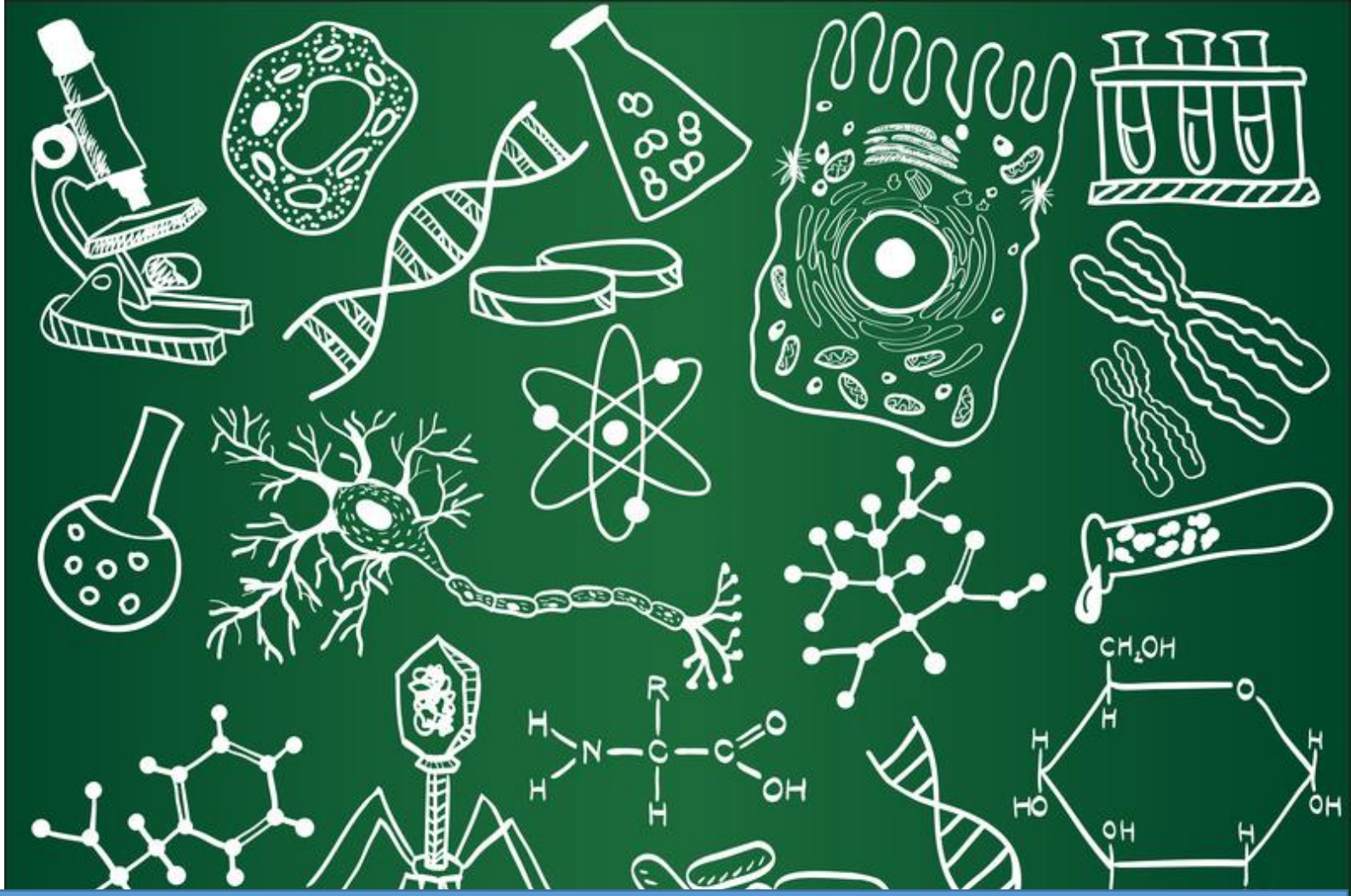
