Very rare
- Often fatal

D) ***Skin***

Cutaneous Amoebiasis (Amoebiasis Cutis)

Occurs due to

- Extension of severe intestinal disease to:
  - Perianal region
- Rupture of:
  - Liver abscess
  - Colonic or appendicular lesions onto skin

Features

- **Painful ulcerative** skin lesions
- Surrounding inflammation

➔ Laboratory Diagnosis of Intestinal Amoebiasis

1. Direct methods – detect the parasite itself
2. Indirect methods – detect antibodies

A) **Direct Diagnosis** (Most Important)

1) ***Macroscopic Stool Examination***

- **Loose, offensive stool**
- **Mixed with mucus & blood**

Suggests invasive amoebic dysentery

2) ***Microscopic Stool Examination***

Most used diagnostic method in endemic settings

Techniques:

- Direct wet smear
- Iodine staining
- Stool culture

What we look for:

Stool Type	Finding
Loose stool	Trophozoites (often with ingested RBCs)
Formed stool	Cysts

3) ***Sigmoidoscopy***

- Allows visualization of:
  - Ulcers
  - Inflamed mucosa
- Trophozoites can be seen in:
  - Ulcer aspirate
  - Biopsy material

4) ***Radiology*** (less commonly used now)

- X-ray after barium enema may show:
  - Ulcers
  - Strictures
  - Deformities

B) **Indirect Diagnosis – Serology**

Tests include:

- CFT – Complement fixation test
- IHAT – Indirect hemagglutination
- IFAT – Immunofluorescence
- ELISA
- GDPT – Gel diffusion precipitin test

Very Important Concept:

Serology is:

- **Positive in invasive amoebiasis**
- **Negative in asymptomatic carriers**

Because antibodies form only when tissue invasion occurs.

➔ Diagnosis of Extra-Intestinal Amoebiasis

Diagnosis depends on the organ affected, and is divided into:

1. Direct methods – show the lesion or parasite
2. Indirect methods – show immune response or supportive changes

A) **Direct Methods**

1) ***Imaging***

- X-ray findings
  - Liver: space-occupying lesion
  - Lung:
    - Pleuritis
    - Elevation of the right diaphragm
    - Basal lung opacity (if rupture from liver)
- Ultrasonography / CT / MRI → **MOST USEFUL**
  - Best for detecting amoebic liver abscess
  - Shows:
    - Single or multiple cavities
    - Usually in the right lobe

Ultrasound = first-line investigation.

2) ***Aspiration of Liver Abscess***

- Aspiration may show:
  - Thick brown “anchovy-paste” material
  - Trophozoites at the wall of the abscess

Trophozoites are often few in the center → sample from the edge is better.

B) **Indirect Methods**

1) ***Serological Tests***

- Same tests as intestinal amoebiasis: ELISA, IHAT, IFAT, CFT

Key point:

- Positive in invasive disease
- Antibodies may persist for years

2) ***Molecular Diagnosis***

- PCR from:
  - Blood
  - Aspirate
- Highly sensitive and specific

3) ***Blood Examination***

- Leukocytosis
- Elevated ESR

4) ***Liver Function Tests***

- Elevated liver enzymes: Increased in amoebic liver abscess.

➔ **Treatment of Amoebiasis**

Treatment depends on the clinical category:

1. Asymptomatic carrier
2. Intestinal amoebiasis
3. Extra-intestinal amoebiasis

1) **Asymptomatic Intestinal Carrier**

Goal

- **Eradicate cysts from the intestinal lumen**
- **Preventing transmission**

Drugs = **Luminal** amoebicides

- **Paromomycin**
- Diloxanide furoate
- Iodoquinol

Metronidazole is **NOT** enough alone for carriers.

2) ***Intestinal Amoebiasis (Symptomatic)***

Drug of Choice = **Tissue** amoebicide

- **Metronidazole** (Flagyl) or Tinidazole

3) ***Extra-Intestinal Amoebiasis*** (e.g., liver abscess)

Combination Therapy

- **Metronidazole**

  +

- **Luminal** amoebicide (Paromomycin or Diloxanide)

Because metronidazole kills tissue trophozoites but not luminal cysts, as **it does not achieve adequate cysticidal levels** in the intestinal lumen.

➔ **Prevention of Amoebiasis**

Key Principle: Break the fecal–oral cycle

Measures

- Safe water supply
- Proper sewage disposal
- Washing vegetables (especially lettuce)
- Hand hygiene for food handlers
- Boiling water kills cysts
- Amoebic cysts:
  - *E. histolytica* cysts can be viable in the environment for at least 3 weeks.
  - Not killed by low chlorine or iodine doses.
  - **Killed by boiling**.

[GIARDIA DUODENALIS]

(Also known as *Giardia lamblia*)

➔ **Overview**

- **Flagellated** protozoan parasite
- One of the most common causes of intestinal infection worldwide
- Disease caused: **Giardiasis**
- **Non-invasive** organism
- Transmitted via the fecal–oral route

“Beaver fever” is a common nickname in Canada due to **waterborne outbreaks**, although the name is informal.

➔ **Classification & General Features**

- Belongs to Flagellates
- Reproduces by **binary fission**
- Exists in two stages:
  - **Trophozoite** (active form)
  - **Cyst** (infective form)

Pathogenesis occurs **in the small intestine** (duodenum and jejunum), unlike *Entamoeba histolytica*, which affects the large intestine.

➔ **Habitat**

- Most commonly found in the **duodenal crypts**
- Trophozoites attach to intestinal villi using a **ventral adhesive** (sucking) disc
- Does not invade the intestinal mucosa

Cyst Formation

- Occurs as organisms move down to the **jejunum**
- Triggered by **exposure to bile secretions**

➔ **Morphology**

1) **Trophozoite** (Active Form)
Characteristics
- Pear-shaped / teardrop-shaped
- Bilaterally symmetrical
- Heart-shaped appearance
- 10–20 µm in size

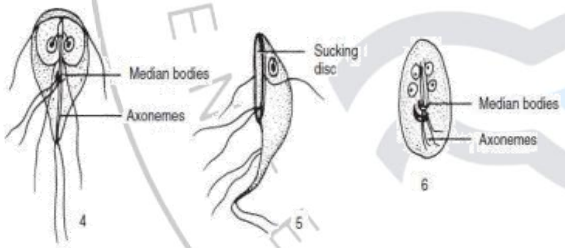
Key Features
- 2 nuclei → "face-like appearance."
- 4 pairs of flagella (8 total)
- Ventral adhesive disc for attachment
- Axonemes (axostyles)
- Characteristic "falling leaf" motility under microscopy

Trophozoites do **not** survive outside the body.

2) **Cyst** (Infective Stage)
Characteristics
- Oval shape
- Thick protective wall
- 8–12 µm

Contains
- 2 nuclei (immature cyst)
- 4 nuclei (mature cyst → infective stage)
- Internal axonemes

The mature quadrinucleate cyst is the infective stage.



➔ **Epidemiology of *Giardia duodenalis***

Transmission
- Infection occurs by ingestion of viable cysts
- Route: fecal–oral
  - Contaminated drinking water
  - Raw food
  - Person-to-person (day-care centers)

Risk Groups
- High incidence in:
  - Children in crowded settings
  - Immunodeficient patients (especially IgA deficiency)
  - Areas with poor sanitation

Incubation & Infectious Dose
- Incubation period: 1–2 weeks
- Infectious dose: as low as 10 cysts

➔**Pathogenesis**

Giardia does **not** invade intestinal tissue.
Disease occurs due to:
- **Mechanical attachment** to mucosa
- **Disruption of brush border enzymes**
- **Villous atrophy** (mild)
- **Impaired fat absorption**

Leads to:
- Fat malabsorption
- Protein malabsorption
- Deficiency of fat-soluble vitamins (A, D, E, K)
- Possible B12 and folate deficiency in chronic cases

➔**Clinical Features of Giardiasis**

1) ***Asymptomatic Infection***
- Many people have no symptoms
- Often, **treatment is not required** unless high-risk or persistent shedding

2) ***Symptomatic Infection***
Typical Diarrhea Pattern
- Starts as **watery diarrhea**
- Later becomes:
  - **Greasy**
  - **Foul-smelling**
  - **Floating stool** (steatorrhea)

Because Giardia causes **fat malabsorption**.
Steatorrhea is stool with fat in it.

Associated Symptoms
- Abdominal cramps
- Bloating & flatulence
- Malaise, fatigue
- Weight loss
- Malabsorption of:
  - **Fats**
  - **Vitamins (A, D, E, K)**

Uncommon Features
- Bloody diarrhea
- Tenesmus
- Severe vomiting

These are uncommon because Giardia is **non-invasive**.

➔ **Life Cycle of *Giardia duodenalis***

Infective Stage

- Mature quadrinucleate cyst is the only **infective** form
- Humans get infected by: contaminated water, food, hands/fomites.

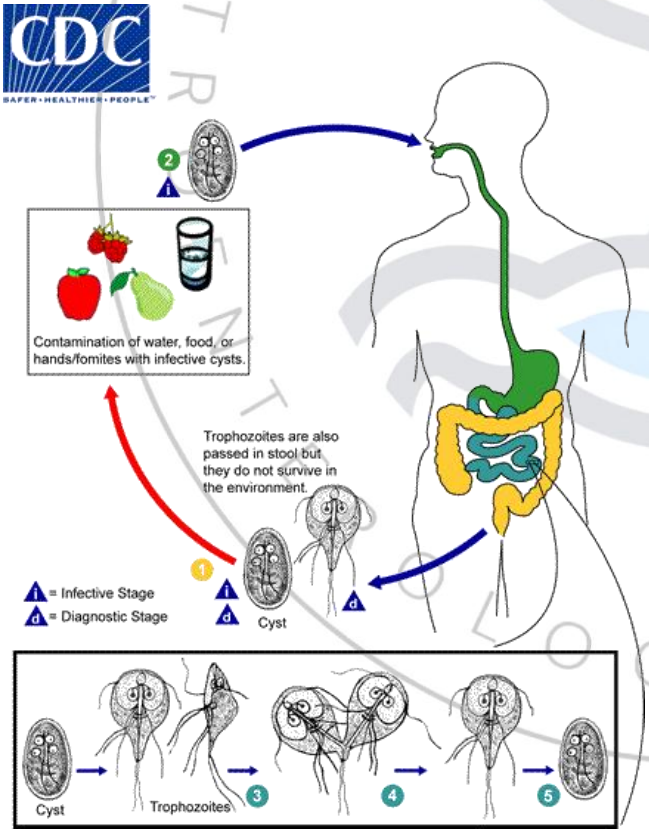
Steps

1) **Ingestion of cysts** via contaminated water, food, or hands
2) Cyst survives gastric acid
3) **Excystation occurs in the duodenum**
4) Each cyst releases **two trophozoites**
5) Trophozoites **attach** to the mucosa using the ventral disc
6) Multiply by **binary fission**
7) **Encystation** occurs as organisms move toward the **colon**
8) Cysts are passed in stool

Diagnostic Stage

- Cysts (most common finding)
- Trophozoites (in fresh diarrheal stool)

Trophozoites do **NOT** survive outside the body.



➔ **Laboratory Diagnosis of Giardia**

1) ***Routine Stool Examination***

- Cysts → most common finding.
- Sometimes trophozoites in fresh watery stool.

Methods

- Direct wet mount
- Iodine staining
- Concentration techniques

2) ***Antigen Detection*** (Preferred Method)

- Stool antigen **ELISA**
- Immunochromatographic assays
- Detection of Giardia-specific antigens (e.g., GSP65)

Advantages

- **Highly sensitive & specific**
- Detects infection even when microscopy is negative.
- Single sample often enough.

3) Other Methods (less common)

- **PCR** from stool
- **Duodenal aspirate** in difficult cases
- **String test** (Enterotest):
  A capsule attached to a string swallowed
  After 4–5 hours, the string is withdrawn,
  Trophozoites may be detected.

