قسم التقنيات الاحيائية
مادة الاحياء المجهرية ٢
المرحلة الثانية
الكورس الثاني

أ.م.د. عائدة حسين ابراهيم
م.د. رشا طاب السهلاني

Lecture one

Host defenses and Immunity system

The main function of the immune systems is to prevent or limit infections by microorganism such as bacteria, viruses, fungi and parasite.

The immune system relies on an overlapping network of host defenses that operate on several levels.

1- Intrinsic: Physical and chemical barriers.

- Skin, mucous, tears, low pH, surface cleansing

2- Innate immune system., natural defenses,: First line of immune defense

3- Adaptive immune system: Tailored to pathogen.

The first line of defense includes any barrier that blocks invasion at the portal of entry. This mostly nonspecific line of defense limits access to the internal tissues of the body.

Physical or Anatomical Barriers at the Body's Surface: includes:

1- The skin : The outer layer (stratum corneum) of the skin is composed of epithelial cells compacted and impregnated with an insoluble protein, keratin prevent the penetration of pathogens.

2- The mucocutaneous membranes of the digestive, urinary, and respiratory tracts and of the eye are moist and permeable. The mucous coat on the free surface of some membranes impedes the entry and attachment of bacteria.

3- Blinking and tear production (lacrimation) flush the eye's surface with tears and rid it of irritants.

Immune system

The immune system, which is made up of special cells, proteins, tissues, and organs, defends people against germs and microorganisms every day.

The primary functions of a healthy functioning immune system can be summarized as

1. Surveillance of the body.
2. Recognition of foreign material such as pathogens.
3. Attack and destruction of foreign invaders.

- **The Immune Response**

The response generated against a potential pathogen is called an immune response.

The first line of defense, which is nonspecific to the invading pathogen, is rapidly mobilized at the initial site of infection but lacks immunologic memory and is called innate immunity.

The second defense system is called adaptive immunity. It is specific for the pathogen and confers protective immunity to reinfection with that pathogen. Adaptive immunity can specifically recognize and destroy the pathogen because lymphocytes carry specialized cellular receptors and produce specific **antibodies.** A protein that is produced in response to a particular pathogen is called the antibody, and the substance that induces the production of antibodies is called the **antigen.**

- **Innate immunity**

Innate immunity is an immediate response to a pathogen that does not confer long-lasting protective immunity. It is a nonspecific defense system and includes barriers to infectious agents, such as the skin (epithelium) and mucous membranes.

It also includes many immune components important in the adaptive immune response, including phagocytic cells, natural killer (NK) cells, Toll-like receptors (TLRs), cytokines, and complement.

Few microorganisms can penetrate body surfaces. These surfaces have an epithelial cell layer as their barrier, which is present in the skin, airways, gastrointestinal (GI) tract, and genitourinary tract. The epithelial cell layer has tight junctions and produces a number of powerful antimicrobial peptides that help and provide protection against invading pathogens. Lysozyme is an example of an antimicrobial peptide that dissolves some bacterial cell walls.

Mucus, a complex mixture of mucins, proteins, proteases, and protease inhibitors, is a major component of the mucosal epithelium. Some bacteria attach to the

surface epithelial cells by means of adhesive bacterial surface proteins (eg, the pili of gonococci and *Escherichia coli*). But, the presence of mucus limits bacterial adhesion to these cell surfaces and, once entrapped in the mucus, the bacteria are removed by ciliary clearance. Thus, the mucosal surface and the ciliated epithelial cells tend to inhibit microbial adhesion and limit exposure time. Likewise, the GIT has mechanisms to inhibit bacteria. The acidity of the stomach and the proteolytic enzymes of the small intestine make this environment hostile to many bacteria.

Also, the presence of an acidic pH in sweat and sebaceous secretions and, the low pH of the stomach have antimicrobial properties. Moreover, the production of fatty acids on the skin also tends to eliminate pathogenic organisms.

In addition, to the physiologic barriers of protection, the Innate system has both cells and proteins (such as, Cytokines and complement) at its disposal. Phagocytic leukocytes, such as polymorphonuclear neutrophilic leukocytes (neutrophils), and macrophages along with NK cells are the primary cellular components to combat microbes.

The inter- action of the invading microbe with these cells and other cells throughout the body triggers the release of complement and numerous cytokines. Many of these cytokines are proinflammatory molecules such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and the interferons,

- **Cellular Components and Phagocytosis**

  The key elements of effective innate immunity are responses that are rapid, nonspecific, and of short duration. These features are the hallmark of the phagocytic process. During infection, circulating phagocytic cells increase and can participate in chemotaxis, migration, ingestion, and microbial killing. Any antigen (microorganism) that enters the body through the lymphatics, lung, or bloodstream is engulfed by phagocytic cells.

Therefore, phagocytes, present in the blood, lymphoid tissue, liver, spleen, lung, and other tissues are the cells responsible for the uptake and removal of foreign antigen.

Phagocytic cells include :

(1) Monocytes and macrophages: Monocytes are small leukocytes that circulate in the blood and mature into macrophages that can be found in almost all tissues (figure 1). For example, they are known as Kupffer cells in the liver and microglial cells in the nervous tissue.

While Macrophages are critical cells that engulf and kill pathogens, process and present antigen, and regulate immune reactivity by producing a variety of molecules (eg,\ cytokines).

(2) Granulocytes, including neutrophils, eosinophils, and basophils: Granulocytes are leukocytes that contain densely staining granules. Neutrophils have a short half-life and are important phagocytic cells that destroy pathogens within intracellular vesicles. Eosinophils and basophils are less abundant and store granules containing enzymes and toxic proteins that can be released upon activation of the cells.

(3) Dendritic cells.

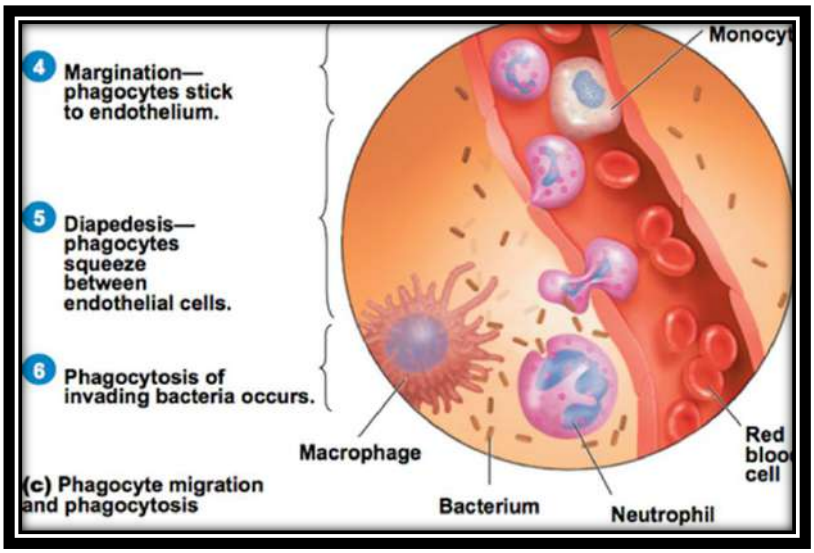


Figure (1): migration and differentiation of the monocytes.

Lecture 2

2. Acquired immunity (Adaptive Immunity):

When an infectious organism is not eliminated by innate immune mechanisms, acquired immune responses ensue with generation of antigen – specific lymphocytes (effector cells) and memory cells that can prevent reinfection with the same organism. Adaptive immunity which occurs after exposure to an antigen (eg, an infectious agent) is specific and is mediated by either antibody or lymphoid cells. This type of immune response take more time to develop (>96 h) because the rare B and T cells specific for the invading microorganism must undergo clonal expansion before they can differentiate into effector cells to help eliminate the infection.

Adaptive immunity can be:

1. Passive immunity.
2. Active immunity.

Passive Immunity

Passive immunity is transmitted by antibodies or lymphocytes preformed in another host. The main advantage of passive immunization with preformed antibodies is the prompt availability of large amounts of antibody; disadvantages are the short life span of these antibodies and possible hypersensitivity reactions if antibodies (immunoglobulins) from another species are administered.