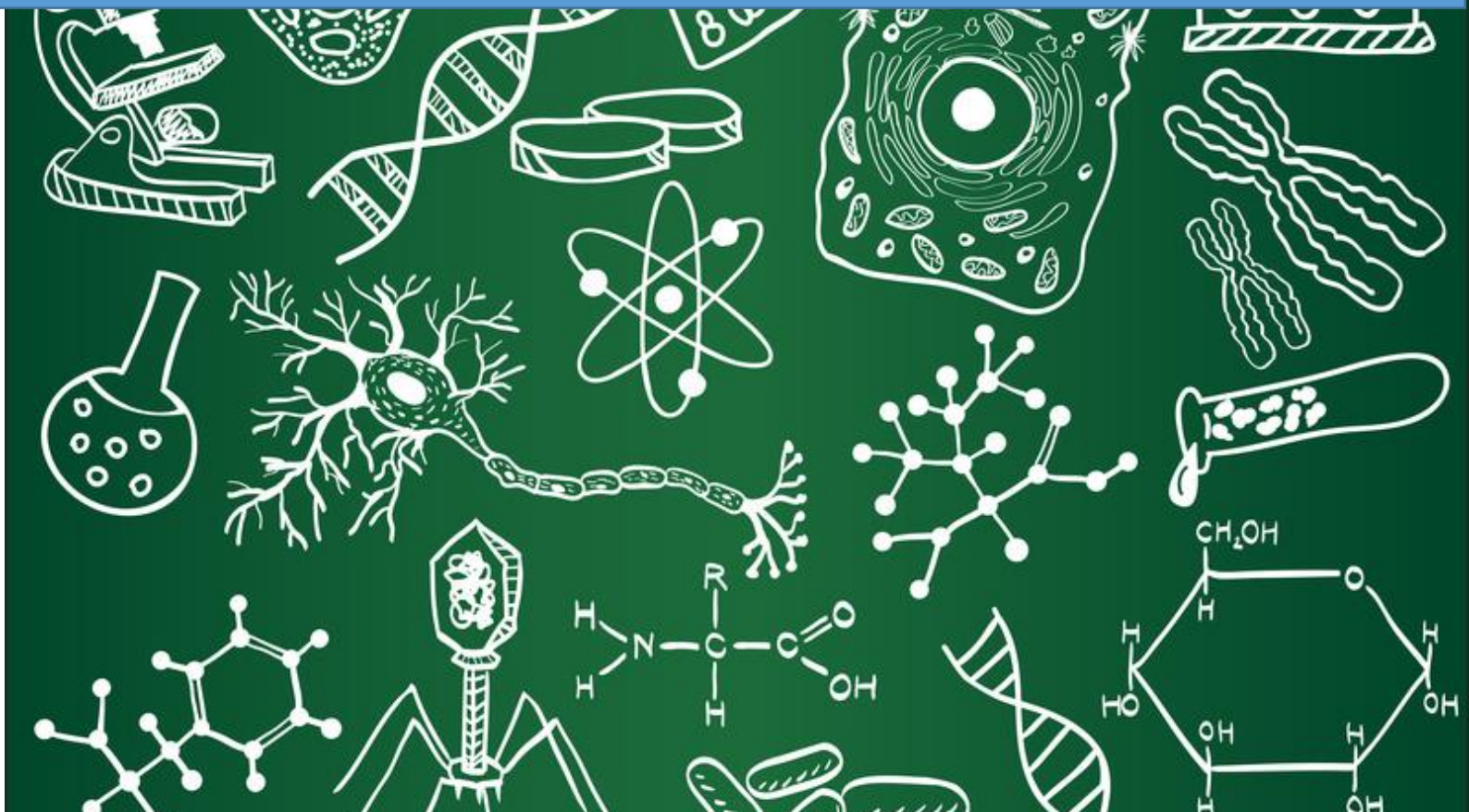