➔ **Treatment of Giardiasis**

Drugs of Choice

- **Metronidazole – first line**
- **Tinidazole** – alternative (shorter course)

Other options

- Nitazoxanide
- Albendazole (sometimes used)

[Cryptosporidium species]

➔ **Overview**

- Intracellular protozoan parasites
- Belong to the coccidia subclass (Sporozoa)
- Infect epithelial cells of:
  - **Small intestine** (main site)
  - Stomach (rare)
  - Biliary ducts (especially in immunocompromised)

Main Species

Cryptosporidium parvum → infects humans and other mammals
Cryptosporidium hominis → primarily infects humans

➔ **Life Cycle**

Infective Stage: Thick-walled oocyst

Oocysts are:

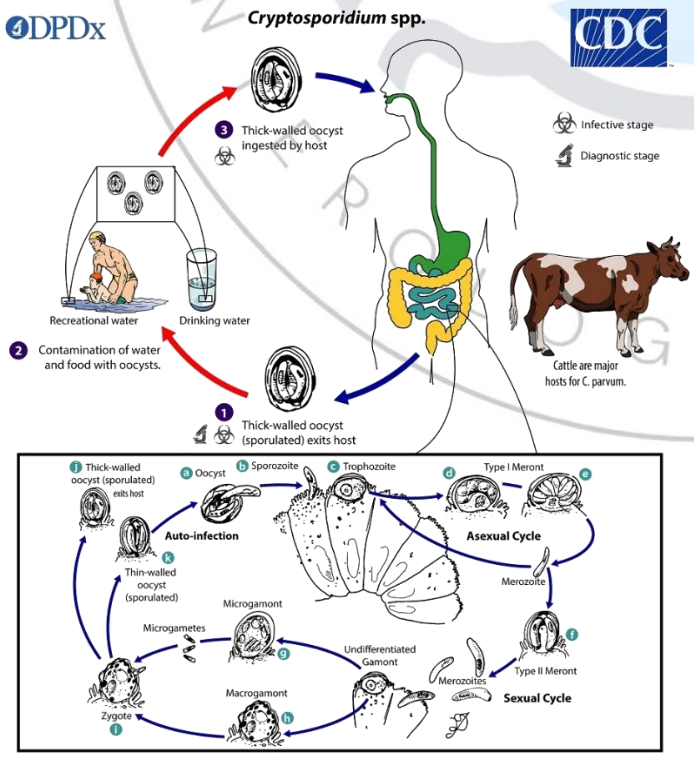
- Oocysts are immediately infective when passed in stool (no external maturation required).
- Resistant to environmental stress
- Resistant to standard chlorination levels

Steps

1) Infection begins by **ingestion** of viable oocysts (contaminated water, food, hands)
2) Oocyst reaches the small intestine
3) Each oocyst releases **4 sporozoites**
4) Sporozoites **invade epithelial cells** of the small intestine (brush border)
5) Develop into trophozoites
6) Undergo **asexual reproduction → Type I meronts**
7) Can:
   - Reinfect cells (autoinfection), or
   - Develop into **Type II meronts**
8) **Type II meronts** undergo **sexual reproduction**
9) Formation of oocysts:
   - **Thick-walled oocysts** → passed in stool (infective stage).
   - **Thin-walled oocysts** → cause internal autoinfection.

Internal Autoinfection

- Occurs due to thin-walled oocysts
- Leads to **persistent infection**
- Particularly significant in immunocompromised patients.



➔ Epidemiology

- Prevalence of fecal oocysts: approximately 3–10%
- Major transmission sources:
  - **Contaminated drinking water**
  - Recreational water (swimming pools, water parks)
  - Person-to-person spread
- Cryptosporidium is well known for **causing outbreaks from recreational water** because oocysts are **chlorine resistant**.

➔ Clinical Features

In Immunocompetent Patients

- Often asymptomatic
- Or **mild, self-limited watery** diarrhea
- Symptoms last 1–2 weeks

In Immunocompromised Patients (e.g., HIV/AIDS)

- **Profuse** (Copious) watery diarrhea
- Stool volume may reach 3–17 liters/day
- **Severe dehydration**
- **Abdominal pain**
- **Nausea and vomiting**
- **Weight loss**
- Chronic, debilitating disease

Difference by Immune Status

Patient Type	Course
Immunocompetent	Self-limited (1-2 weeks)
Immunocompromised (HIV/AIDS)	Chronic, severe, life-threatening

➔ Diagnosis

1) *Stool Microscopy*
Modified Acid-Fast Stain (Kinyoun Technique)
Historically, it was considered the gold standard.

- Oocysts appear red/pink against a blue background
- Used for:
  - Cryptosporidium
  - Cyclospora
  - Isospora
  - Nocardia (partially acid-fast)

2) Direct Fluorescent Antibody Test (DFA)

- Highly sensitive
- Considered **the gold standard** in many settings

3) Other Methods

- **Stool antigen** detection tests
- **PCR** (high sensitivity and specificity)

➔ **Treatment**

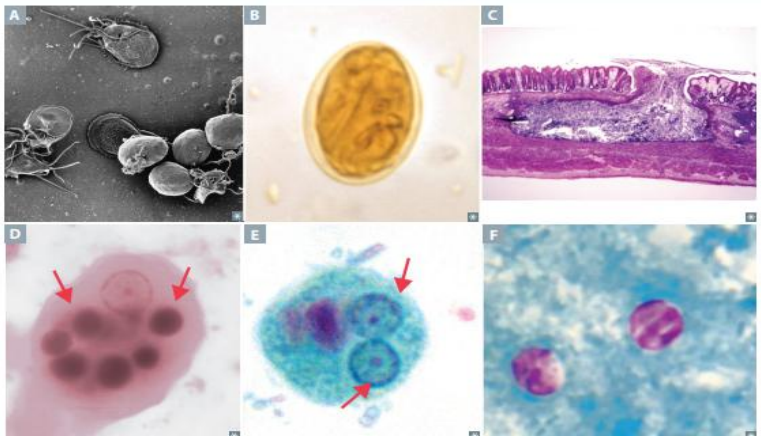
Immunocompetent Patients
- Usually **self-limited**
- Management:
  - **Oral rehydration**
  - **IV fluids if severe**
- **Nitazoxanide** can be used and is effective in many cases

Immunocompromised Patients
- **Nitazoxanide** and other antiparasitic agents may be tried
- Response is variable
- Most important management:
  - **Optimization of immune status**
  - For HIV patients → **Effective antiretroviral therapy** (ART/HAART)
- Metronidazole is **not** effective.

Quick **Revision**

Protozoa—gastrointestinal infections

ORGANISM	DISEASE	TRANSMISSION	DIAGNOSIS	TREATMENT
Giardia lamblia	**Giardiasis**—bloating, flatulence, foul-smelling, nonbloody, fatty diarrhea (often seen in campers/hikers)—think fat-rich Ghirardelli chocolates for fatty stools of *Giardia*	Cysts in water	Multinucleated trophozoites A or cysts B in stool, antigen detection, PCR	Tinidazole, nitazoxanide, or metronidazole
Entamoeba histolytica	**Amebiasis**—bloody diarrhea (dysentery), liver abscess ("anchovy paste" exudate), RUQ pain; histology of colon biopsy shows flask-shaped ulcers C	Cysts in water	Serology, antigen testing, PCR, and/or trophozoites (with engulfed RBCs D in the cytoplasm) or cysts with up to 4 nuclei in stool E; *Entamoeba* Eats Erythrocytes	Metronidazole; paromomycin for asymptomatic cyst passers
Cryptosporidium	Severe diarrhea in AIDS Mild disease (watery diarrhea) in immunocompetent hosts	Oocysts in water	Oocysts on acid-fast stain F, antigen detection, PCR	Prevention (eg, filtering); nitazoxanide (severe disease and/or immunocompromised)



Questions:

Q1: Which of the following protozoa causes flask-shaped ulcers in the colon?
A) Giardia lamblia
B) Cryptosporidium parvum
C) Strongyloides stercoralis
D) Entamoeba histolytica
E) Shigella sonnei

Q2: Amoebic liver abscess most commonly involves which hepatic lobe?
A) Left lobe via lymphatic drainage
B) Right lobe via portal vein
C) Both lobes via systemic circulation
D) Caudate lobe via hepatic artery
E) All lobes equally

Q3: Which organism causes steatorrhea (greasy, foul-smelling stool)?

A) Entamoeba histolytica
B) Giardia lamblia
C) Cryptosporidium parvum
D) Shigella dysenteriae
E) Campylobacter jejuni

Q4: What is the most appropriate lab diagnostic method for Cryptosporidium?

A) Blood smear
B) CSF analysis
C) Modified acid-fast stain of stool
D) ELISA of serum
E) Sputum culture

Q5: What is the definitive host for Entamoeba histolytica?

A) Dogs
B) Rats
C) Pigs
D) Sheep
E) Humans

Q6: What is the infective stage of Giardia lamblia?

A) Trophozoite
B) Flagellated cyst
C) Cyst
D) Oocyst
E) Sporozoite

Q7: Which protozoan presents as an asymptomatic carrier with only cysts in the stool?

A) Entamoeba histolytica
B) Giardia lamblia
C) Cryptosporidium parvum
D) Balantidium coli
E) Cyclospora cayetanensis

Q8: Which of the following is a complication of amoebic colitis?

A) Esophageal varices
B) Appendicitis
C) Pancreatitis
D) Cholangitis
E) Pericarditis

Q9: Which drug combination is most effective for invasive amoebiasis?

A) Tinidazole + doxycycline
B) Metronidazole + paromomycin
C) Albendazole + mebendazole
D) Nitazoxanide + erythromycin
E) Doxycycline + Metronidazole

Q10: Which protozoa is associated with 3–17 liters of watery diarrhea per day in HIV+ patients?

A) Giardia lamblia
B) Entamoeba histolytica
C) Cryptosporidium hominis
D) Balantidium coli
E) Cyclospora cayetanensis

Q11: A patient presents with abdominal pain, bloody diarrhea, and later develops a liver abscess. Which organism is the most likely cause?

A) Giardia lamblia
B) Clostridium difficile
C) Cryptosporidium parvum
D) Entamoeba histolytica
E) Strongyloides stercoralis

Q12: Which of the following protozoa has a cyst stage with four nuclei (quadrinucleate)?

A) Giardia lamblia
B) Balantidium coli

C) Entamoeba histolytica
D) Cryptosporidium
E) Cyclospora

Q13: Which of the following diagnostic tools is most specific for amoebic liver abscess?

A) Stool culture
B) Antigen ELISA from feces
C) Sigmoidoscopy
D) Liver function test
E) CT-guided aspiration

Q14: What is the most reliable method to identify Cryptosporidium oocysts in stool?

A) Gram stain
B) India ink stain
C) Modified acid-fast stain
D) Direct culture
E) PCR of blood

Q15: Which of the following drugs is used in immunocompromised patients with cryptosporidiosis?

A) Metronidazole
B) Nitazoxanide
C) Paromomycin
D) Vancomycin
E) Cefixime

Q16: Which of the following is NOT typically seen in giardiasis?

A) Greasy floating stool
B) Tenesmus
C) Malabsorption
D) Flatulence and bloating
E) Watery diarrhea

Q17: Which protozoan is associated with lesions in the duodenum and steatorrhea?

A) Cryptosporidium parvum
B) Giardia lamblia
C) Entamoeba histolytica
D) Cyclospora cayetanensis
E) Trichomonas hominis

Q18: The infective stage of Cryptosporidium parvum is:

A) Sporozoite
B) Merozoite
C) Oocyst
D) Trophozoite
E) Cyst

Q19: The most common complication of untreated acute amoebic dysentery is:

A) Malabsorption syndrome
B) Giardiasis
C) Hydronephrosis
D) Esophagitis
E) Amoebic liver abscess

Q20: What characteristic feature helps Giardia attach to intestinal epithelium?

A) Flagella
B) Cilia
C) Suction disk
D) Spicules
E) Pseudopodia

Q21: Which of the following is wrong about Giardia?

A) Malabsorption
B) Dorsal sucker
C) Two prominent nucleoli
D) 4 pairs of flagella
E) Causes bloody diarrhea

Q22: Which of the following produces massive watery diarrhea, especially in immunocompromised patients?

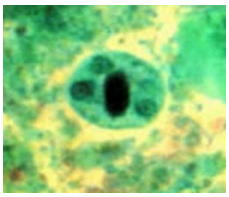
A) Entamoeba histolytica
B) Giardia lamblia
C) Cryptosporidium parvum
D) Trichuris trichiura
E) E. Coli

Q23: A protozoan that causes intermittent episodes of diarrhea alternating with constipation is most likely:

A) Cryptosporidium
B) Entamoeba histolytica
C) Giardia lamblia
D) Balantidium coli
E) Trichomonas hominis

Q24: Patient presents with a history of recurrent episodes of diarrhea alternating with constipation, and the microscopic examination of the stool sample shows the picture. Which of the following is the cause:

A) Entamoeba histolytica
B) Yersinia enterocolitica
C) Giardia lamblia
D) Clostridium difficile
E) Enterotoxigenic Escherichia coli.