Active Immunity

Active immunity is induced after contact with foreign antigens (eg, microorganisms or their products). This contact may consist of :

1- Clinical or subclinical infection

2- Immunization with live or killed infectious agents or their antigens

3- Exposure to microbial products (eg, toxins, toxoids)

4- Transplantation of foreign cells.

In all these instances the host actively produces antibodies, and lymphoid cells acquire the ability to respond to the antigens.

Advantages of active immunity include:

1- Long-term resistance (based on memory of prior contact with antigen

2- The capacity to respond faster and to a greater extent on subsequent contact with the same antigen.

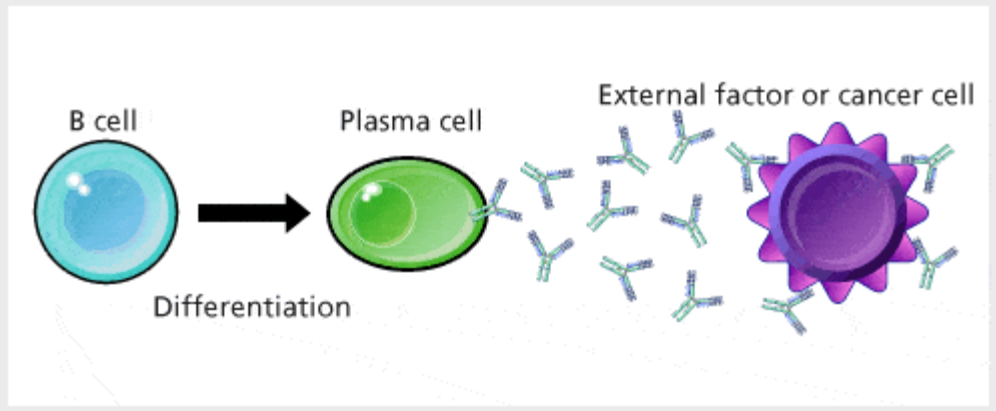
Disadvantages include:

1-The slow onset of resistance.

2- The need for prolonged or repeated contact with the antigen.

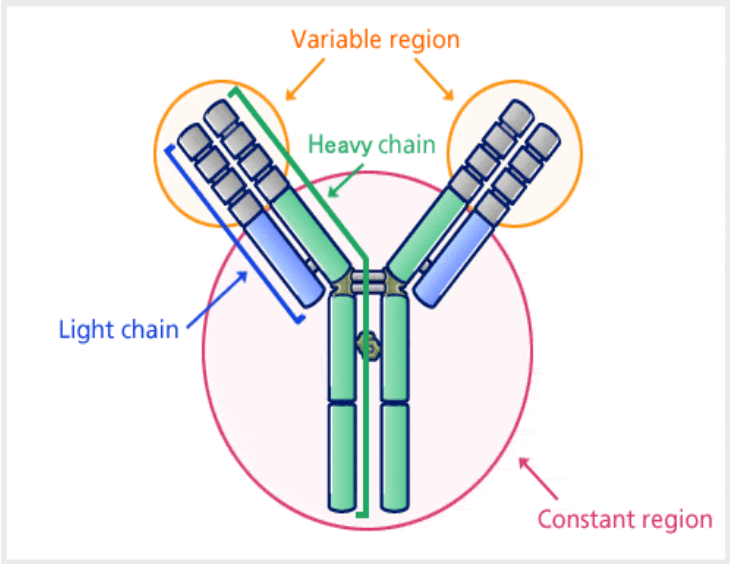
Antibody- Structure, Classes and Functions

Antibody (Ab) also known as Immunoglobulin (Ig) is the large Y shaped protein produced by the body's immune system when it detects harmful substances, called antigens like bacteria and viruses. The production of antibodies is a major function of the immune system and is carried out by a type of white blood cell called a B cell (B lymphocyte), differentiated B cells called plasma cells. The produced antibodies bind to specific antigens express in external factors and cancer cells (figure 1).



Structure of Antibody

Antibodies are heavy (~150 kDa) globular plasma proteins. The basic structure of all antibodies are same. There are four polypeptide chains: two identical **heavy chains** and two identical **light chains** connected by disulfide bonds. Light Chain (L) consists polypeptides of about 22,000 Da and Heavy Chain (H) consists larger polypeptides of around 50,000 Da or more. There are five types of Ig **heavy chain** (in mammal) denoted by the Greek letters: α, δ, ε, γ, and μ. There are two types of Ig **light chain** (in mammal), which are called lambda (λ) and kappa (κ) (Figure 2).



An antibody is made up of a variable region and a constant region, and the region that changes to various structures depending on differences in antigens is called the **variable region**, and the region that has a constant structure is called the **constant region (Figure 3).**

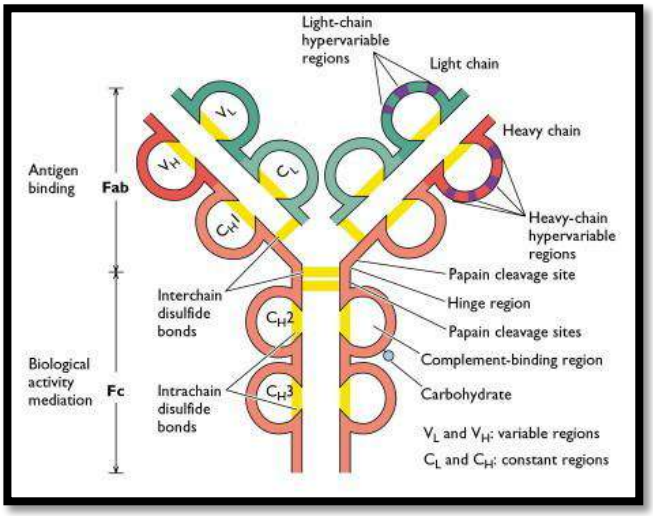
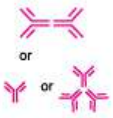


Figure 3 : Structure of antibody in details

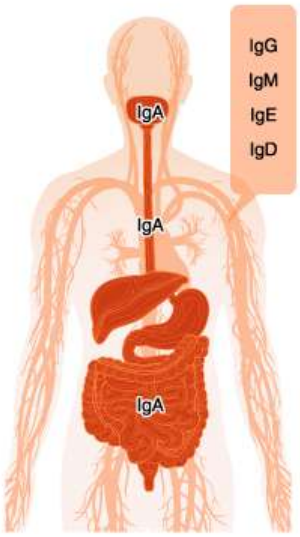
Classes/Types of Antibody

Serum containing antigen-specific antibodies is called antiserum. The 5 types – IgG, IgM, IgA, IgD, IgE – (isotypes) are classified according to the type of heavy chain constant region, and are distributed and function differently in the body (Figure 4).

Types and characteristics of antibodies

IgG		• Highest opsonization and neutralization activities. • Classified into four subclasses (IgG1, IgG2, IgG3, and IgG4).
IgM		• Produced first upon antigen invasion. Increases transiently.
IgA		• Expressed in mucosal tissues. Forms dimers after secretion.
IgD		• Unknown function.
IgE		• Involved in allergy.

Distribution in the body



Lecture three

• Host-Parasite Relationships

Parasitology is defined as the study of parasites and their relationship to their host. It is one of the most fascinating and rewarding phase of biology.This discipline actually involves several approaches to the study of parasitic organisms.

Parasitism is one of those aspects of biology and in particular ecology, which deals with the relationship of organisms to one another and to their habitat.

Parasitism involves an association between animals of different species where one, the host, is indispensable to the other, the parasite; while the host can quite well do without the parasite. In other words Parasitism is a heterospecific type of an association between two individuals in which one of the partners called parasite is metabolically dependent on another referred to as host. There are many parasitic agents or organisms among the viruses, bacteria, fungi, plants, and animals . By convention, when the word parasite is used without qualification, it refers specifically to a protozoan or helminthic (nematode, trematode, cestode) organism.

The relationship may be permanent as in the case of tapeworms found in the intestines of mammals, or temporary, as during the feeding of mosquitoes, leeches, and ticks on their host's blood.