MEDICAL BIOLOGY

TEXTBOOK



CHUVASH STATE UNIVERSITY by I.Y. ULYANOV name

MEDICAL BIOLOGY

Modul N 2.
CELLS AND TISSUES

Cheboksary 2015

ВВС 28.0я73
UDC 57

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2. Text Book of Human Parasitology Edited by Lu Gang. 256 p.

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The manual describes the basic technology of the microscope and cell biology. Given the reference questions for self-checking, and test output control of knowledge. The manual is intended for medical students studying a medical discipline in English.

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RULES

The terms in **bold**, you need to write in an alphabetic dictionary and memorize it.

Sections marked **It is necessary to remember** with must memorize.

LESSON 1. PRINCIPLES OF MICROSCOPY

Microscopes make small objects appear bigger. A light microscope will magnify an image up to **1500** times its original size. Electron microscopes can achieve magnifications up to 1 million times. However, bigger is only better when more details are revealed. The fineness of detail that a microscope can reveal is its resolving power. This is defined as the smallest distance that two objects can approach one another yet still be recognized as being separate. The resolution that a microscope achieves is mainly a function of the wavelength of the illumination source it employs. The smaller the wavelength, the smaller the object that will cause diffraction and the better the resolving power. The light microscope, because it uses visible light of wavelength around **500** nanometers (nm, where **1000** nm = **1** /xm), can distinguish objects as small as about half this: **250** nm. It can therefore be used to visualize the smallest cells and the major intracellular structures or organelles. The microscopic study of cell structure organization is known as **cytology.** An electron microscope is required to reveal the **ultrastructure** (the fine detail) of the organelles and other cytoplasmic structures (Fig. 1).

The wavelength of an electron beam is about 100,000 times less than that of white light. In theory, this should lead to a corresponding increase in resolution. In practice, the electron microscope can distinguish structures about 1000 times smaller than is possible in the light microscope, that is, down to about 0.2 nm in size.

The Light Microscope

A light microscope (Fig. 1.) consists of a light source, which may be the sun or an artificial light, plus three glass lenses: a **condenser lens** to focus light on the specimen, an **objective lens** to form the magnified image, and a **projector lens,** usually called the eyepiece, to convey the magnified image to the eye. Depending on the focal length of the various lenses and their arrangement, a given magnification is achieved. In **bright-field microscopy,** the image that reaches the eye consists of the colors of white light less that absorbed by the cell. Most living cells have little color (plant cells are an obvious exception) and are therefore largely transparent to transmitted light. This problem can be overcome by **cytochemistry,** the use of colored stains to selectively highlight particular structures and organelles. However, many of these compounds are highly toxic and to be effective they often require that the cell or tissue is first subjected to a series of harsh chemical treatments.

A different approach, and one that can be applied to living cells, is the use of **phase-contrast microscopy.** This relies on the fact that light travels at different speeds through regions of the cell that differ in composition. The phase-contrast microscope converts these differences in refractive index into differences in contrast, and considerably more detail is revealed (Fig. 2). Light microscopes come in a number of physical orientations (upright, inverted, etc.) but whatever the orientation of the microscope the optical principles are the same.

RULES OF WORK WITH THE MICROSCOPE

It is necessary to remember

When working with a microscope is necessary to observe operations in the following order:

1. To work with the microscope should be sitting;
2. Microscope to view, to wipe the dust with a soft cloth the lens, eyepiece, mirror;
3. Microscope set in front of him, slightly to the left at 2-3 cm from the edge of the table. During operation it does not move;
4. Fully open the aperture, raise the condenser to its highest position;
5. Work with the microscope always start with a small increase;
6. Lower the lens 8 x in operating position, i.e. at a distance of 1 cm from the slide;
7. Looking one eye in the eyepiece and using the mirror with the concave side, to guide the light from the window in the lens, and then maximally and uniformly illuminate a field of view;
8. Put microreport on the object table so that the object under study was under the lens. Looking from the side, lower the objective lens using macavinta as long as the distance between the lower lens and micropreparation becomes 4-5 mm;
9. One eye looking into the eyepiece and rotate the coarse focusing screw on, smoothly picking up the lens to the position at which the good will be seen the image of the object. You cannot look into the eyepiece and lower the lens. The front lens can crush the coverslip, and show scratches;
10. Moving the drug hand to find the right place, place it in the center of the field of view of the microscope;
11. If the image does not appear, it is necessary to repeat the operations of paragraphs 6, 7, 8, 9;
12. To examine the object at high magnification first you need to put the selected area in the center of the field of view of the microscope at low magnification. Then to change the lens 40 on x by rotating the revolver, so he took a working position. Using micropatronage screw to get a good image of the object. On the box micropatronage mechanism, there are two risks, and micrometres the screw - point, which is between the risks. If it goes beyond that, it must be returned to the normal position. Failure to comply with this rule, micrometry the screw may stop working;
13. After working with a large increase, set a small increase, to raise the lens to remove from the work table agent, then wipe with a clean cloth all the parts of the microscope, cover it with a plastic bag and put in the closet.