Q25) Patient presents with a history of watery diarrhea of several weeks' duration, and the microscopic examination of the stool sample shows the picture. Which of the following is the cause:

A) Giardia lamblia
B) Clostridium difficile
C) Entamoeba histolytica
D) Yersinia enterocolitica
E) Enterotoxigenic Escherichia coli.

Answers:

Q1: D
Q2: B
Q3: B
Q4: C
Q5: E
Q6: C
Q7: A
Q8: B
Q9: B
Q10: C
Q11: D
Q12: C
Q13: E
Q14: C
Q15: B
Q16: B
Q17: B
Q18: C
Q19: E
Q20: C
Q21: E
Q22: C
Q23: B
Q24: A
Q25: A

The University of Jordan
Gastroenterology Interest Group (UJ-GIG)
Booklet

Microbiology

Common Helminthic infections of the GI tract

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[Introduction to Helminths]

Helminths are **multicellular** parasitic organisms that belong to the group Metazoa and represent one of the two major categories of human parasites studied in medical microbiology.

To recall from general microbiology, parasites are broadly divided into:

1) **Protozoa** (Unicellular Parasites)
   - Single-celled eukaryotic organisms
   - Multiply within the human host
   - Their number increases inside the body
   - Therefore, their life span in the host can be indefinite.

2) **Metazoa** (Helminths – Multicellular Parasites)
   - Multicellular organisms (worms)
   - Do **not** multiply significantly within the human host (with rare exceptions such as *Strongyloides stercoralis* and *Echinococcus* species).
   - The parasite burden depends on the number of **infective larvae or eggs ingested**.
   - They have a definite life span
   - Worms eventually degenerate and disintegrate over time

Unlike protozoa, helminths generally cause disease due to:
- Mechanical obstruction
- Nutritional competition
- Tissue invasion
- Host immune response

Classification of Helminths

Helminths are divided into two major phyla:

1) **Nematodes (Roundworms)**
   - **Cylindrical** in shape
   - Unsegmented
   - **Dioecious** (male and female worms are separate)
   - Complete digestive tract

They are further classified into:
- Intestinal nematodes
- Extra-intestinal (tissue/blood) nematodes

2) **Platyhelminthes (Flatworms)**

These are **flattened**, leaf-like worms and include:
- Cestodes (Tapeworms)
- Trematodes (Flukes)

Most platyhelminths are **hermaphroditic** (except schistosomes).

[Ascaris lumbricoides]

➔ **Overview**
- A **nematode** (roundworm).
- The **largest intestinal** helminth infecting humans.
- The **most common** helminthic infection worldwide.
- Estimated prevalence: >1 billion people
- Disease caused: Ascariasis
- Does **not** multiply inside the human body (parasite burden depends on the number of ingested eggs).

➔ **Morphology**

- **Cylindrical**
- Unsegmented
- Yellow–golden in color
- Dioecious (separate male and female)

Male Worm
- Length: 15–20 cm
- Posterior end: **Curved/coiled**
- Contains copulatory spicules (mating organs)

Female Worm
- Length: 20–40 cm
- Posterior end: **Straight**
- **Larger** than male
- Produce approximately 200,000 eggs per day

Eggs (Diagnostic Form)

Eggs are:
- Oval
- Thick-shelled
- **Mammillated** (bumpy outer coat)
- Chitinous shell
- **Bile-stained** (because they pass through the small intestine)

Types:
- Fertilized eggs (can become infective after embryonation)
- Unfertilized eggs (cannot develop further)

Important:
- Eggs passed in stool are **not immediately infective**.
- They require an **incubation period in the soil** to embryonate and become infectious.

➔ **Transmission**

1) ***Mode of Transmission***
- **Fecal–oral** transmission by ingestion of eggs, which are present in:
  - Contaminated soil
  - Unwashed vegetables
  - Dirty hands
  - Contaminated water
- **Reinfection** is common (No lasting immunity develops)

2) ***Habitat in Humans***
Adult worms live in the **small intestine**
- Mainly jejunum
- Ileum

3) ***Infective Stage***
- **Embryonated (fertilized) egg** is the infective form.
- Not the adult worm or larva.
- Each female produces ≈ 200,000 eggs/day

Eggs are highly **resistant**:
- Survive dry conditions
- Tolerate freezing
- Remain viable in soil for months

➔ **Life Cycle**

A) ***In the Environment***
1) Eggs are passed in feces
2) In **soil** → eggs become embryonated (infective)

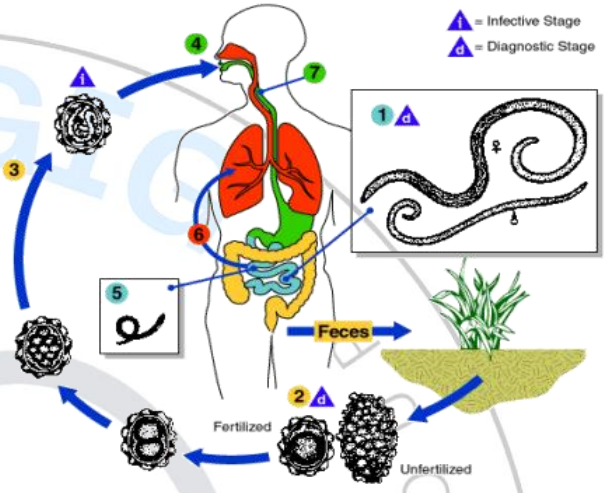
B) ***After Ingestion***
1) An embryonated egg is swallowed
2) Egg **survives gastric acidity**
3) Larva hatches in the **small intestine**
4) Larva penetrates the intestinal wall → **enters venous circulation**

C) ***Lung Migration*** (Transpulmonary Phase)
1) Larva travels to: Liver → Heart → Lungs
2) In lungs:
   - Breaks into alveoli
   - Ascends the bronchial tree
   - Reaches pharynx
   - Is swallowed again

This stage may cause: **Löffler Syndrome**
- **Cough**
- **Wheezing**
- **Fever**
- **Eosinophilia**
- **Transient pulmonary infiltrates** on X-ray

Mechanism: **Hypersensitivity** reaction to migrating larvae

D) Back to Intestine
3. Larva returns to the small intestine
4. Mature into an adult worm
5. Females produce eggs
6. Eggs passed in stool



➔ **Diagnostic Stage**

- Fertilized or unfertilized eggs in **stool**
- Adult worms (or parts) in **stool**
- Larvae in **sputum** (during pulmonary phase)
- Occasionally, gastric aspirates

➔ **Pathogenesis & Spectrum of Disease**

Disease Name: Ascariasis
Most affected
- Children
- Young adolescents

due to poor hygiene & soil exposure
- Many infections are asymptomatic, particularly with low worm burden, because helminths do not multiply within the human host.

1) Symptomatic Disease
A) **Pulmonary Phase**: **Löffler Syndrome**
Occurs during larval lung migration
Features: Wheezing, Fever, Eosinophilia, Transient lung infiltrates on X-ray.

B) **Intestinal Phase**: Adult Worm Effects
GI Manifestations
- Malnutrition
- Anemia
- Malabsorption
- Steatorrhea

Mechanical Complications
- **Intestinal obstruction** (worm bolus)
- **Biliary obstruction** → jaundice
- **Appendicitis**
- **Pancreatitis**

➔Ectopic Ascariasis

Worm migration outside the normal habitat may occur. Possible sites:
- Bile ducts
- Pancreatic duct
- Appendix
- Female genital tract
- Peritoneum

Triggers for wandering:
- **Anti-helminthic drugs**
- **Steroids**
- **Anesthesia**
- **Fever**

➔ Laboratory Diagnosis

1) ***Blood Findings***
Eosinophilia (an increase in eosinophils), especially during migration.

2) ***Stool Examination*** — Main Diagnostic Method
Microscopic Examination (Definitive Diagnosis)
Identification of characteristic eggs in stool:
- Fertilized eggs
- Unfertilized eggs

Techniques:
- Direct saline wet smear
- Iodine preparation
- Concentration methods (increase sensitivity)

3) Other Samples
- **Adult worms may be seen in feces**
- Larvae may be found in:
  - **Sputum**
  - **Gastric aspirate (**during lung phase)

➔ Treatment

Drug of Choice
- **Albendazole 400 mg orally STAT** (STAT = immediately)

Alternatives
- Mebendazole
- Pyrantel pamoate

[Enterobius vermicularis (Pinworm)]

➔ General Characteristics

- Small, thin, white **nematode** (roundworm)
- **Most common helminthic infection in children** (especially 5–14 years)
- Worldwide distribution
- Most common helminthic infection in the United States
- Disease caused: Enterobiasis
- Also called “seatworm” due to perianal itching
- Lives mainly in the large intestine (cecum & appendix)

➔ Morphology

Female Worm
- Length: 8–13 mm
- Posterior end: **pointed** (“pin-shaped”) → gives name pinworm
- Lays approximately 11,000 eggs
- Lives about 1 month

Male Worm
- Smaller: 2–5 mm
- Posterior end **curved**
- Dies after fertilization
- May be passed in stool

➔ Mode of Transmission

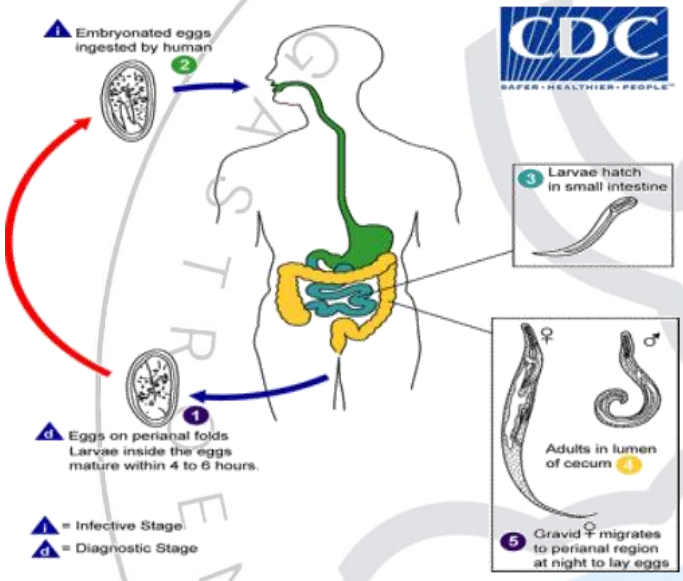
Main Routes
- **Fecal–oral** (most common)
- **Inhalation** of airborne eggs from contaminated dust
- **Autoinfection** by scratching the perianal area (hand-to-mouth after scratching)
- **Direct person-to-person** spread
- Indirect spread via:
  - Bedding
  - Clothing
  - Toys
  - Door handles
- Spread common in:
  - Families
  - Schools
  - Crowded institutions

Infective Stage: Embryonated egg
Becomes infective within hours after being laid

➔ Life Cycle

1) At night, the gravid female migrates to the **perianal region**
2) Lays thousands of eggs → causes **itching**
3) Eggs embryonate within 4–6 hours
4) Scratching **transfers eggs to fingers**
5) Eggs **reach the mouth** → swallowed
6) Larvae **hatch** in **the small intestine** → **mature** in **the large intestine**.

Important:
- No lung migration (unlike Ascaris)
- Retroinfection may occur (larvae hatch on perianal skin and migrate back)



➔ Clinical Features

- Most Infections: Often asymptomatic
- Hallmark Symptom: **Perianal Pruritus** (itching), especially at **night** due to migration of female worm to lay eggs
- Leads to:
  - scratching
  - sleep disturbance
  - irritability in children

Possible Complications are rare but important:
- Appendicitis
- Oophoritis (inflammation of the ovaries), in females.
- Vulvovaginitis
- Ulcerative bowel lesions

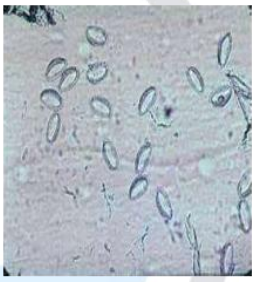
➔ Diagnosis

Method of Choice
Cellophane (Scotch) Tape Test

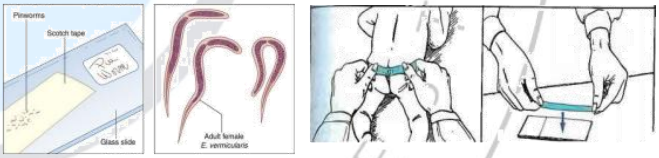
Procedure:
- Performed early morning
- Before bathing or defecation
- Tape applied to the perianal region
- Examined microscopically

Findings:
Egg Characteristics
- Oval
- **Flattened** on one side → **D-shaped** / planoconvex (Diagnostic)
- Colorless
- Transparent shell

Important:
- Stool examination is usually negative
- Eggs may sometimes be found under fingernails



Enterobius vermicularis eggs



➔ Treatment

Drug of Choice
- **Albendazole** orally
- Repeat dose after 2 weeks

Important Measures
- Treat **all family members**
- Wash bedding & clothes
- Cut fingernails
- Improve hand hygiene

[Echinococcus granulosus- Hydatid cyst]

➔ General Characteristics

- One of the **smallest** tapeworms infecting humans.
- Adult length: 3–9 mm
- Causes **hydatid disease** (echinococcosis)

➔ Morphology (Adult Worm)

- Has a scolex (head) with:
  - **Hooks**
  - **Suckers**
- Body composed of ONLY 3 proglottids → Therefore, it is called a **3-segment** tapeworm

Very small compared to other cestodes like Taenia.