Several types of parasitism are recognized.

1. Ectoparasite – a parasitic organism that lives on the outer surface of its host, e.g. lice, ticks, mites etc.

2. Endoparasites – parasites that live inside the body of their host, e.g. Entamoeba histolytica Trypanosoma, Plasmodium etc.

3. Obligate Parasite - This parasite is completely dependent on the host during a segment or all of its life cycle, e.g. Plasmodium spp.

4. Facultative parasite – an organism that exhibits both parasitic and non-parasitic modes of living and hence does not absolutely depend on the parasitic way of life, but is capable of adapting to it if placed on a host. E.g. Naegleria fowleri.

5. Accidental parasite – when a parasite attacks an unnatural host and survives. E.g. Hymenolepis diminuta (rat tapeworm).

6. Erratic parasite - is one that wanders in to an organ in which it is not usually found. E.g. Entamoeba histolytica in the liver or lung of humans

Types of hosts:

1. Definitive host – a host that harbors a parasite in the adult stage or where the parasite undergoes a sexual method of reproduction.

2. Intermediate host - harbors the larval stages of the parasite or an asexual cycle of development takes place. In some cases, larval development is completed in two different intermediate hosts, referred to as first and second intermediate hosts.

3. Paratenic host – a host that serves as a temporary refuge and vehicle for reaching an obligatory host, usually the definitive host, i.e. it is not necessary for the completion of the parasites life cycle.

4. Reservoir host – a host that makes the parasite available for the transmission to another host and is usually not affected by the infection.

5. Natural host – a host that is naturally infected with certain species of parasite.

6. Accidental host – a host that is under normal circumstances not infected with the parasite.

Classification of Parasites :

Parasites of medical importance come under the kingdom called protista and animalia. Protista includes the microscopic single-celled eukaroytes known as protozoa. In contrast, helminthes are macroscopic, multicellular worms possessing well differentiated tissues and complex organs belonging to the kingdom animalia. Medical Parasitology is generally classified into: Biotechnology Dept. Dr. Aida Alsaedy Univ. of Baghdad / College of Science Dr. Rasha Al- Sahlanee Lecture 3 2nd semester 2020-2021

• Medical Protozoology - Deals with the study of medically important protozoa.

• Medical Helminthology - Deals with the study of helminthes (worms) that affect man.

• Medical Entomology - Deals with the study of arthropods which cause or transmit disease to man.

Describing animal parasites follow certain rules of zoological nomenclature and each phylum may be further subdivided as follows:

Super class Super family

Phylum Subphylum Class Order Family Genus Species

Subclass Subfamily

Effects of parasites on the host

The damage which pathogenic parasites produce in the tissues of the host may be described in the following two ways;

(a) Direct effects of the parasite on the host

• Mechanical injury - may be inflicted by a parasite by means of pressure as it grows larger, e.g. Hydatid cyst causes blockage of ducts such as blood vessels producing infraction.

• Deleterious effect of toxic substances- in Plasmodium falciparum production of

toxic substances may cause rigors and other symptoms.

• Deprivation of nutrients, fluids and metabolites -parasite may produce disease by competing with the host for nutrients. Biotechnology Dept. Dr. Aida Alsaedy Univ. of Baghdad / College of Science Dr. Rasha Al- Sahlanee Lecture 3 2nd semester 2020-2021

(b) Indirect effects of the parasite on the host:

Immunological reaction: Tissue damage may be caused by immunological response of the host, e.g. nephritic syndrome following Plasmodium infections. Excessive proliferation of certain tissues due to invasion by some parasites can also cause tissue damage in man, e.g. fibrosis of liver after deposition of the ova of Schistosoma.

Lecture four

I - Intestinal protozoa:

a. Entamoeba histolytica:

Entamoeba histolytica is a common parasite in the large intestine in the humans, uses amoebic dysentery and liver abscess.

 Geographical distribution:

Infection by E histolytica is found worldwide but occurs most frequently in tropical countries, essentially in areas with poor sanitation.

 Habitat: large intestine.

 Morphology :

The life cycle of E. histolytica has two stages : the motile ameba (trophozoites) and non-motile cyst. The trophozoites is found within the intestinal and extraintestinal lesions and diarrheal stools. The cyst has four nuclei an important diagnostic criterion upon excystation in the intestinal tract, an ameba with four nuclei emerges and then divides to form eight trophozoites .The mature trophozoites has a single nucleus.

 Infective stage: cyst.

 Life cycle :

The organism is acquired by ingestion of cysts that are transmitted primarily by the fecal – oral route in contaminated food and water. There is no animal reservoir. The ingested cyst differentiate into tophozoites in the ileum but tend to colonize the cecum and colon. Biotechnology Dept. Dr. Aida Alsaedy Univ. of Baghdad / College of Science Dr. Rasha Al- Sahlanee Lecture 3 2nd semester 2020-2021

The trophozoites invade the colonic epithelium and secret enzymes that cause localized necrosis (teardrop). Progression into the submucosa leads to invasion of the portal circulation by the trophozoites.

 Acute manifestations :

Acute intestinal amoebiasis present as dysentery (bloody, mucus containing diarrhea) accompanied by lower discomfort.

Amoebic abscess of liver is characterized by right – upper quadrant pain, weight loss, fever and enlarged liver.

 Laboratory diagnosis :

1. Diagnosis of intestinal amoebiasis rests on finding either trophozoites in diarrheal stools or cysts in formed stools. Diarrheal stools should be examine within 1 hour of collection to see the amoeboid motility of the trophozoites.

2. Serology testing is helpful for the diagnosis of invasive amebiasis such as ELISA and IFAT.

Lecture five

Genito – urinary tract

Trichomonas vaginalis

Trichomonas vaginalis is an anaerobic, flagellated protozoan parasite and the causative agent of trichomoniasis.

Transmission usually occurs via direct, skin-to-skin contact with an infected individual, most often through vaginal intercourse.

Geographical distribution:

It is the most common pathogenic protozoan infection of humans in industrialized countries. the WHO has estimated that 160 million cases of infection are acquired annually worldwide.

Habitat: Trichomonas vaginalis in women inhabits vagina, vulva and urethra, while in man inhabits prostate and urethra.

Morphology

Unlike other parasitic protozoa (Giardia lamblia, Entamoeba histolytica etc.), Trichomonas vaginalis exists in only one morphological stage, a trophozoite, and cannot encyst.

 T. vaginalis is a pear-shaped protozoan 10-23μm (about the size of a white blood cell).

 Four flagella produce movement and a fifth may help with direction.

 High motility contributes to its pathogenicity .

 Life cycle : Trichomonas vaginalis Reproduces through binary fission.

Diagnosis: includes the following steps:

 take a sample of vaginal or penile discharge for examination under a microscope

 send a sample to the lab for a test.

T. vaginalis was traditionally diagnosed via:

1. a wet mount preparation, in which "corkscrew" motility of trophozoites was observed.

2. Currently, the most common method of diagnosis is via overnight culture, with a sensitivity range of 75–95%.

3. The presence of T. vaginalis can also be diagnosed by PCR. Biotechnology Dept. Asst. prof. Dr. Aida Alsaedy Univ. of Baghdad / College of Science Dr. Rasha Al-Sahlanee Lecture 4 2nd semester 2020 -2021

Treatment

Infection is treated and cured with metronidazole or tinidazole. The CDC recommends a onetime dose of 2 grams of either metronidazole or tinidazole as the first-line treatment; the alternative treatment recommended is 500 milligrams of metronidazole, twice daily, for seven days if there is failure of the single-dose regimen.

Prevention:

To prevent infection or reinfection, any sexual partners should also receive treatment.

Blood and tissue protozoan

Plasmodium :

Malaria is a mosquito-borne infectious disease most commonly spread by an infected female Anopheles mosquito that affects humans and other animals. four plasmodia species that causes malaria :

- Plasmodium ovale.
- Plasmodium falciparum.
- Plasmodium vivax.

Plasmodium malariae. P. falciparum is by far the most lethal in humans, resulting in hundreds of thousands of deaths per year.