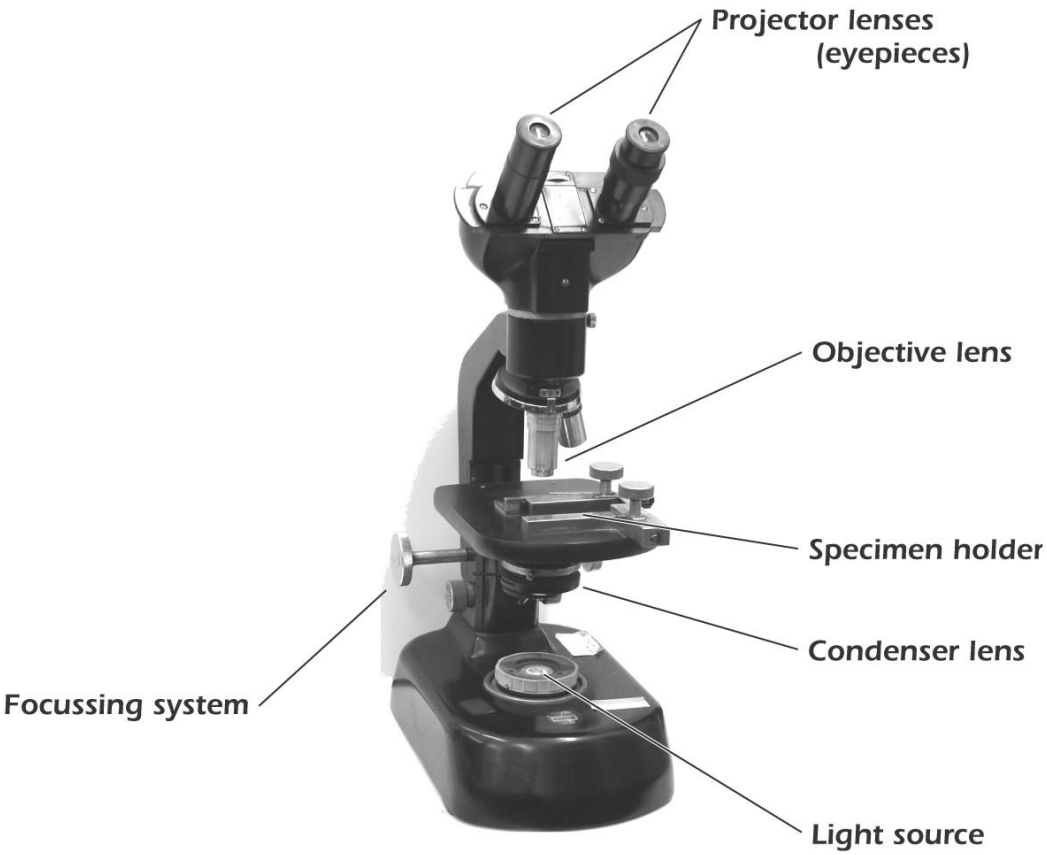


Figure 1. A simple upright light microscope.