➔ Life Cycle & Hosts

Unlike nematodes (Ascaris, Enterobius), this parasite **requires two hosts**.

Definitive Host (where the adult worm lives)
- **Dog/canine**
- Worm lives in the small intestine of dogs

Intermediate Hosts (Larval Stage)
- **Sheep**
- **Cattle**
- Other herbivores

Humans: **Accidental** intermediate host

Important Concept
- Humans are a **dead-end** host
- Parasite **cannot** complete its cycle in humans
- Leads to the formation of hydatid cysts

➔ Hydatid Disease in Humans

Where cysts occur
- **Liver**: most common
- **Lungs**: second most common
- Less common:
  - brain
  - bone
  - Spleen

➔ Clinical Features

- Often **asymptomatic** for years
- Symptoms depend on:
  - **Size**
  - **Location**
  - **Mass effect**

Possible problems
- Mass effect on organs
- Cyst rupture → **anaphylaxis**
- Secondary spread

Anaphylaxis is a life-threatening type I hypersensitivity reaction that is characterized by soft tissue swelling, itchy rash, respiratory wheezes due to airway narrowing and low blood pressure.

➔ Growth Rate

- Hydatid cysts are **slow-growing**
- Average**:** 2–3 cm per year

➔ Diagnosis

Usually **incidental on radiology**

Imaging (Main)
- **Ultrasound**
- **CT scan**
- **MRI**

Serology
- **ELISA**
- **Indirect hemagglutination** (IHA)

➔ Treatment

Main Treatment, depends on the size:

Surgery (careful removal)

Medical:
- **Albendazole**
  - Before surgery
  - After surgery
  - Sometimes alone if inoperable

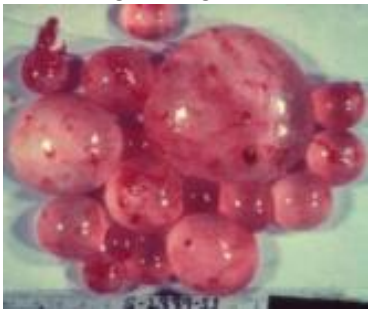
➔ Gross Appearance of the cyst

On naked-eye examination, the cyst looks like a **"bunch of grapes."**

Why?

Main cyst contains:
- **Daughter cysts**
- **Hydatid fluid**
- **Protoscolices**, which represent the infective stage

Fluid-filled vesicles → give a grape-like appearance



[Schistosomiasis]

Schistosomiasis is a human disease syndrome caused by infection with blood flukes of the genus *Schistosoma*

- They are trematodes
- The **ONLY** trematodes with **separate** sexes (dioecious)
- Adult worms live **in the venous circulation**

It is the second most important parasitic disease worldwide after malaria.

➔ Main Species Causing Human Disease

1) ***Schistosoma mansoni***
- Mainly affects the **GIT**
- Adults live in **mesenteric** veins
- Eggs found in **stool**
- Causes **intestinal & hepatic disease**

2) ***Schistosoma japonicum***
- Also, **GIT** involvement
- Lives in mesenteric veins
- Eggs in **stool**
- Produces **more eggs** → more severe disease
- Common in East Asia

3) ***Schistosoma haematobium***
- Affects the **urinary** tract
- Adults live in the **vesical venous plexus**
- Eggs found in **urine**
- Causes **hematuria & bladder complications**
- Discovered by Theodor Bilharz in Cairo (1861)
- Called Bilharzia

➔ Epidemiology

- ~200 million infected worldwide
- 500–600 million at risk
- Endemic in tropical & subtropical regions
- Endemic in Egypt (especially S. haematobium)
- In Jordan:
  - S. haematobium (especially Egyptian expatriates)
  - S. mansoni is also present

Geographic distribution depends on **freshwater snail** species.

➔ Habitat in Humans

Adult worms reside in:

Species	Location
S. mansoni	Mesenteric venous plexus
S. japonicum	Mesenteric venous plexus
S. haematobium	Vesical venous plexus

➔ Life Cycle of *Schistosoma* (Blood Flukes)

Step 1 – **Eggs Leave Human**
- Eggs are passed from an infected person:
  - In **stool** → *S. mansoni* & *S. japonicum*
  - In **urine** → *S. haematobium*
- Eggs must reach fresh water to continue the cycle.

Step 2 – **Miracidium Stage**
- In water, the egg hatches → releases ciliated miracidium
- Miracidium must find a freshwater snail (**intermediate** host)

Without snail → life cycle stops.

Step 3 – **Development in Snail**

Inside the snail:
- Miracidium multiplies **asexually**
- Produces thousands of cercariae

Snail = intermediate host & **site of multiplication**.

Step 4 – **Cercariae in Water** (*Infective Stage*)
- Fork-tailed cercariae are released into water
- This is the **infective stage for humans**

Step 5 – **Human Infection**
- Cercariae penetrate intact human skin during:
  - Swimming
  - Bathing
  - Working in freshwater

Unlike other trematodes, there is no need for ingestion → infection is through **SKIN**.

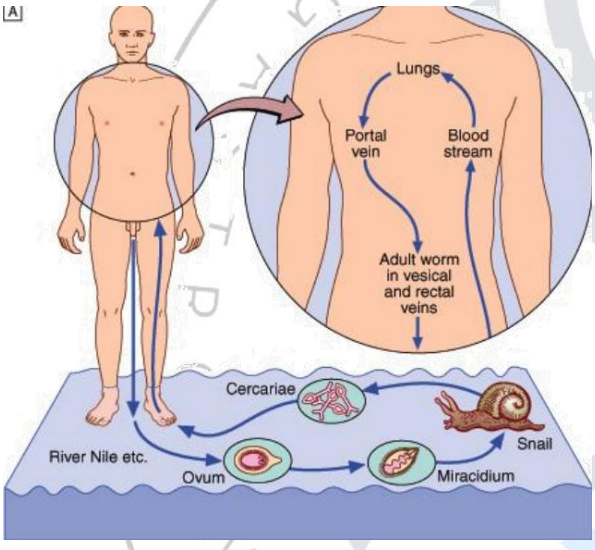
Step 6 – **Migration in Human**

After penetration

1) Cercaria → loses tail → becomes schistosomula
2) Travels via bloodstream to:
   - **lungs**
   - **liver**
3) Finally reaches the **portal venous system**

Step 7 – **Adult Worm Stage**

1) Male & female worms pair
2) Settle in veins:
   - mesenteric veins → *mansoni/japonicum*
   - vesical plexus → *haematobium*
3) Females lay eggs → cycle repeats.



➔ **Morphology**

Schistosoma are the only trematodes with separate sexes → male and female worms are distinct.

1) Suckers

Both male & female have:

- **Oral sucker** → around the mouth (anterior)
- **Ventral sucker** (acetabulum) → on ventral surface

These suckers **help the worm attach to the wall of blood vessels** where it lives.

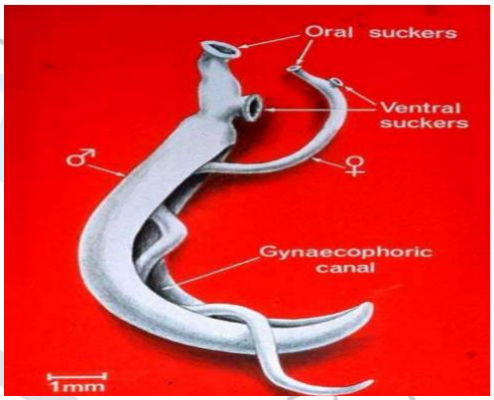
2) Male Worm

- Shape: **Flat**, leaf-like body
- **Edges are folded** to form a groove called the Gynecophoric Canal, which is:
  - A longitudinal channel
  - The **male holds the slender female inside** it for almost the entire length
- Organs: Testes present

This **permanent pairing** is characteristic of Schistosoma.

3) Female Worm

- **Slender**, **cylindrical**
- Lives inside the male's gynecophoric canal
- Contains:
  - Ovary
  - Uterus → lays eggs continuously



➔ **Pathogenesis & Manifestations**

1) **Early Stage: Skin Phase**

Cercarial **Dermatitis** ("Swimmer's Itch")

- Occurs at the time of skin penetration
- Itchy rash
- Local inflammation

2) **Migration Phase**

- Parasites travel via the lungs → liver
- Can cause:
  - **Cough**
  - **Respiratory symptoms**
  - **Fever & eosinophilia**

Part of acute schistosomiasis (Katayama fever).

3) **Chronic Phase: MAIN DAMAGE**

Intestinal / Hepatic (*mansoni* & *japonicum*)

Cause of Pathology = **EGGS** (not adult worms)

- Eggs get trapped in tissues
- Cause:
  - Granulomatous inflammation
  - Fibrosis
  - Sclerosis

Consequences in the Portal System

- Portal hypertension
- Hepatosplenomegaly (HSM)
- Esophageal varices
- Liver failure (periportal fibrosis)

The most common cause of mortality in abdominal schistosomiasis:
→ **Esophageal varices**

Esophageal vaices are dilated veins in the distal esophagus. They veins are formed by anastamoses between the systemic and portal circulation. An increase in portal pressure leads to dilation of these veins which makes them at the risk of rupture, causing fatal bleeding.

➔ **Diagnosis**

1) **Clinical Suspicion**
- History of freshwater exposure
- Symptoms + eosinophilia

2) **Laboratory Confirmation**
Definitive Diagnosis (Gold Standard)
Detection of characteristic **eggs**:(ova in stool, urine, or tissue biopsy)

Species	Where Found	Spine Type
S. mansoni	Stool	**Lateral** spine
S. haematobium	Urine	**Terminal** spine
S. japonicum	Stool	Small **rudimentary** spine

- Eggs are **non-operculated** (unlike other trematodes).
- Tissue biopsy if needed
- Supportive Tests
  - Hematological
  - Biochemical
  - Serology in early disease

➔ **Treatment**
Drug of Choice
- **Praziquantel** 40 mg/kg orally
- Single dose effective for all species

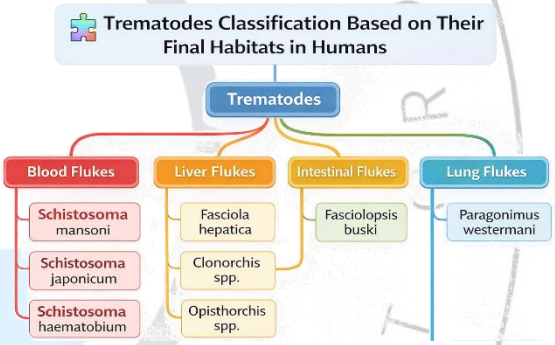
➔ **Important Comparison With Intestinal & Liver Flukes**
Unlike schistosoma:

Intestinal Flukes
Examples:
- *Fasciolopsis buski*
- *Heterophyes heterophyes*
- *Metagonimus yokogawai*

Infection
→ Ingestion of metacercaria (on water plants or fish)

Liver Flukes
Examples
- *Fasciola hepatica*
- *Clonorchis sinensis*
- *Opisthorchis viverrini*
- *Dicrocoelium dendriticum*

Infection
→ Ingestion of metacercaria



Questions:

Q1: A white worm that migrates to the perianal area at night, causing intense itching, is:
A) Giardia lamblia
B) Ascaris lumbricoides
C) Enterobius vermicularis
D) Trichuris trichiura
E) Fasciola hepatica

Q2: Ascaris lumbricoides infective stage is:
A) Unembryonated egg
B) Larva
C) Embryonated egg
D) Adult worm
E) Trophozoite

Q3: Loeffler's syndrome is associated with which parasite?