Geographical distribution:

Species of Plasmodium are distributed globally wherever suitable hosts are found. Insect hosts which is the vector and the definitive host are most frequently mosquitoes of the genera Culex and Anopheles. Vertebrate hosts include reptiles, birds, and mammals. Plasmodium parasites were first identified in the late 19th century by Charles Laveran.

Habitat : Blood.

Acute manifestation:

A malaria infection is generally characterized by the following signs and symptoms:

 Fever, chills, headache, nausea and vomiting, muscle pain and fatigue other signs and symptoms may include: sweating, chest or abdominal pain and cough.

 In severe malaria (primarily caused by Plasmodium falciparum), clinical findings (confusion, coma, neurologic focal signs, severe anemia, respiratory difficulties) are more striking and may increase the index of suspicion for malaria.

Diagnosis:

1.Clinical diagnosis :is based on the patient's symptoms and on physical findings at examination. However, Clinical findings should always be confirmed by a laboratory test for malaria. Biotechnology Dept. Asst. prof. Dr. Aida Alsaedy Univ. of Baghdad / College of Science Dr. Rasha Al- Sahlanee Lecture 4 2nd semester 2020 -2021

2**. Microscopic examination**: Malaria parasites can be identified by examining under the microscope a drop of the patient's blood, spread out as a "blood smear" on a microscope slide. Prior to examination, the specimen is stained (most often with the Giemsa stain) to give the parasites a distinctive appearance. This technique remains the gold standard for laboratory confirmation of malaria.

3. Antigen Detection

Various test kits are available to detect antigens derived from malaria parasites. Such immunologic ("immunochromatographic") tests most often use a dipstick or cassette format, and provide results in 2-15 minutes. These "Rapid Diagnostic Tests" (RDTs) offer a useful alternative to microscopy in situations where reliable microscopic diagnosis is not available.

4. Molecular Diagnosis :

PCR is most useful for confirming the species of malarial parasite after the diagnosis has been established by either smear microscopy or RDT.

5. **Serology** : Serology detects antibodies against malaria parasites, using either indirect immunofluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA). Serology does not detect current infection but rather measures past exposure.

Treatment : A number of drugs have been developed to treat Plasmodium infection; however, the parasites have evolved resistance to each drug developed. Chloroquine is the drug of choice for acute malaria.

Prevention:

Malaria can be prevented by: 1) Avoiding mosquito bites by using mosquito repellants, mosquito nets .

2) Chemoprophylaxis – 2 weeks before entering to 4 weeks after leaving an endemic area Chemoprophylaxis is defined as the use of medications or chemical agents to prevent a disease.

Lecture six

2. Toxoplasma gondii:

Is an obligate intracellular parasitic one-celled eukaryote, This protozoan parasite is a zoonotic parasite infects human and other warm-blooded animals and causes the disease toxoplasmosis.

Morphology : This parasite presents at a three main stages, tachyzoites, bradyzoites and sporozoites.

1. Tachyzoites : Crescent or oval in shape, Blunted at posterior end and tapered at anterior end. Reside in a vacuole, fast replicating and highly invasive and destructive for tissues can be eliminated by drug treatment because it represent the acute infection figure (1).

2. Bradyzoites : Reside in a tissue cyst characterized by slow growth and persistent in host, resistant to current drug treatment because it represent the chronic infection figure

3. Oocyst :

- The oocyst is non infectious before sporulation .

- Unsporulated oocysts are subspherical to spherical.

- Sporulated oocysts are sub- spherical to ellipsoidal .

- Each oocyst has two ellipsoidal sporocysts (figure 3).

- Each sporocysts contains four sporozoites.

- Shedding occurs 3-5 days after ingestion of tissue cysts.

- Sporulated oocysts remain infective for months.

Geographical distribution:

T. gondii is a cosmopolitan parasite with a variable frequency rate worldwide. It is estimated that *T.gondii* infects one third of world population.

Habitat:

The most common location is the brain, eye , lungs , heart and skeletal muscles. This parasite presents at a three main stages, tachyzoites, bradyzoites and sporozoites.

Life cycle.

The life cycle of T.gondii is complex. It comprises a phase of sexual reproduction in definitive hosts, especially cats. It also comprises a phase of asexual reproduction that occurs in intermediate hosts (birds and mammals) as well as in definitive hosts.

1- The asexual cycle

After ingestion of oocysts by the intermediate hosts (birds and mammals), the sporozoites release from the oocysts and penetrate into the intestinal epithelial cells, where they transform into tachyzoites. The tachyzoites are able to infect any nucleated cell type, and disseminate throughout the body via infected blood cells. After a few days, some of these tachyzoites transform into bradyzoites, gathered in cysts mainly found in nerve and muscle cells. The cysts of T. gondii could persist throughout the host life. The mechanism of tissue cyst persistence is unknown. However, tissue cysts could break down periodically: bradyzoites transform to tachyzoites and reinvade host cells to form new tissue cysts figure (4,5).

2. The sexual cycle

The sexual cycle occurs only in felines. After the ingestion by a cat of cysts present in tissues of an infected intermediate host, the parasites invade its intestinal epithelial cells. They firstly undergo a limited number of asexual multiplications (schizogony). Merozoites resulting from these first steps of asexual multiplications in enterocytes produce male or female gametocytes that will mature into gametes. The fertilization between male and female gametes leads to the production of unsporulated oocysts or non-infecting oocysts. These oocysts are excreted in the faeces of Felidae and become sporulated in the environment, after a meiotic reduction leading to the production of sporozoites. The shedding of oocysts begins 4 to 6 days after the ingestion of tissue cysts and may continue up to 20 days. Cats can excrete several millions of oocysts that disseminate in the environment. The oocysts are very resistant and infective for both intermediate and definitive hosts (figure 4). Biotechnology Dept. Asst. prof. Dr. Aida Alsaedy Univ. of Baghdad / College of Science Dr. Rasha Al- Sahlanee Lecture 5 : 2nd semester 2020 -2021

How do humans become infected?

The possible ways of contamination are:

1. Ingestion of tissue cysts by eating raw meat, undercooked or insufficiently frozen (lamb, pork, cow, beef, chicken, horse,...)

2. Ingestion of oocysts present in an environment contaminated by cat feces: plants (fruit, vegetable from the garden...), water, soil (gardening or farming activities), animal fur …

3. Direct contamination by cat by handling dropping litter in the absence of a proper hygiene.

5. Contamination through blood transfusion or organ transplant is quite possible although much infrequent.

6. Transplacental transmission leading to a congenital infection of fetus when a woman acquires infection during pregnancy.

The diagnosis of T. gondii infection or toxoplasmosis may be established by:

1. Demonstration of the Toxoplasma gondii organism in blood, body fluids, or tissue.

2. Detection of Toxoplasma gondii antigen in blood or body fluids by enzyme-linked immunosorbent assay (ELISA) technique

3. The Sabin-Feldman dye test: is a sensitive and specific neutralization test. It measures IgG antibody and is the standard reference test for toxoplasmosis. High titters suggest acute disease. Serologically: IgM fluorescent antibody test detects IgM antibodies within the first week of infection, but titters fall within a few months.

4. Amplification of specific nucleic acid sequences (i.e., Polymerase Chain Reaction :on body fluids, including CSF, amniotic fluid, and blood) .

5. Skin test results : showing delayed skin hypersensitivity to Toxoplasma gondii antigens. Antibody levels in aqueous humor or CSF may reflect local antibody production and infection.

6. Animal inoculation: inoculation of suspected infected tissues into experimental animals

7. Isolation of the organism: inoculation of suspected infected tissues into tissue culture.

lecture seven

1. Trypanosoma spp.

Human African trypanosomiasis (HAT), also called sleeping sickness, is a parasitic infection that almost invariably progresses to death, unless treatment is provided. HAT caused devastating epidemics during the 20th century.

a. Trypanosoma Brucei

T. brucei belongs to the Trypanosomatidae, a family consisting of exclusively parasitic organisms found world-wide in vertebrates and insects .

These unicellular parasites have co-evolved with their hosts to such an extent that most of them are commensal rather than pathogenic.

The species T. brucei includes three morphologically indistinguishable subspecies .