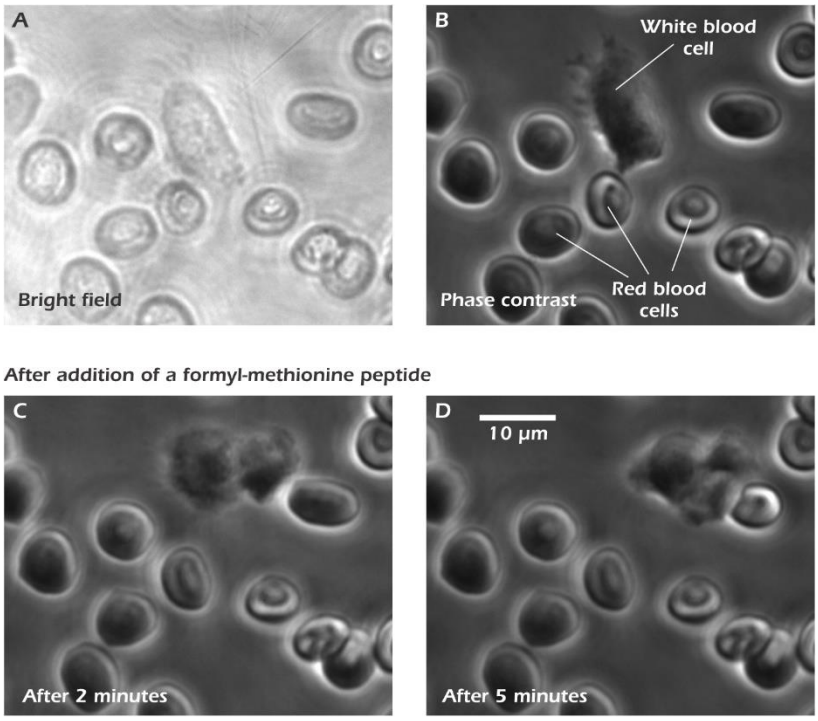


Figure 2. Human blood cells viewed by bright-field (A) and phasecontrast (B) light microscopy. Thin extensions of the white blood cell are clear in the phase contrast image but invisible in the bright field image. (C) and (D) are phase contrast images acquired 2 and 5 minutes after addition of a formyl methionine peptide (see page 128). The white blood cell is activated and begins crawling to the right.

The Fluorescence Microscopy

Fluorescent molecules emit light when they are illuminated with light of a shorter wavelength (Fig. 3). Familiar examples are the hidden signature in bank passbooks, which is written in fluorescent ink that glows blue (wavelength about 450 nm) when illuminated with ultraviolet light (UV) (wavelength about 360 nm), and the whitener in fabric detergents that causes your white shirt to glow blue when illuminated by the ultraviolet light in a club. The fluorescent dye Hoechst 33342 has a similar wavelength dependence: It is excited by UV light and emits blue light. However, it differs from the dyes used in ink or detergent in that it binds tightly to the DNA in the nucleus and only fluoresces when so bound. Diagram a shows the optical path through a microscope set up so as to look at a preparation stained with Hoechst. White light from an arc lamp passes through an excitation filter that allows only UV light to pass. This light then strikes the heart of the fluorescent microscope: a special mirror called a dichroic mirror that reflects light of wavelengths shorter than a designed cutoff but transmits light of longer wavelength. To view Hoechst, we use a dichroic mirror of cutoff wavelength 400 nm, which therefore reflects the UV excitation light up through the objective lens and onto the specimen. Any Hoechst bound to DNA in the preparation will emit blue light. Some of this will be captured by the objective lens and, because its wavelength is greater than 400 nm, will not be reflected by the dichroic mirror but will instead pass through. An emission filter, set to pass only blue light, cuts out any scattered UV light. The blue light now passes to the eye or camera in the usual way. Image b shows a field of cells cultured from rat brain (gift of Dr. Charles Krieger, Simon Fraser University) after staining with Hoechst. Only the nuclei are seen, as bright ovals.

Although some of the structures and chemicals found in cells can be selectively stained by specific fluorescent dyes, others are most conveniently revealed by using **antibodies.** In this technique an animal (usually a mouse, rabbit, or goat) is injected with a protein or other chemical of interest. The animal's immune system recognizes the chemical as foreign and generates antibodies that bind to (and therefore help neutralize) the chemical. Some blood is then taken from the animal and the antibodies purified. The antibodies can then be labeled by attaching a fluorescent dye. Images c and d show the same field of brain cells but with the excitation filter, dichroic mirror, and emission filter changed so as to reveal in c a protein called ELAV that is found only in nerve cells; then in d an intermediate filament protein (page 000) found only in glial cells. The antibody that binds to ELAV is labeled with a fluorescent dye that is excited by blue light and emits green light. The antibody that binds to the glial filaments is labeled with a dye that is excited by green light and emits red light. Because these wavelength characteristics are different, the location of the three chemicals—DNA, ELAV, and intermediate filament—can be revealed independently in the same specimen. See the CBASC website for an image of all three signals in color and superimposed.