A) Enterobius vermicularis
B) Trichuris trichiura
C) Ascaris lumbricoides
D) Echinococcus granulosus
E) Taenia solium

Q4: Which helminth is diagnosed using the cellophane tape test?

A) Ascaris lumbricoides
B) Echinococcus granulosus
C) Trichinella spiralis
D) Enterobius vermicularis
E) Schistosoma mansoni

Q5: The most common symptom of Enterobius vermicularis infection is:

A) Diarrhea
B) Perianal itching
C) Rectal bleeding
D) Abdominal distention
E) Cough

Q6: Which of the following is true about the female Enterobius vermicularis?

A) Migrates to the liver
B) Lays eggs around the perianal area
C) Produces 200,000 eggs/day
D) Lives in lungs
E) Causes jaundice

Q7: The drug of choice for hydatid cyst is:

A) Albendazole
B) Nitazoxanide
C) Ivermectin
D) Praziquantel
E) Mebendazole

Q8: The definitive host for Echinococcus granulosus is:

A) Human
B) Pig
C) Sheep
D) Dog
E) Cow

Q9: In hydatid disease, humans are:

A) Intermediate hosts
B) Definitive hosts
C) Part of a natural cycle
D) Reservoir hosts
E) Only transmitters

Q10: Schistosomiasis diagnosis is confirmed by:

A) Blood smear
B) Chest X-ray
C) Detection of ova in stool or tissue
D) Serology only
E) Stool antigen detection

Q11: Portal hypertension and esophageal varices in schistosomiasis are due to:

A) Liver necrosis
B) Egg granulomas in portal circulation
C) Immune hypersensitivity
D) Snail toxin
E) Adult worm damage

Q12: Which of the following schistosome species primarily affects the GI tract?
A) Schistosoma japonicum
B) Schistosoma mansoni
C) All of the above
D) B AND A
E) Schistosoma haematobium

Q13: Albendazole is effective against all the following EXCEPT:

A) Hookworm
B) Ascaris
C) Hydatid cyst
D) Schistosoma
E) Enterobius

Q14: Eosinophilia and larvae in sputum are characteristic of:

A) Fasciola hepatica
B) Trichuris trichiura
C) Ascaris lumbricoides
D) Taenia solium
E) Enterobius vermicularis

Q15: What is the habitat of Ascaris lumbricoides in humans?

A) Lungs
B) Colon
C) Stomach
D) Liver
E) Small intestine

Q16: Diagnosis of Echinococcus granulosus is best confirmed by:

A) X-ray and serology
B) Stool microscopy
C) Liver biopsy
D) Blood eosinophil count
E) Sputum smear

Q17: Which one of the following is incorrect about Ascaris lumbricoides?

A) A humid environment is needed for infectivity
B) Reinfection is possible
C) Eggs become infective 2 to 6 weeks after deposition
D) Larvae may be found in sputum or gastric aspirates
E) An unembryonated egg is the infective stage

Q18: Wrong about Schistosoma:
A) Pathology is mostly due to eggs
B) Associated with urinary diseases
C) The female is shorter and wider than the male
D) Transmission via freshwater snails
E) Involves the portal venous system

Q19: Which of the following parasites causes obstruction of the intestine and biliary tract?

A) Trichuris trichiura
B) Ascaris lumbricoides
C) Hookworm
D) Schistosoma mansoni
E) Giardia lamblia

Q20: Eggs of which parasite are most likely detected in stool by microscopy and are linked to portal fibrosis?

A) Echinococcus granulosus
B) Taenia saginata
C) Schistosoma mansoni
D) Enterobius vermicularis
E) Strongyloides stercoralis

Q21: Which parasite causes biliary obstruction and jaundice in severe cases?
A) Echinococcus granulosus
B) Fasciola hepatica
C) Schistosoma haematobium
D) Ascaris lumbricoides
E) Giardia lamblia

Q22: Snail is the intermediate host of which parasite?

A) Schistosoma mansoni
B) Enterobius vermicularis
C) Ascaris lumbricoides
D) Taenia saginata
E) Echinococcus granulosus

Answers:
Q1: C
Q2: C
Q3: C
Q4: D
Q5: B
Q6: B
Q7: A
Q8: D
Q9: A
Q10: C
Q11: B
Q12: D
Q13: D
Q14: C
Q15: E
Q16: A
Q17: E
Q18: C
Q19: B
Q20: C
Q21: D
Q22: A


UJ - GIG
GASTROENTEROLOGY INTEREST GROUP

The University of Jordan
Gastroenterology Interest Group (UJ-GIG)
Booklet

Microbiology

Hepatitis Viruses

Written by: Ammar Alirani

Edited by: Lujain Badarneh

Reviewed by: Amr Abdallah

[Hepatitis]

- **Hepatitis** = inflammation of the liver.
- Characterized by **the presence of inflammatory cells in liver** tissue.

➔ Causes of Hepatitis

Hepatitis can result from many factors:

1) Infections
   - Viruses (most common)
   - Bacteria
   - Protozoa
2) Toxins & Drug Causes
   - Isoniazid
   - Carbon tetrachloride
   - Ethanol (alcohol)
   - Many medications

➔ Acute vs Chronic

Acute Hepatitis
- Symptoms last **< 6 months**

Chronic Hepatitis
- Inflammation lasting **> 6 months**
- Risk of:
  - **Cirrhosis**
  - **Liver failure**
  - **Hepatocellular carcinoma**

Viral Hepatitis: Key Concept
- Is the inflammation of the liver induced by viral infections, by specific hepatotropic viruses.
- Clinical picture of acute hepatitis is often similar regardless of the virus.
- Exact cause is identified by laboratory tests (serology, PCR).

Types of Viral Hepatitis

Type	Virus Family	Genome	Envelope
A	Picornavirus	+ssRNA	Non-enveloped
B	Hepadnavirus	dsDNA (partial)	☑ Enveloped
C	Flavivirus	+ssRNA	☑ Enveloped
D	Deltavirus	–ssRNA (defective)	Needs HBV
E	Hepevirus	+ssRNA	Non-enveloped

[Hepatitis A virus (HAV)]

➔ Virology

- A typical Enterovirus, and a distinct member of the Picornaviridae
- Previously called Enterovirus 72
- Structure:
  - **Naked** (non-enveloped)
  - **Icosahedral** capsid
  - **+ss**RNA genome
- No virion polymerase
- Only **one serotype**

Being non-enveloped makes it **resistant** to:
- Detergents
- Acid
- Bile → survives in the GI tract

➔ Mode of Transmission

- **Fecal–ora**l route (enterically transmitted)
- Sources:
  – Contaminated water
  – Food handlers
  – Poor sanitation
  – Outbreaks in communities

➔ Pathogenesis

1) Ingestion of the virus
2) Replicates in:
   - **oropharynx**
   - **intestinal epithelial cells**
3) Enters bloodstream → viremia
4) Reaches liver → infects hepatocytes

- **Excretion**: Newly replicated virus is **excreted into the bile** and then released into the feces.
- **Shedding** Window: Infected persons typically excrete the virus in their feces for approximately **two weeks before the onset** of symptoms and for up to one week after symptoms appear.
- **Transmission**: Asymptomatic individuals, particularly children, can still transmit the virus through the fecal-oral route.

➔ Liver Damage Mechanism

- Virus itself is not directly cytopathic.
- Liver injury caused by:
  Cell-mediated immunity (CMI)
  → T-cell attack on infected hepatocytes

- Histology:
  - **Periportal** necrosis
  - **Mononuclear** cell infiltrates

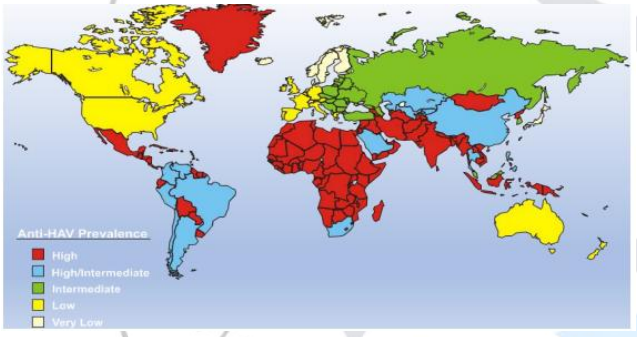
➔ **Epidemiology**

- Globally, HAV is the most common cause of **acute** viral hepatitis.
- Worldwide distribution
- High prevalence in:
  - **Developing** countries
  - Areas with poor sanitation

Pattern

- Often childhood infection
- Outbreaks related to:
  - contaminated food
  - water supplies
  - daycare centers

- **Traveler Susceptibility**: Because residents of developed countries often lack natural immunity or vaccination, travelers are particularly susceptible to infection when visiting endemic areas.



➔ **Clinical Manifestations**

- Incubation Period: **2–6 weeks**
- Disease Presentation: HAV causes **infectious hepatitis**, which is typically an acute, self-limiting disease. It is often clinically milder or asymptomatic in young children, whereas adults are more likely to experience symptomatic illness.

- Many infections are**:**

1- **Asymptomatic**, especially in children

2- Symptomatic Disease:
  - General Symptoms: **Fever, Anorexia, Nausea & vomiting, and Fatigue**
  - Hepatic Symptoms: **Jaundice, Abdominal pain, Hepatomegaly ± splenomegaly, Dark urine, and Clay-colored stools**.

➔ **Course of Disease**

- Usually self-limited
- Recovery within 2–4 weeks. “Resolve spontaneously.”
- **No Chronicity**: There is no carrier state or chronic infection associated with HAV; once the acute phase passes, the virus is cleared from the body.
- Fulminant hepatitis = rare

➔ **Diagnosis of Hepatitis A**

1) **Clinical**
2) **Lab Findings:** Liver Enzymes
- High AST & ALT
- Mild bilirubin elevation

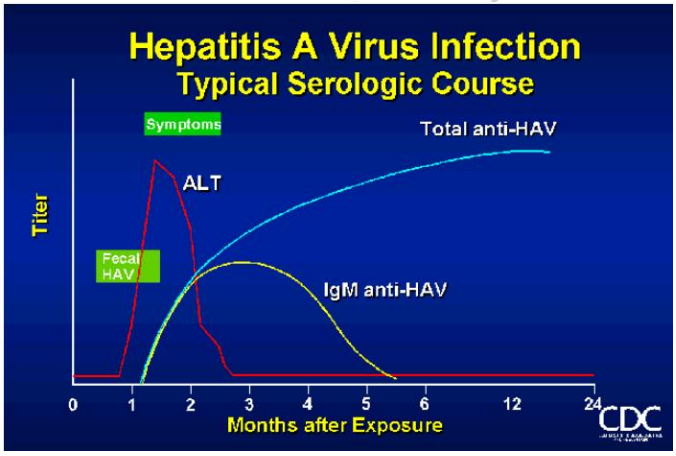
3) **Serology**: **GOLD STANDARD**

1- **IgM** Anti-HAV
- Indicates acute infection
- Remains positive for 3–6 months

2- **IgG** Anti-HAV
- Indicates:
  - **Past infection**
  - **Vaccination**
- Gives lifelong immunity

➔ **Serologic Course**

1. Fecal HAV appears first → patient is infectious.
2. Then:
   - **Rise in ALT**
   - Onset of symptoms
3. IgM anti-HAV appears during the acute phase
4. Later → Total anti-HAV (IgG) persists long term



➔ **Treatment**

- Usually full recovery in 90% of patients within 3–6 months

Acute management

- Supportive treatment
- Do NOT give:
  - **Paracetamol**
  - **Alcohol**
- Immunoglobulins may be used

Fulminant hepatitis

- Supportive treatment
- May need liver transplantation

➔ Prevention

- Hygiene measures
- **Vaccine**: **killed** vaccine
  - Intramuscular
  - 2 doses separated by 3–6 months

[Hepatitis E virus (HEV)]

➔ Virus Characteristics

- Hepatitis E virus is:
  - **Non-enveloped** → more **resistant** in the environment
  - **Single-stranded** RNA virus
- Viral particles seen in stool:
  - **Spherical** shape
  - Size 27–34 nm
  - Have **spikes** on the surface

These features explain why the virus can survive in contaminated water and spread in communities.

➔ Mode of Transmission

- **Fecal–oral** route
- Typically, through contaminated water supplies
- Responsible for waterborne epidemics of hepatitis

Clinical Importance: Infection is acute only.