1- T. b. brucei, which causes AAT, is not infective to humans.

2- T. b. rhodesiense.

3- T. b. gambiense can infect humans as they developed the ability to resist apolipoprotein A (ApoL1), a serum protein that triggers death in other trypanosomes.

EPIDEMIOLOGY

Endemic in rural sub-Saharan Africa. T. b. rhodesiense is found in eastern and southeastern Africa, mainly Tanzania, Uganda, Malawi, Zambia, and Zimbabwe. T. b. gambiense is found in central Africa and in limited areas of West Africa, primarily in Democratic Republic of the Congo, Central African Republic, Angola, South Sudan, Guinea, Cameroon, Gabon, Côte d'Ivoire, Congo, Chad, and northern Uganda. World Health Organization.

In 2014, 3,796 sleeping sickness cases were reported to the World Health Organization; T. b. gambiense accounted for >98% of cases. Tsetse flies inhabit rural, densely vegetated areas; people who only travel to urban areas are not at risk. Flies bite during the day, and <1% are infected. Risk of infection increases with the number of fly bites, which is not always directly correlated with duration of travel.

Morphology:

T. brucei cells contain one central nucleus, one single mitochondrion with its own DNA comprising the kinetoplast situated at the posterior end of the cell, and a flagellum attached to the cell by an undulating membrane. Univeristy of Baghdad / college of Sciences Dr Aida Al Saedy Biotechnology Dept. Dr. Rasha AL-SAHLANEE Microbiology II / Lecture 6 : Parasitology 2nd semester 2020-2021

LIFE CYCLE

Trypanosoma brucei needs two hosts to live and reproduce. Its life cycle starts, when an infected tsetse fly bites human skin. While it is feeding on blood, metacyclic trypomastigotes are transmitted to the skin from the salivary glands of the fly. The parasites get into the bloodstream by entering lymphatic or blood vessels. They travel in different body fluids (such as blood, lymphatic or spinal fluid), transform into bloodstream trypomastigotes and multiply by binary fission. The disease can be spread by another tsetse fly that drinks the infected blood. Inside the fly the life cycle takes about three weeks. Ingested bloodstream trypomastigotes transform into procyclic trypomastigotes in the fly's midgut and multiply. They transform into epimastigotes, migrate to the salivary glands, then transform into metacyclic trypomastigotes and multiply once again by binary fission.

In the blood and tissues of mammals, trypanosomes can be observed as spindle shaped cells, 20-30 μm long (about 3x the diameter of a human erythrocyte), 2-5 μm wide and characterised by their wriggling movement. Sometimes, shorter forms

can also be seen which are metabolically pre-adapted to survival in the tsetse intestines.

In the mammalian host, the trypanosome cell membrane is covered by a dense coat of identical glycoprotein dimers shielding the underlying membrane against innate immunological attacks, e.g. by complement. These highly immunogenic glycoproteins induce a specific antibody response that triggers destruction of all the trypanosomes opsonised with these antibodies. To survive this antibody mediated immune response, trypanosomes developed "antigenic variation", by which the glycoprotein coat on the cell membrane is replaced by an antigenically different coat.

Infection of mammalian hosts starts with the injection of metacyclic trypanosomes, together with tsetse saliva, into the skin. After several days of local multiplication, the trypanosomes spread via the lymph and blood to a variety of peripheral organs and tissues. Later on, the parasites invade the brain parenchyma where they trigger local inflammation and neurological damage. The parasites' journey through the mammalian host is accompanied and regulated by important immunological reactions, some of which are pathogenic, induced by components of the parasite and the tsetse fly saliva .

Tsetse flies become infected with T. brucei when they ingest trypanosomes residing in the blood or, as shown in experimental infections, in the skin of mammals . Once ingested, the short stumpy Univeristy of Baghdad / college of Sciences Dr Aida Al Saedy Biotechnology Dept. Dr. Rasha AL-SAHLANEE Microbiology II / Lecture 6 : Parasitology 2nd semester 2020-2021

trypanosomes undertake a complex journey through the fly tissues, until they reach the salivary glands and develop into the human-infective metacyclic forms . Under natural conditions, only a small fraction of the tsetse flies carry mature infection of

T. brucei (about 0.01 %, 38,39) but one single tsetse fly, feeding every 3 days, is able to infect several persons during its two- to three-month life-span. Eliminating the tsetse or reducing tsetse/human contact is one way to reduce or interrupt HAT transmission.

Lecture eight

b. *Trypanosoma cruzi* :

Trypanosoma cruzi, is a parasitic protozoan that is the causative agent of Chagas disease (American trypanosomiasis). Currently, six distinct lineages of *T. cruzi* are classified into discrete typing units (TcI-VI), which vary in their geographic occurrence, host specificity, and pathogenicity.

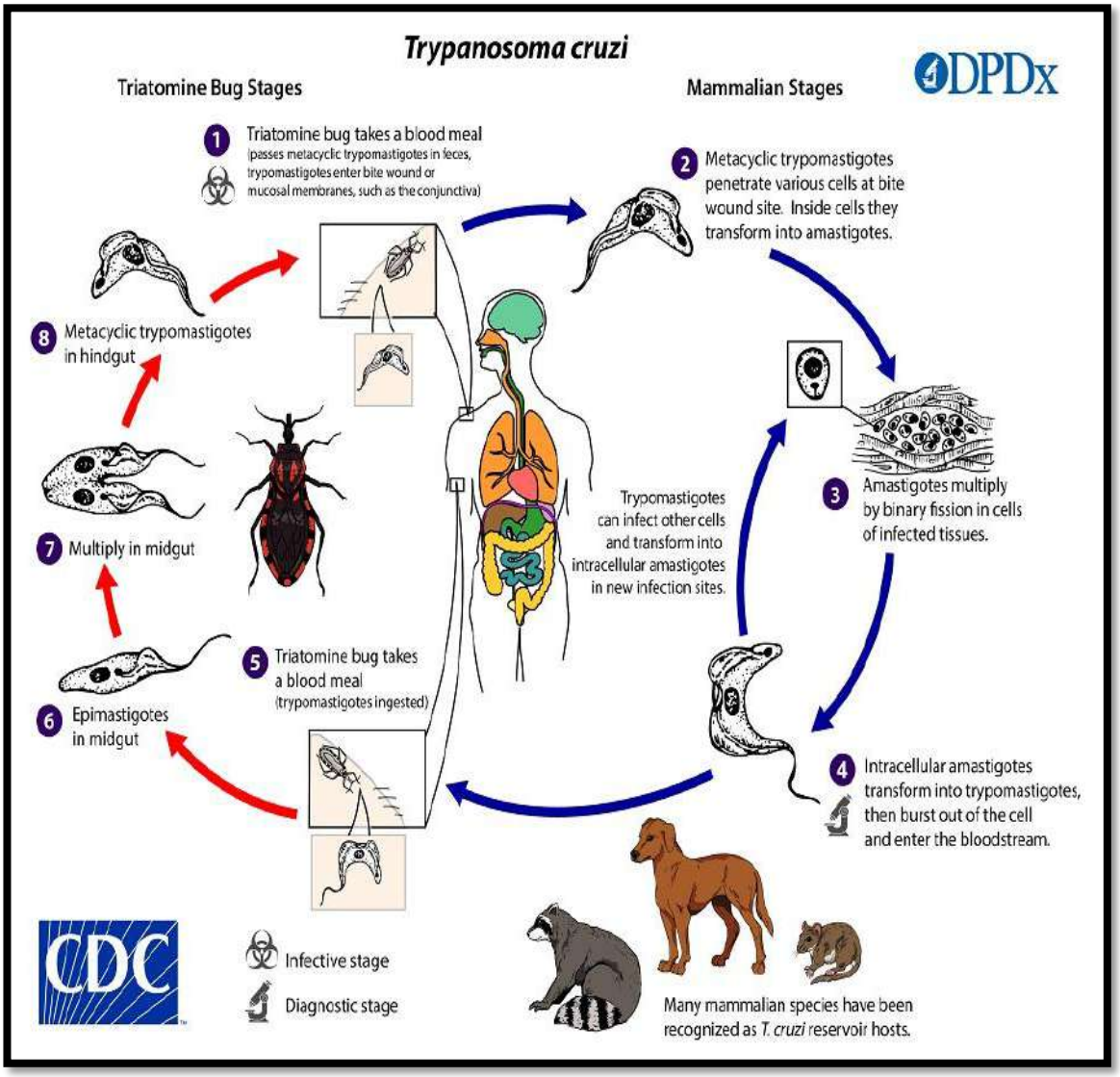
Hosts /Vectors:

Apart from humans, a number of mammals serve as reservoir hosts for *T. cruzi,* e.g. armadillos, opossums, raccoons, woodrats, some other rodents, and domestic dogs. Common triatomine vector species for trypanosomiasis belong to the genera *Triatoma*, *Rhodnius*, and *Panstrongylus*.