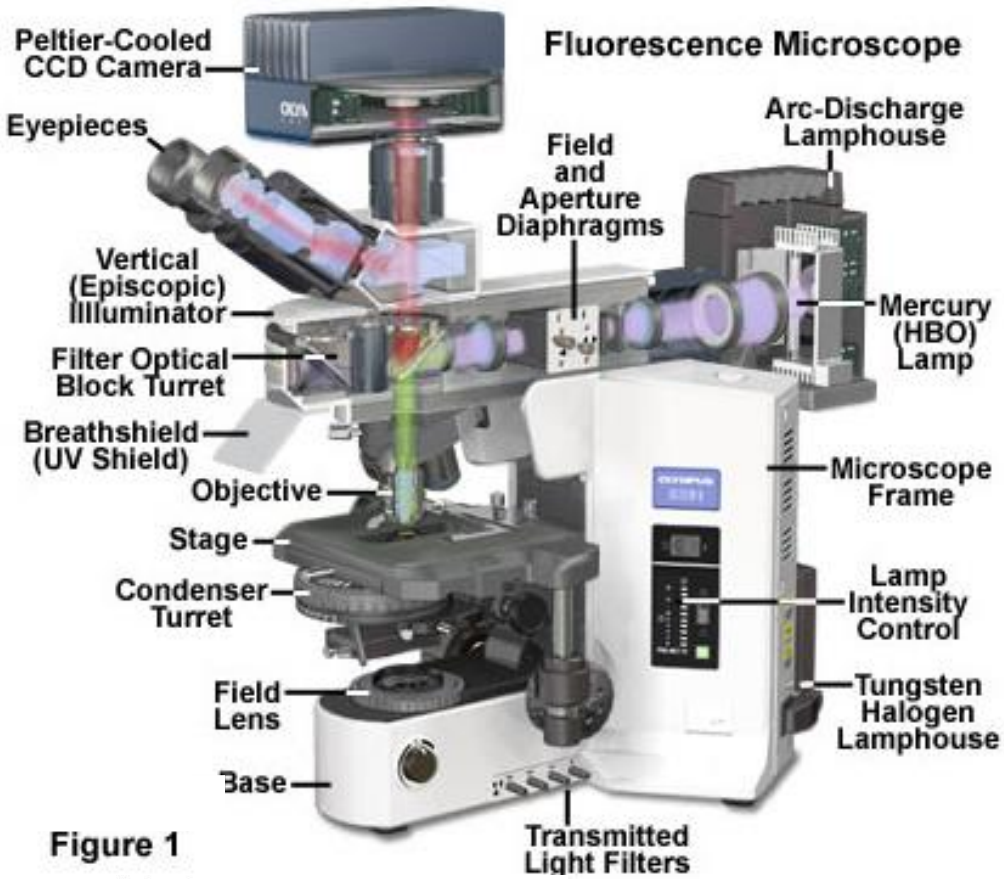
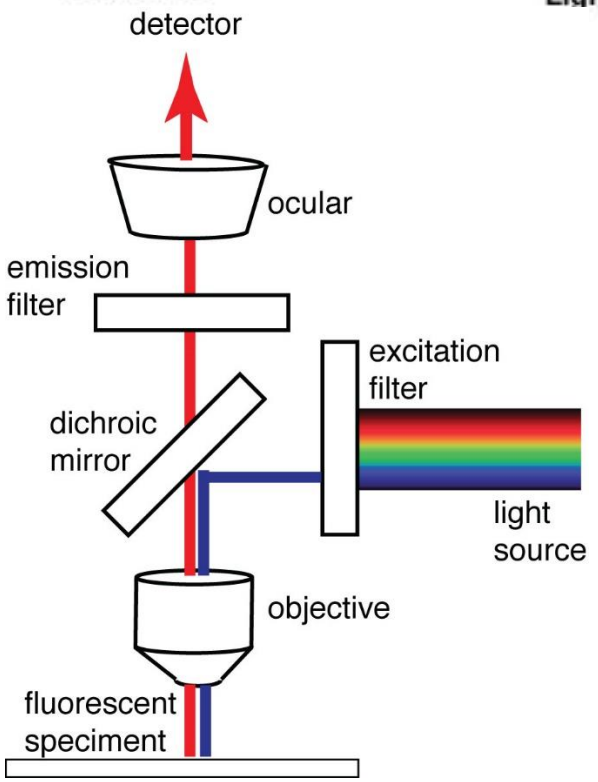
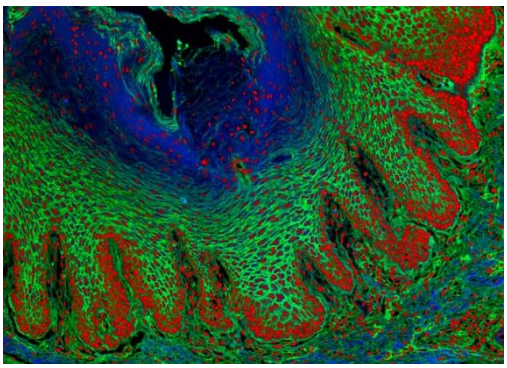