- **No chronic** infection
- No carrier state

High-Mortality Group: **Pregnant women**

[Hepatitis B virus (HBV)]

➔General Characteristics

- Hepatitis B is caused by Hepatitis B virus, a member of the Hepadnaviridae family.
- It is one of the **smallest DNA** viruses infecting humans, with a genome of approximately 3.2 kb (3200 nucleotides).
- The viral genome is **partially double-stranded circular DNA** (relaxed circular DNA).
- The virus is **enveloped**.
- The nucleocapsid has an **icosahedral** symmetry.

➔ Genome Structure

- The genome consists of:
  - One **complete negative DNA** strand
  - One **incomplete positive DNA** strand (partially single-stranded region)
- The virus replicates through **reverse transcription**, which is unusual for DNA viruses.

➔ Viral Components / Antigens

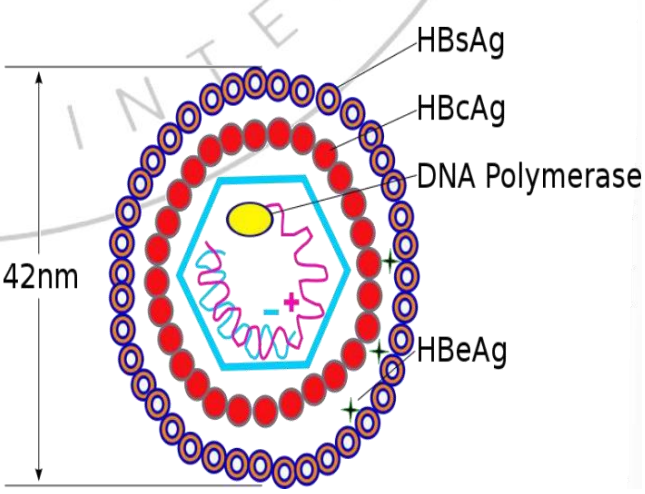
HBV has three major antigen systems:

- **Core antigen** (HBcAg)
- **Precorehepatitis B e antigen** (HBeAg)
- **Surface antigen** (HBsAg)

➔Structural Organization of HBV

The virus consists of:

1. Envelope
   - Contains HBsAg
2. Nucleocapsid (core particle)
   - Contains HBcAg
   - Viral DNA
   - Viral polymerase (reverse transcriptase)
3. Secreted antigen
   - HBeAg



➔ Transmission

- **Parenteral via blood or plasma**: Needle stick injury
- **Vertically**: mother → baby
- Through **body fluids**

Risk Groups

- Health care workers
- Drug abusers
- Recipients of blood or blood products
  - Blood should ideally be screened
- Dialysis patients
- Homosexual men

➔ Pathogenesis

- Virus is **blood borne** → reaches liver cells
- Causes:
  - Hepatocyte injury and necrosis
  - Described as **piecemeal** necrosis
- Damage is largely **cell-mediated** (immune response)

➔ Clinical Features

Incubation

- 1–4 months after infectious dose

Asymptomatic Infection

- 90% of children → asymptomatic
- 50% of adults → asymptomatic
- May show only increased liver enzymes

Symptomatic Infection:

1) **Pre-icteric phase**
- **Flu-like symptoms**
- **Nausea**
- **Anorexia**
- **Malaise**

2) **Icteric phase** (icteric means the presence of jaundice due to elevated bilirubin)
- **Jaundice**
- **Pale stool**
- **Dark-coloured urine**
- **Increased liver enzymes**
- **Increased bilirubin**

➔ Outcome of HBV Infection

A) **Most patients recover**: 90–95% → complete recovery (The immune system clears the virus.)

B) **Some become chronic carriers**
- 5–10% → chronic carriers
- Defined as: HBsAg (surface antigen) present for more than 6 months

This means the virus stays in the body long term.

C) **Serious complications**

1) **Chronic active hepatitis**
   - More severe
   - More fatal than simple acute infection
2) **Mortality**
   - About 1% fatality from acute infection
3) **Cancer risk**
   - 20% of chronic carriers develop hepatocellular carcinoma (HCC)

➔ Diagnosis of HBV

1) **Clinical Picture**: Symptoms and signs:
- Jaundice
- Dark urine
- Pale stool
- Flu-like symptoms

This only suggests hepatitis but does not prove HBV.

2) **Laboratory Tests**
- **Liver function tests** → show liver damage
- **Kidney function tests**
- **Tests to exclude other causes**, such as:
  - CMV
  - EBV

3) **Specific HBV Tests** (Most Important)

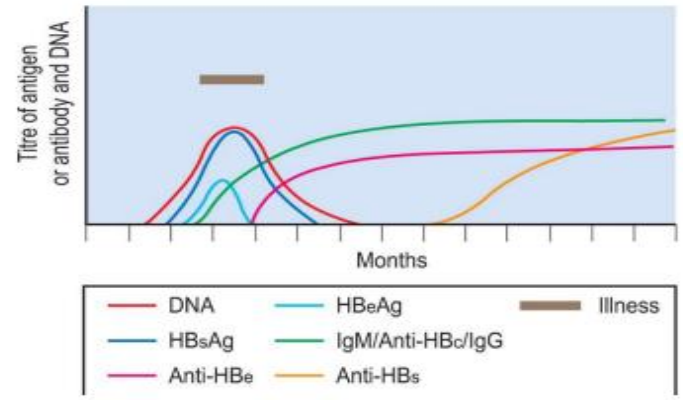
1) **S and e antigens and antibodies**
   - Surface antigen (HBsAg)
   - e antigen (HBeAg) and their antibodies
2) **Anti-core antibodies**
3) **DNA detection**
   - Detect the actual virus genetic material

These are the tests that CONFIRM it is Hepatitis B

HBsAg	Antigen found on surface of HBV; indicates hepatitis B infection.
Anti-HBs	Antibody to HBsAg; indicates immunity to hepatitis B due to vaccination or recovery from infection.
HBcAg	Antigen associated with core of HBV.
Anti-HBc	Antibody to HBcAg; IgM = acute/recent infection; IgG = prior exposure or chronic infection. IgM anti-HBc may be the sole ⊕ marker of infection during window period.
HBeAg	Secreted by infected hepatocyte into circulation. Not part of mature HBV virion. Indicates active viral replication and therefore high transmissibility and poorer prognosis.
Anti-HBe	Antibody to HBeAg; indicates low transmissibility.

TABLE 41–4 Serologic Test Results in Four Stages of HBV Infection

Test	Acute Disease	Window Phase	Complete Recovery	Chronic Carrier State
HBsAg	Positive	Negative	Negative	Positive
HBsAb	Negative	Negative	Positive	Negative[1]
HBcAb	Positive[1]	Positive	Positive	Positive



	HBsAg	Anti-HBs	Anti-HBc	HBeAg	Anti-HBe
Incubation	+				
Acute infection	+		+ (IgM)	+	
Window			+ (IgM)		+
Recovery		+	+ (IgM)		+
Chronic infection (high infectivity)	+		+ (IgG)	+	
Chronic infection (low infectivity)	+		+ (IgG)		+
Immunized		+			

1) **Acute Hepatitis B Infection**
- Virus present → **HBsAg (+)**
- Early immune response → **IgM anti-HBc (+)**
- No protective immunity yet → **Anti-HBs (–)**

2) **Window phase**
Only marker = **HBcAb**
This happens when:
- Surface antigen disappeared (HBsAg)
- Surface antibody not formed yet (Anti-HBs)

Note: Only HBcAb positive = window period.

3) **Complete recovery**
- Virus cleared → **HBsAg (–)**
- Protective immunity → **Anti-HBs (+)**

4) **Chronic Carrier state**
Virus persists → patient is a carrier.
- Occurs when infection persists > 6 months.
- Serology:
  - **HBsAg (+)**
  - **Anti-HBs (–)**
  - **IgG anti-HBc (+)**
- Additional markers determine activity:
  - HBeAg (+) → **high replication**
  - Anti-HBe (+) → **lower replication**

➔ **Treatment of HBV**

Main medications
1. **Pegylated Interferon alpha**
2. **Oral antivirals**:
   - Lamivudine
   - Tenofovir
   - Entecavir

These are used to **control viral replication in chronic** HBV.

➔ **Prevention**

A) **Passive Immunization** (Immunoglobulin)
Used in:
- **Accidental exposure in non-vaccinated** persons
- **Newborns** of infected mothers

Gives immediate **short-term** protection.

B) **Active Immunization** – Vaccine
- Recombinant HBsAg vaccine
- **3 intramuscular doses at:**
  - **0 month**
  - **1 month**
  - **2 OR 6 months**

Important practical points
- Fridge storage required
- Check response:
  - Measure anti-HBs antibodies 2 months after the last dose
  - Level >10 mIU/mL = protective
- Part of the Ministry of Health program at:
  - 2, 3, 4 months of age

[Hepatitis D virus (HDV)]

HDV **needs HBV** to replicate
- HBV **provides the envelope** for HDV

Therefore, HDV can occur only in patients who have HBV.

➔ Transmission

- Same routes as HBV**: parenteral, blood, body fluids, vertical**

➔ Types of Infection

A) **Co-infection**
- Patients get HBV and HDV at the same time

B) **Superinfection**
- Patient already has **chronic HBV**
- Then becomes infected with HDV

This is **more dangerous**: High risk of liver failure.

➔ Diagnosis

By **serology** ➞ blood tests to detect HDV markers.

➔ Treatment

Same as HBV treatment

[Hepatitis C virus (HCV)]

➔ Virology

- Member of the Flavivirus family
- **Enveloped** virus
- **Single-stranded** RNA, **positive** sense
- **No polymerase** in the virion

Genotypes
- HCV has 6 genotypes
- Genotyping is needed for:
  - Treatment decisions (Rx)
  - Medicolegal purposes

➔ Transmission
- Spread via:
  - **Infected blood**
  - **Sexual contact**

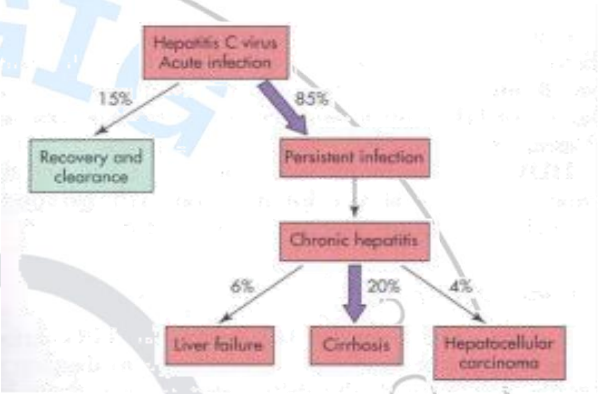
➔ Incubation & Clinical Course

- Incubation period: 6–8 weeks
- Most infections are **sub-clinical** (mild or no symptoms)

Severity
- Clinical disease is generally less severe than HBV
- Liver damage occurs due to a cell-mediated immune response

Chronicity:
- HCV has a much higher rate of chronic disease than HBV
- 70–80% remain viremic for more than 1 year

Means: the majority become chronic carriers.
Epidemiology: About 170 million cases worldwide



➔ Diagnosis of HCV

- **Anti-HCV IgM: Detecting antibodies** to HCV
- **RNA detection: Detecting the actual viral RNA** (Viral load).

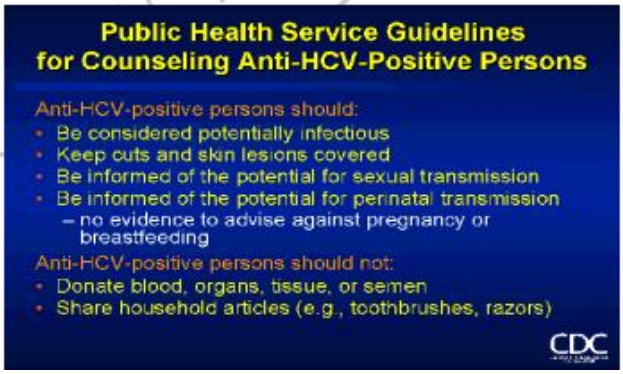
➔ Treatment

- **Antivirals**

➔ HCV prevention

Hepatitis C virus / prevention

- No vaccine
- Blood screening



Postexposure Prophylaxis

1) HBV

HBV PEP for occupational exposure [5]			
HCP vaccination status		Source patient	
		HBsAg negative	HBsAg positive or unknown
Complete series	Anti-HBs ≥ 10 mIU/mL	• No intervention required	
	Anti-HBs < 10 mIU/mL	• Administer 1 dose of HBV vaccine.	• Administer 1 dose of HBV vaccine, PLUS 1 dose of HBIG, simultaneously at two different anatomical sites. • Complete vaccine series
Unvaccinated or incomplete series		• Administer 1 dose of HBV vaccine. • Complete vaccine series.	