Geographic Distribution:

T. cruzi is endemic in vectors and wildlife reservoirs throughout the Americas from the southern half of the United States down to Argentina. Chagas disease cases have been reported from South and Central American countries, particularly in rural, impoverished areas.

Life cycle : An infected triatomine insect vector (or "kissing" bug) takes a blood meal and releases trypomastigotes in its feces near the site of the bite wound. Trypomastigotes enter the host through the bite wound or intact mucosal membranes, such as the conjunctiva ❶ . Inside the host, the trypomastigotes invade cells near the site of inoculation, where they differentiate into intracellular amastigotes ❷ . The amastigotes multiply by binary fission ❸ and differentiate into trypomastigotes, and then are released into the circulation as bloodstream trypomastigotes ❹ . Trypomastigotes infect cells from a variety of tissues and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle. The bloodstream trypomastigotes do not replicate (different from the African trypanosomes). Replication resumes only when the parasites enter another cell or are ingested by another vector. The "kissing" bug becomes infected by feeding on human or animal blood that contains circulating parasites ❺. The ingested trypomastigotes transform into epimastigotes in the vector's midgut ❻. The parasites multiply and differentiate in the midgut ❼ and differentiate into infective metacyclic trypomastigotes in the hindgut ❽. Other less common routes of transmission include blood transfusions, organ transplantation, transplacental transmission, and foodborne transmission (via food/drink contaminated with the vector and/or its feces).



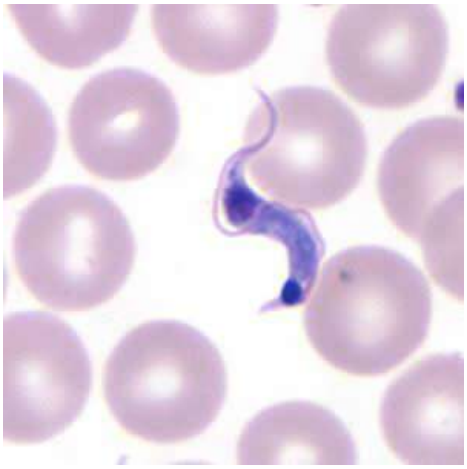
Clinical Presentation :

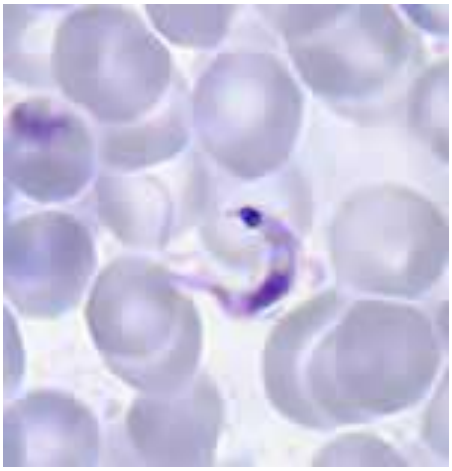
Chagas disease has an acute phase and chronic phase. The acute phase is usually asymptomatic, but can present with nonspecific somatic symptoms. Rarely, the acute phase may be more severe with potential cardiac or neurologic symptoms and signs. Nodular lesions or furuncles, usually called chagomas, may develop around the vector's feeding site. Chagomas occurring on the on the eyelids are commonly referred to as palpebral and periocular firm swelling. Most acute cases resolve over a period of a few weeks or months into a subclinical chronic form of the disease ("indeterminate form"). Reactivation of Chagas disease from this asymptomatic form may occur in patients with HIV or those receiving immunosuppressive drugs.

The symptomatic chronic form ("determinate form") may not occur for years or even decades after initial infection. This may include cardiac or gastrointestinal involvement, which occasionally occur together. The many complications of chronic Chagas disease can be fatal. Amastigote invasion of smooth muscle can lead to megaesophagus, megacolon, and dilated cardiomyopathy.

Diagnosis

During the acute phase of infection, parasites may be seen circulating in the blood. The diagnosis of Chagas disease can be made by observation of the parasite in a blood smear by microscopic examination. A thick and thin blood smear are made and stained for visualization of parasites.





Diagnosis of chronic Chagas disease is made after consideration of the patient's clinical findings, as well as by the likelihood of being infected, such as having lived in a country where Chagas disease is common. Diagnosis is generally made by testing for parasite specific antibodies.

Treatment : The two drugs used to **treat** infection with ***T. cruzi*** are benznidazole and nifurtimox (Lampit®). Benznidazole is approved by FDA for use in children 2–12 years of age and is available from benznidazole tablets.com

Prevention & Control :

Improvement of the housing and spraying insecticide inside housing to eliminate the bugs has significantly decreased the spread of Chagas disease. In the United

States and in other regions where Chagas disease is now found but is not widespread, control strategies are focused on preventing transmission from blood transfusion, organ transplantation, and mother-to-baby.

Lecture nine

4. Leshmania spp:

Leishmaniasis is a vector-borne disease that is transmitted by sandflies and caused by obligate intracellular protozoa of the genus *Leishmania*.

Causative agents :

Human infection is caused by about 21 of 30 species that infect mammals.

These include the

1. *L. donovani* complex with 3 species (*L. donovani*, *L. infantum*, and *L. chagasi*).
2. *L. mexicana* complex with 3 main species (*L. mexicana*, *L. amazonensis*, and *L. venezuelensis*).
3. *L. tropica*; *L. major*; *L. aethiopica*.

The different species are morphologically indistinguishable, but they can be differentiated by isoenzyme analysis, molecular methods, or monoclonal antibodies.

Transmission : Leishmaniasis is transmitted by the bite of infected female phlebotomine sandflies . Their primary hosts are vertebrates; *Leishmania* commonly infects hyraxes, canids, rodents, and humans.

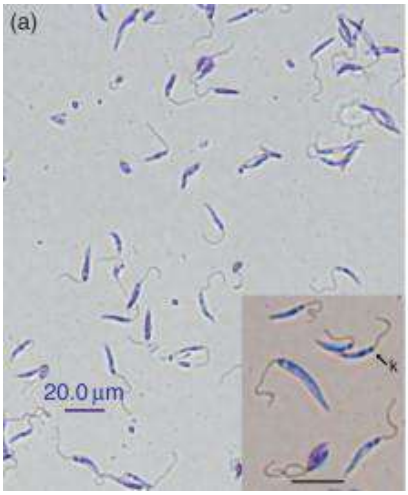
Epidemiology

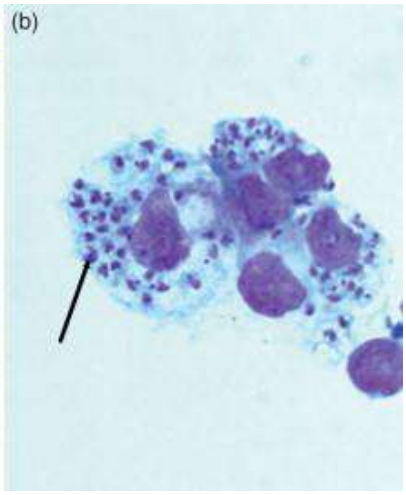
Leishmania currently affects 6 million people in 98 countries. About 0.9-1.6 million new cases occur each year, and 21 species are known to cause disease in humans.

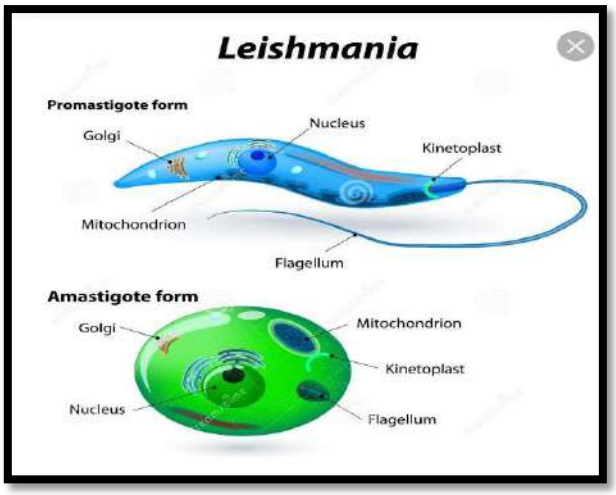
Morphology and structure :

Leishmania species are unicellular eukaryotes having a well-defined nucleus and other cell organelles including kinetoplasts and flagella. Depending on their life

cycle there are two stages of leishmania including Promastigote (infective stage) and amastigote.

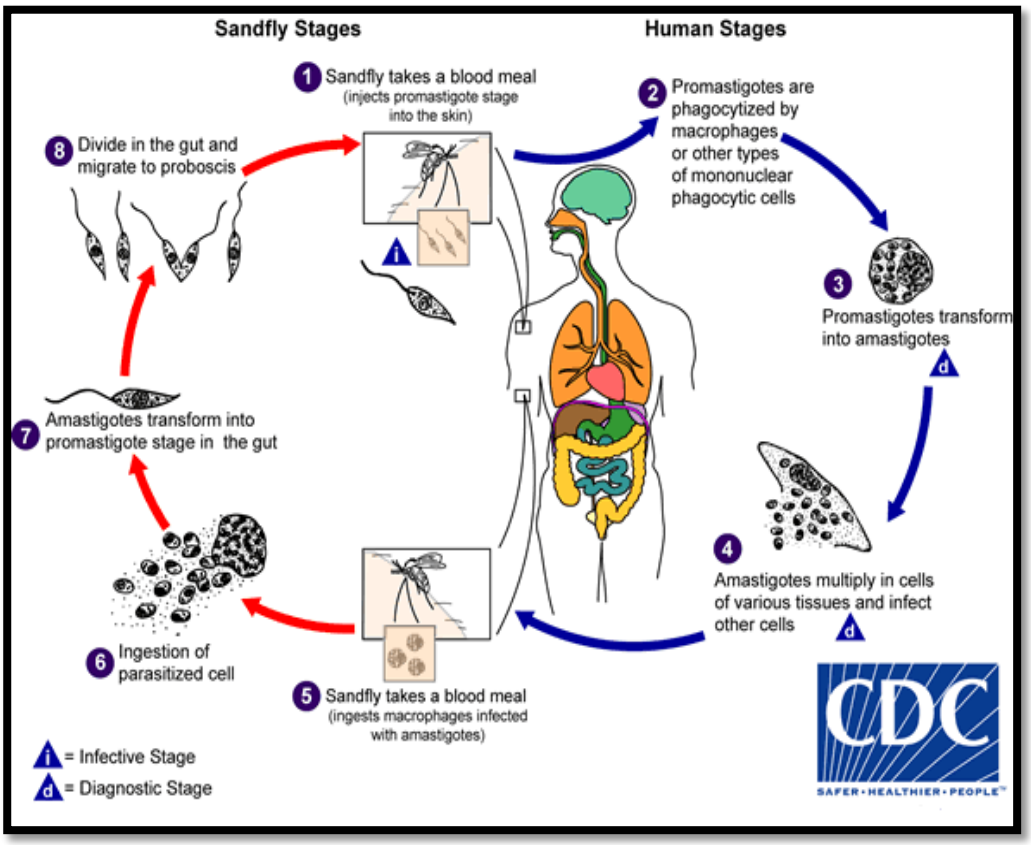






Life cycle :

Leishmaniasis is transmitted by the bite of infected female phlebotomine sandflies. The sandflies inject the infective stage (i.e., promastigotes) from their proboscis during blood meals ❶. Promastigotes that reach the puncture wound are phagocytized by macrophages ❷ and other types of mononuclear phagocytic cells. Promastigotes transform in these cells into the tissue stage of the parasite (i.e., amastigotes) ❸, which multiply by simple division and proceed to infect other mononuclear phagocytic cells ❹. Parasite, host, and other factors affect whether the infection becomes symptomatic and whether cutaneous or visceral leishmaniasis results. Sandflies become infected by ingesting infected cells during blood meals (❺, ❻). In sandflies, amastigotes transform into promastigotes, develop in the gut ❼ (in the hindgut for leishmanial organisms in the *Viannia* subgenus; in the midgut for organisms in the *Leishmania* subgenus), and migrate to the proboscis ❽



Clinical signs :

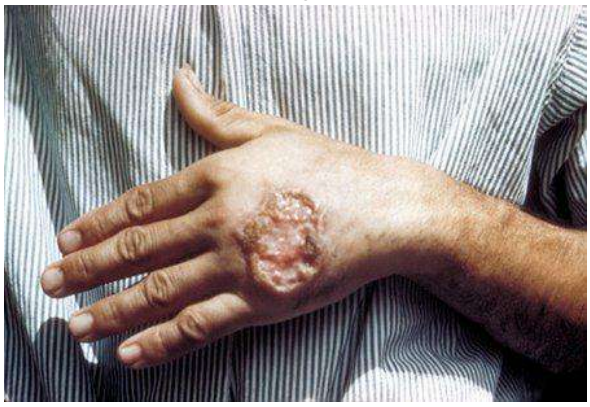
Type	Pathogen	Location
1. *Cutaneous leishmaniasis* (localised and diffuse) infections appear as obvious skin reactions.	The most common is the *Oriental Sore* (caused by Old World species *L. major, L. tropica*,.	Cutaneous infections are most common in Iraq (Baghdad boils) Iran, Peru, Saudi Arabia and Syria.
2. *Mucocutaneous leishmaniasis* infections start off as a reaction at the bite, and can go by metastasis into the mucous membrane and become fatal.	*L. braziliensis*	Mucocutaneous infections are most common in Bolivia, Brazil and Peru.
3. *Visceral leishmaniasis* (*kala azar*)	Caused exclusively by	visceral infections are most common in

species of the *L. donovani*	Bangladesh, Brazil, India, Nepal, and Sudan.

Diagnosis : We recommend using multiple diagnostic approaches to maximize the likelihood of a positive *Leishmania* result, using methods such as visualization of the characteristic amastigote in smears or tissue (histopathology); parasite isolation by *in vitro* culture; molecular detection of parasite DNA; and, for VL, serologic testing . Simultaneous testing for other diagnoses (e.g., by histopathology and culture) should be considered**.**

Treatment :

The skin sores of **cutaneous leishmaniasis** usually heal on their own, even without treatment. But this can take months or even years, and the sores can leave ugly scars.