HBV PEP following nonoccupational exposure [5][45]			
Patient vaccination status		Source individual	
		HBsAg unknown	HBsAg positive
Complete series	Anti-HBs ≥ 10 mIU/mL	• No intervention required	
	Anti-HBs < 10 mIU/mL or unknown	• No intervention required	• Administer 1 dose of HBV vaccine.
Unvaccinated or incomplete series		• Administer 1 dose of HBV vaccine. • Complete vaccine series.	• Administer 1 dose of HBV vaccine PLUS 1 dose of HBIG, simultaneously at two different anatomical sites. • Complete vaccine series.

2) HCV

- There is currently no effective preexposure or postexposure prophylaxis and no vaccine against HCV infection.
- Use standard precautions for bloodborne pathogens in healthcare settings.
- Educate individuals with HCV infection about preventing transmission.

3) HIV postexposure prophylaxis (PEP):

A short course of ART taken by patients after a potential exposure to HIV

- **Timing:** Initiate **as soon as possible** (ideally within 1–2 hours of exposure).

- **Indications for HIV PEP:**
  1) Injury with HIV-contaminated instruments or needles
  2) Contamination of open wounds or mucous membranes with HIV-contaminated fluids
  3) Unprotected sexual activity with a known or potentially HIV-infected person

- **Regimens**: a **three-drug regimen** is recommended (similar to ART). Typically, this includes a **nucleoside/nucleotide combination NRT**I plus an **integrase** inhibitor, e.g., Tenofovir disoproxil fumarate PLUS **emtricitabine** (may be given as a single combined tablet of Truvada®) PLUS one of the following: Dolutegravir, Raltegravir.

Hepatitis viruses

Signs and symptoms of all hepatitis viruses: episodes of fever, jaundice, ↑ ALT and AST. Naked viruses (HAV and HEV) lack an envelope and are not destroyed by the gut: the vowels hit your bowels.

HBV DNA polymerase has DNA- and RNA-dependent activities. Upon entry into nucleus, the polymerase completes the partial dsDNA. Host RNA polymerase transcribes mRNA from viral DNA to make viral proteins. The DNA polymerase then reverse transcribes viral RNA to DNA, which is the genome of the progeny virus.

HCV lacks 3′-5′ exonuclease activity → no proofreading ability → antigenic variation of HCV envelope proteins. Host antibody production lags behind production of new mutant strains of HCV.

Virus	HAV	HBV	HCV	HDV	HEV
FAMILY	RNA picornavirus	DNA hepadnavirus	RNA flavivirus	RNA deltavirus	RNA hepevirus
TRANSMISSION	Fecal-oral (shellfish, travelers, day care)	Parenteral (Blood), sexual (Bedroom), perinatal (Birthing)	Primarily blood (injection drug use, posttransfusion)	Parenteral, sexual, perinatal	Fecal-oral, especially waterborne
INCUBATION	Short (weeks)	Long (months)	Long	Superinfection (HDV after HBV) = short Coinfection (HDV with HBV) = long	Short
CLINICAL COURSE	Acute and self limiting (adults), Asymptomatic (children)	Initially like serum sickness (fever, arthralgias, rash); may progress to carcinoma	May progress to Cirrhosis or Carcinoma	Similar to HBV	Fulminant hepatitis in Expectant (pregnant) patients
PROGNOSIS	Good	Adults → mostly full resolution; neonates → worse prognosis	Majority develop stable, Chronic hepatitis C	Superinfection → worse prognosis	High mortality in pregnant patients
HCC RISK	No	Yes	Yes	Yes	No
LIVER BIOPSY	Hepatocyte swelling, monocyte infiltration, Councilman bodies	Granular eosinophilic "ground glass" appearance due to accumulation of surface antigen within infected hepatocytes; cytotoxic T cells mediate damage	Lymphoid aggregates with focal areas of macrovesicular steatosis	Similar to HBV	Patchy necrosis
NOTES	Absent (no) carrier state	Carrier state common	Carrier state very common	Defective virus, Depends on HBV HBsAg coat for entry into hepatocytes	Enteric, Epidemic (eg, in parts of Asia, Africa, Middle East), no carrier state

Questions:

Q1: Which of the following is transmitted by the fecal-oral route:

A. Hepatitis A virus
B. Hepatitis B virus
C. Hepatitis C virus
D. Hepatitis D virus
E. All of the above.

Q2: Which of the following pairs of hepatitis viruses have a vaccine:

A. HAV, HBV, HCV
B. HAV, HBV
C. HAV, HBV, HEV
D. HAV, HBV, HDV
E. HAV, HEV

Q3: All are true regarding hepatitis B virus (HBV) infection EXCEPT:

A. It is a blood-borne pathogen
B. HBV vaccine AND HBV immunoglobulin should be given to infants born to mothers carriers of HBV infection
C. HBV vaccine is given to all health care workers
D. It causes only the chronic form of hepatitis
E. Can be transmitted through a vertical way from mother to infant.

Q4: Which of the following is most associated with hepatocellular carcinoma?

A. Chronic Hepatitis C
B. Acute Hepatitis C
C. Acute Hepatitis A
D. Chronic Hepatitis B +D
E. Chronic Hepatitis B

Q5: Which of the following Indicates carrier status of HBV:

A. HBcAg and IgG anti-HBc
B. HBcAg and IgM anti-HBc
C. HBeAg after 3 months
D. HBeAg and absence of HBV DNA
E. HBsAg and HBs antibody

Q6: Which serologic marker, if found, indicates resolution of acute HBV infection:

A. HBsAg
B. Anti-HBc IgM
C. HB DNA
D. Anti HBeAg
E. Anti HbC IgD

Q7: Which of the following serologic markers indicate HBV clearance:

A. HbCAg IgG antibody
B. HbSAg Antibody
C. HbCAg
D. HbCAg IgM antibody
E. HbSAg

Q8) Which serologic marker is the most sensitive indicator of HCV:

A. HCV RNA by PCR
B. Anti-HCV
C. HCVAg
D. Anti-HCVsAg
E. HVC DNA by TMA (transcription-mediated amplification).

Q9: Which of the following is deemed a hepatitis B carrier: (Ag=antigen, AB=antibody):

A. HbSAg (+) HbSAb (+) HbEAB(-) HbCIgM AB (+) HbCIgG AB(+)
B. HbAg (-) HbSAb (-) HbEAB(+) HbCIgM AB (+) HbCIgG AB(+)
C. HbSAg (+) HbSAb (+) HbEAB(-) HbCIgM AB (-) HbCIgG AB(+)
D. HbAg (+) HbSAb (-) HbEAB(+) HbCIgM AB (+) HbCIgG AB(+)
E. HbSAg (+) HbSAb (-) HbEAB(+) HbCIgM AB (-) HbCIgG AB(+).

Q10: All are true regarding hepatitis A virus (HAV) infection, EXCEPT:

A. It is transmitted via feco oral route
B. The HAV vaccine can be given to a selected group of patients
C. Can cause acute liver failure
D. Usually causes chronic infection
E. Diagnosis is done by a positive HAV IgM SEROLOGY

Q11: A 3-year-old female Gravida 3, Para 2 at 32 weeks of gestation, presents to the emergency department with complaints of severe abdominal pain, fatigue, and nausea, Physical examination is significant for profound jaundice and tenderness to palpation of the right upper quadrant of the abdomen. The patient returned 2 weeks ago from a 1 month-long trip to India. She received sporadic pre-natal care while traveling and reports no known complications in her current pregnancy to date. She denies any past medical problems and states that her prior pregnancy proceeded as a normal vaginal birth without any complications. Infection with which of the following organisms would predispose the worst prognosis with the highest mortality rate for this patient:

A. Hepatitis C
B. Hepatitis A
C. Hepatitis B
D. Hepatitis D
E. Hepatitis E

Q: Which of the following is considered a treatment for HCV?
Answer: Direct-acting antivirals (DAA).

Q: Wrong about HAV:
Answer: It causes chronic infection.

Q: Contaminated water:
Answer: Hepatitis A virus.

Q: Chronic Hepatitis B virus:
Answer: More than 6 months

Answers:

Q1: A
Q2: B
Q3: D
Q4: D
Q5: A
Q6: D
Q7: B
Q8: A
Q9: E
Q10: D
Q11: E

The University of Jordan
Gastroenterology Interest Group (UJ-GIG)
Booklet

Microbiology

Microbiology Lab

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[Stool Collection & Culture]

1- Stool Collection

- Collect stool in a clean, wide-mouth container.
- Non-sterile container.
- Purpose: easy collection and sufficient sample volume.

2- Adding Stool to Selenite Broth

- Stool is inoculated into Selenite broth (enrichment medium).
- Why?
    - Inhibits growth of coliforms (normal gut flora).
    - Enhances the growth of pathogens, especially *Salmonella* and *Shigella*.
- Helps isolate pathogens when present in small numbers.

3- Common Bacterial Pathogens in Stool Culture

- Most common:
    - *E. coli*
    - *Salmonella*
    - *Shigella*
    - *Proteus*
- Others:
    - *Yersinia*
    - *Campylobacter*
    - *Clostridium*
    - *Bacillus*

➔ Stool Culture Media

Culture Method

- Stool sample is cultured using the streak plate method.
- Purpose:
    - Isolate single colonies.
    - Differentiate pathogens based on colony appearance.

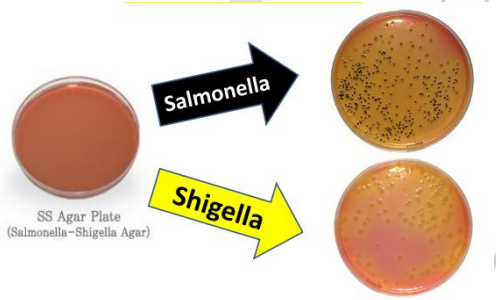
Media Used for Stool Culture

- S-S Agar (Salmonella–Shigella agar)
- Hektoen Enteric Agar
- T.C.B.S. Agar (for *Vibrio* species)

1- S-S Agar (Salmonella–Shigella Agar):

- Selective & differential medium.
- Used to isolate Salmonella and Shigella.

Organism	Appearance on S-S Agar	Reason
Salmonella	Colorless colonies with black centers	H_2S production
Shigella	Colorless colonies	No lactose fermentation, no H_2S

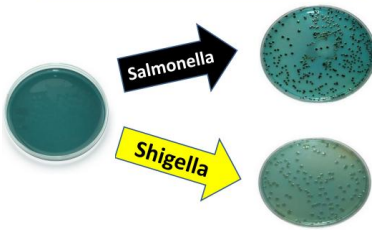


2- Hektoen Enteric Agar:

- Selective & differential medium for enteric pathogens.
- Differentiates lactose fermenters from non-fermenters.

Organism	Appearance on Hektoen Agar	Reason
Salmonella	Green colonies with black centers	H_2S production
Shigella	Green colonies	Non-lactose fermenter

Hekton enteric agar



3- T.C.B.S. (Thiosulfate-Citrate-Bile Salts-Sucrose) Agar:

- Selective medium for Vibrio species.
- High pH inhibits other bacteria.

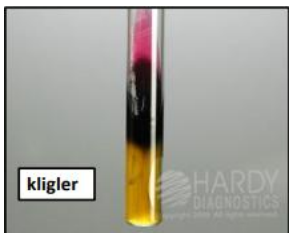
Organism	Appearance
Vibrio cholerae	Yellow colonies (sucrose fermentation)
Other Vibrios	Green colonies

[Salmonella & Proteus: Key Lab Identification]

➔ **Salmonella**

1) Kligler Iron Agar (KIA): Red slant / Yellow + H_2S

Region	Observation	Interpretation
Upper	Pink/red	No lactose fermentation
Lower	Yellow	Glucose fermentation
Between layers	Black precipitate	H_2S production



2) Urease test: Negative

The medium is yellow before inoculation.

After incubation:

Color change	Result
Remains yellow	Urease negative
Turns pink	Urease positive

Result for Salmonella:
Negative urease test (remains yellow).

3) Citrate test**:** Positive

The medium is green before inoculation.