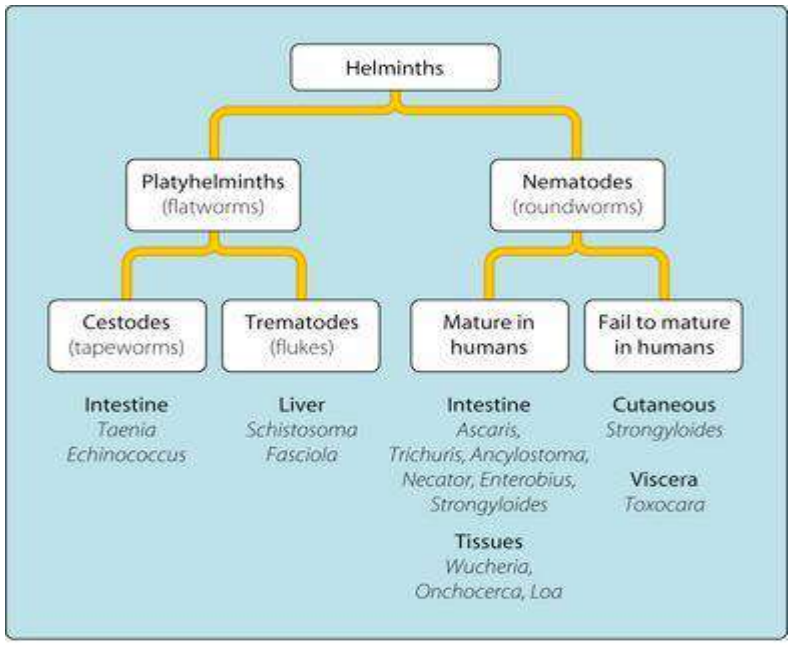
Prevention : The best way to prevent leishmaniasis is to avoid the bite of the sand fly. Simple insect precautions, including protective clothing (long sleeves, shirts tucked into pants, long pants, and socks) and insect repellents containing N, N-diethylmetatoluamide (DEET) reduce the risk of bites.

Lecture ten

Helminthes

The helminths are worm-like parasites. The clinically relevant groups are separated according to their general external shape and the host organ they inhabit. There are both hermaphroditic and bisexual species. The definitive classification is based on the external and internal morphology of egg, larval, and adult stages. The helminths are invertebrates characterized by elongated, flat or round bodies. In medically oriented schemes the flatworms or platyhelminths (platy from the Greek root meaning "flat") include flukes and tapeworms. Roundworms are nematodes (nemato from the Greek root meaning "thread"). These groups are subdivided for convenience according to the host organ in which they reside, e.g., lung flukes, extraintestinal tapeworms, and intestinal roundworms. This chapter deals with the structure and development of the three major groups of helminths.

Classification of helminthes



Flukes (Trematodes)

Adult flukes are leaf-shaped flatworms. Prominent oral and ventral suckers help maintain position in situ. Flukes are hermaphroditic except for blood flukes, which

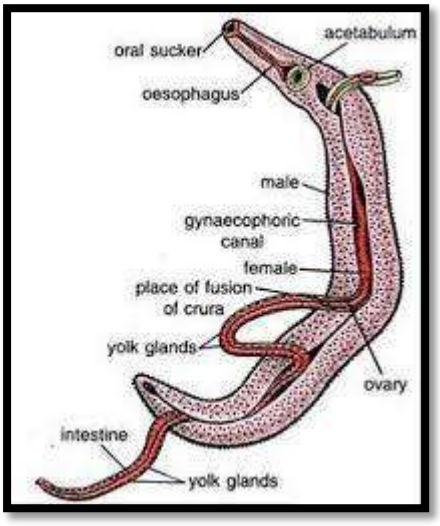
are bisexual. The life-cycle includes a snail intermediate host such as schistosoma spp. And fasciola spp.

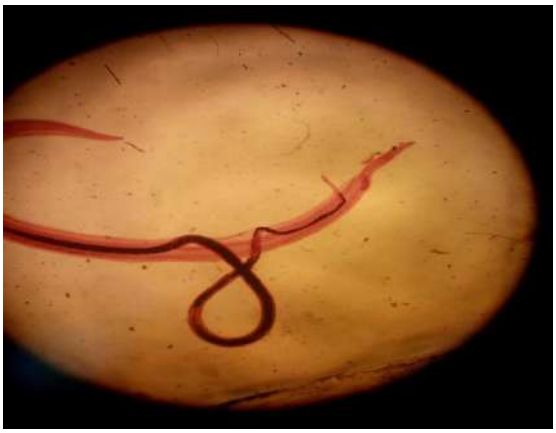
Schistosoma spp.

Schistosoma is a genus of trematodes, commonly known as blood flukes. They are parasitic flatworms responsible for a highly significant group of infections in humans termed schistosomiasis, which is considered by the World Health Organization as the second-most socioeconomically devastating parasitic disease (after malaria), with hundreds of millions infected worldwide. The genus Schistosoma contains six species that are of major pathological importance to man, Schistosoma haematobium (S. haematobium), S. mansoni, S. japonicum, S. mekongi, S. intercalatum, and S. guineensis. The species differ in their final location in the human host, the species of the intermediate (snail) host they use in their life cycle, the pathology they induce, and the number, size and shape of the eggs they produce. This Monograph is restricted to S. haematobium.

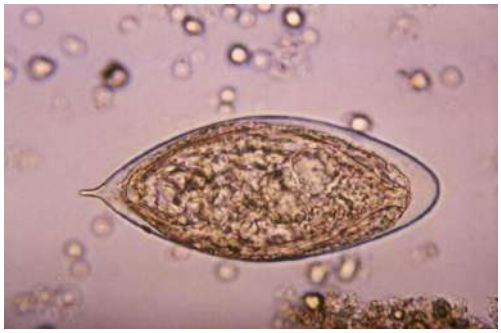
Transmission : via penetration wound skin by infective stage (cercariae) which released by snails.

Structure : Unlike all other pathologically important trematodes, schistosomes are not hermaphroditic, but have separate sexes. The adult worms are 1–2 cm long with a cylindrical body that features two terminal suckers, a complex tegument, a blind digestive tract, and reproductive organs. The male's body forms a groove or gynaecophoric channel, in which it holds the longer and thinner female. As permanently embraced couples, the schistosomes live within the perivesical (S. haematobium) or mesenteric (other species) venous plexus. Schistosomes feed on blood particles through anaerobic glycolysis





Adult worm



Eggs of sch. Haematobium it has terminal spine

Prevalence, geographic distribution

Human schistosomiasis is endemic in large areas of the (sub)tropics. It has been estimated that over 700 million people in 74 countries are exposed to the risk of schistosomal infection

life cycle : The female of S. haematobium worm produces hundreds of eggs per day throughout her life. By the terminal spine penetrate through the bladder wall where they are excreted with urine. Each ovum contains a ciliated larva (miracidium), which secretes proteolytic enzymes that help the eggs migrate into the lumen of the bladder. About half of the eggs produced do not reach the vesical lumen, and are carried away with the bloodstream, and/or trapped in the tissues. These retained eggs provoke a granulomatous inflammatory response, which is the main cause of pathology in the human host. The excreted eggs hatch if they come into contact

with water, and release the miracidium. These remain viable for up to 48 hours and are able to locate a suitable freshwater snail host (i.e. Bulinus spp. for S. haematobium) using external stimuli such as light and snail-derived chemicals. In the snail, asexual multiplication takes place, and several generations of multiplying larvae (sporocysts) develop. Eventually, these sporocysts produce large numbers of infective larvae with a typical bifurcated tail (cercariae). These leave the snail at a rate of thousands per day after a period of weeks. Shedding of these cercariae can continue for months. The cercariae survive for up to 72 hours and use water turbulence and skin-derived chemicals to locate the human host. They attach to and penetrate the human skin within 3–5 minutes.

Clinical signs:

Fever.

Abdominal pain (liver/spleen area)

Bloody diarrhea or blood in the stools.

Cough.

Malaise.

Headache.

Rash.

Body aches

Diagnosis : Serology is the most sensitive and useful test for screening. Among individuals living in endemic areas, the parasite burden should be determined by microscopy for egg detection and antigen detection. The infecting species can be determined via microscopy and molecular tests (polymerase chain reaction [PCR]), although these are less sensitive in the setting of early infection (<3 months).

TREATMENT

Drug of choice: Praziquantel.

PREVENTION and CONTROL

Infection by Schistosomes is acquired when the aquatic cercariae penetrate the skin. Thus, the infection can be prevented by avoiding contact with water known to harbor infected snails shedding the cercariae. Proper sanitation that eliminates

contamination with bathing water or other communal water sources can aid in control by preventing snails from becoming infected.

Tapeworms (Cestodes):

Adult tapeworms are elongated, segmented, hermaphroditic flatworms that inhabit the intestinal lumen. Larval forms, which are cystic or solid, inhabit extraintestinal tissues.

Roundworms (Nematodes)

Adult and larval roundworms are bisexual, cylindrical worms. They inhabit intestinal and extraintestinal sites.