After incubation:

Color change	Result
Green remains	Negative
Turns blue	Positive

Result for Salmonella:
Citrate positive → medium turns blue

SIM test**:**

The SIM test is a biochemical test used to detect three bacterial characteristics in a single medium:

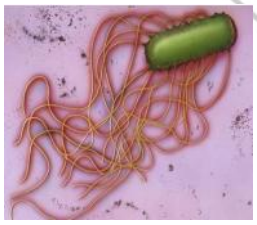
- S (sulfur): Positive (H_2S production)
- I (Indole): Negative
- M (motility): Positive (motile)

Component	What it Detects	Positive Result	Typical Result in Salmonella
S – Sulfur (H_2S production)	Ability to produce hydrogen sulfide from sulfur compounds	Black precipitate forms in the medium	Positive
I – Indole	Ability to break down tryptophan to produce indole	After adding Kovac's reagent → Red ring on top	Negative (yellow/brown ring)
M – Motility	Ability of bacteria to move through the medium	Turbid (cloudy) growth spreading away from stab line	Positive

➔ Proteus

- Gram-negative rods
- Non-lactose fermenter
- Highly motile (flagellated)
- Shows swarming motility on agar plates.
- Prevent swarming by culturing it on CLED or MacConkey media

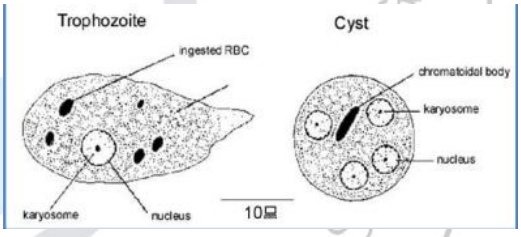
Feature	Description	Lab Significance
Gram stain	Gram-negative rod	Enteric bacteria
Lactose fermentation	Negative	Pale colonies on MacConkey
Motility	Swarming	Concentric rings on agar
Flagella	Present	Causes spreading growth



[Parasitic Infection]

➔ Entamoeba histolytica

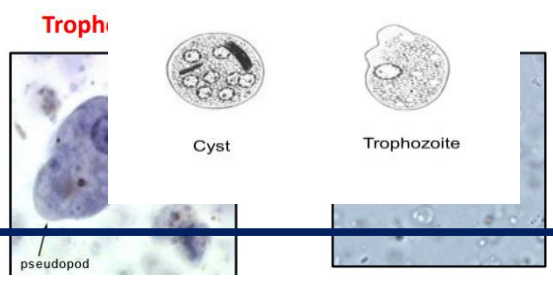
Feature	Trophozoite	Cyst
Size	15–20 µm	12–15 µm
Movement	Progressive motility (pseudopodia)	Non-motile
Nuclei	Single	Up to 4 nuclei (mature cyst)
Chromatoid bodies	Absent	Present (cigar-shaped with blunt ends)
RBC ingestion	Present diagnostic feature	Absent



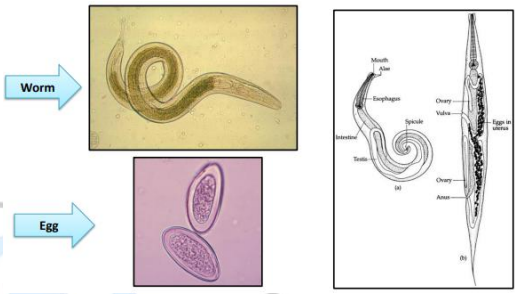
➔ Entamoeba coli (Non-pathogenic)

Feature	Trophozoite	Cyst
Size	20–25 µm	15–25 µm
Movement	Sluggish	Non-motile
Nuclei	Single	Up to 8 nuclei (mature cyst)
Chromatoid bodies	Absent	Present with pointed ends
Pathogenic	✗ Non-pathogenic	✗ Non-pathogenic

Entamoeba coli

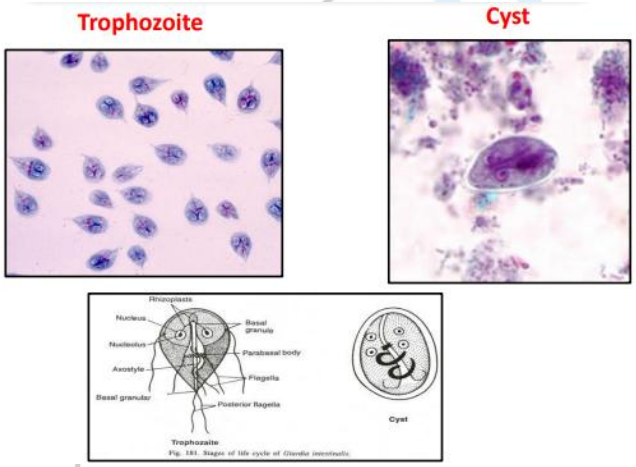


Egg shape	Oval, flattened side D-shaped.
Diagnosis	Scotch tape test
Symptom	Perianal itching



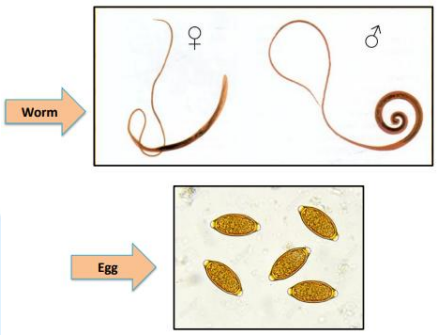
➔ **Giardia lamblia**

Feature	Trophozoite	Cyst
Shape	Pear-shaped	Oval
Nuclei	2	4
Motility	Flagellated	Non-motile
Special feature	Ventral sucking disc	Infective stage



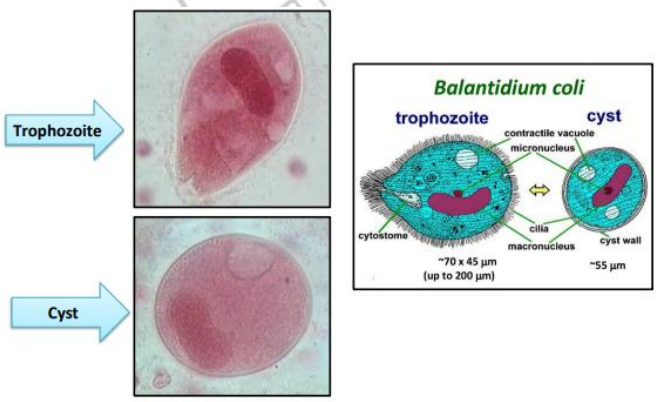
➔ **Trichuris trichiura**

Feature	Description
Worm shape	Whip-like
Egg shape	Barrel-shaped
Special feature	Bipolar plugs
Habitat	Large intestine



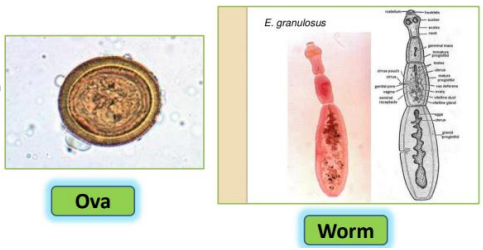
➔ **Balantidium coli**

Feature	Trophozoite	Cyst
Size	Very large	Large
Movement	Cilia	Non-motile
Nucleus	Kidney-shaped macronucleus	Macronucleus visible



➔ **Echinococcus granulosus**

Feature	Description
Type	Cestode (tapeworm)
Disease	Hydatid cyst
Host	Dogs (definitive host)
Human role	Intermediate host

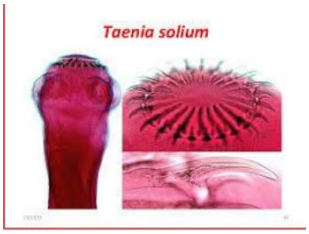


➔ **Enterobius vermicularis (Nematode)**

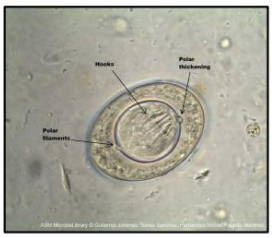
Feature	Description
Worm shape	Thin, thread-like

➔ **Hymenolepis nana (Cestode)**

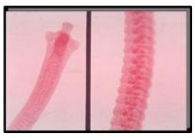
Feature	Description
Size	Smallest tapeworm
Egg feature	Polar filaments
Host	Humans
Unique	No intermediate host



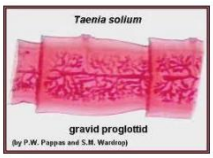
Notice the scolex (the head of the warm)



Ova



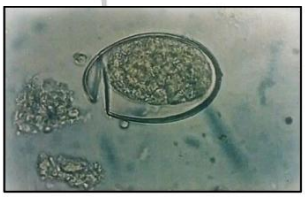
Worm



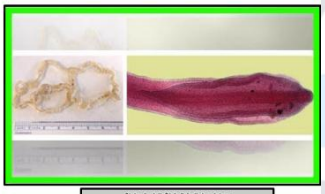
➔ **Taenia saginata (Cestode)**

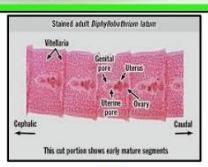
➔ **Diphyllobothrium latum (Cestode)**

Feature	Description
Source	Raw fish
Egg	Operculated + terminal knob
Disease	Vitamin B12 deficiency → megaloblastic anemia
Scolex	Bothria (grooves)



Egg

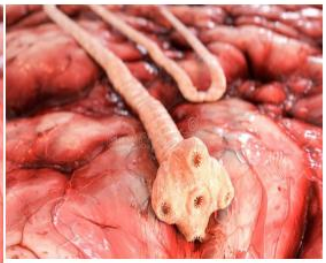


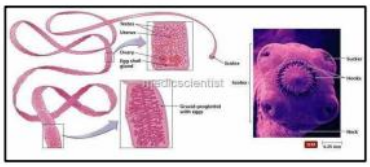


Worm

Taenia Solium

Taenia saginata



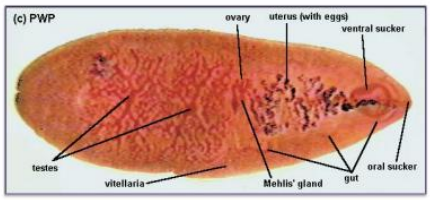


➔ **Taenia solium (Cestode)**

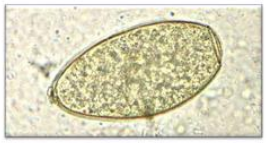
Feature	Description
Source	Pork
Egg	Radially striated shell
Scolex	Hooks + suckers
Major risk	Cysticercosis

Feature	*Taenia solium*	*Taenia saginata*
Intermediate Host	Pork (Pig)	Beef (Cow)
Scolex (Head)	Has 4 suckers and hooks (rostellum)	Has 4 suckers without hooks
Gravid Proglottid	Smaller with fewer uterine branches	Larger with many uterine branches
Length	Shorter	Longer

Worm



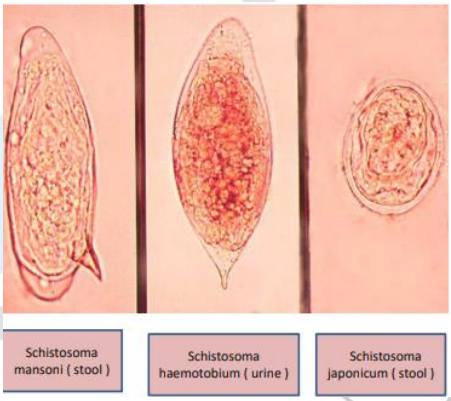
Worm

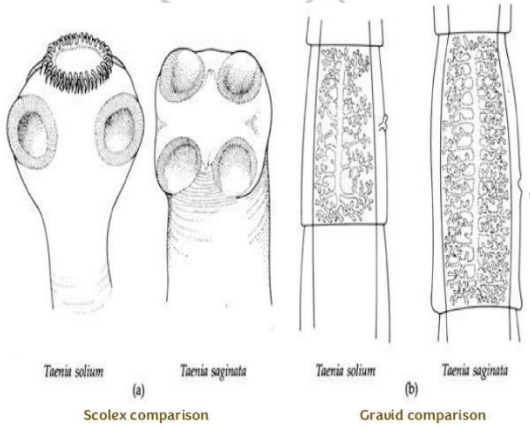


➔ **Fasciolopsis buski (Trematode)**

Feature	Description
Type	Trematode (fluke)
Shape	Leaf-like
Egg	Operculated
Habitat	Small intestine

➔ **Schistosoma (Trematode)**





- S. mansoni:
  Spine: Prominent lateral spine (on the side).
  Sample: Typically found in stool.

- S. haematobium:
  Spine: Distinct terminal spine (at the very tip).
  Sample: Typically found in urine.

- S. japonicum:
  Spine: Very small, inconspicuous lateral knob or spine (often hard to see).
  Sample: Typically found in stool.