Figure 1





Intestine – digital fluorescence microscopy. This method allows obtaining images with a strong color contrast.

Figure 2. Fluorescence microscope & it optical scheme.

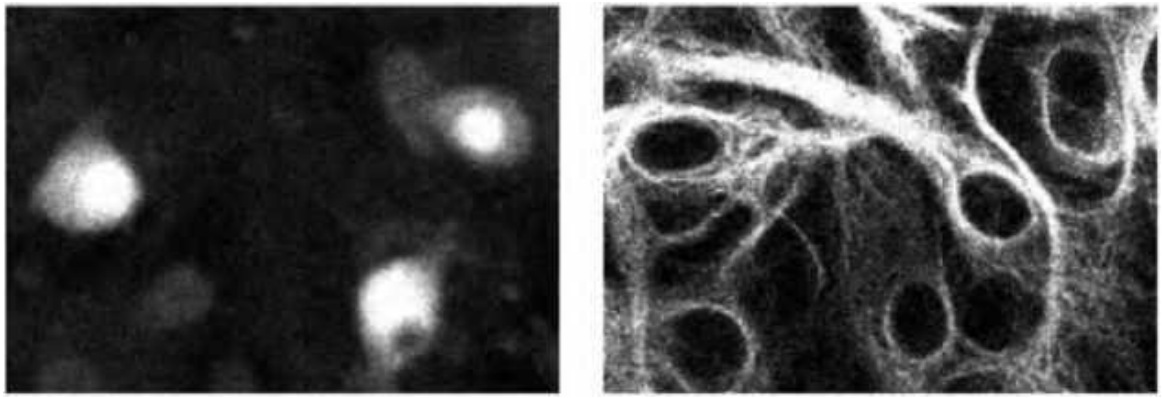


Figure 3. Immunofluorescence microscopy.
On the left is the glow of cell nuclei labeled with fluorescent antibodies to nucleic acids. To the right is the glow of cell stroma.

The technique just described is called **primary immunofluorescence** (Fig. 3) and requires that the antibody to the chemical of interest be labeled with a dye. Only antibodies to chemicals that many laboratories study are so labeled. In order to reveal other chemicals, scientists use **secondary immunofluorescence.** In this approach, a commercial company injects an animal (e.g., a goat) with an antibody from another animal (e.g., a rabbit). The goat then makes "goat anti rabbit" antibody. This, called the **secondary antibody,** is purified and labeled with a dye. All the scientist has to do is make or buy a rabbit antibody that binds to the chemical of interest. No further modification of this specialized, **primary antibody** is necessary. Once the primary antibody has bound to the specimen and excess antibody rinsed off, the specimen is then exposed to the secondary antibody that binds selectively to the primary antibody. Viewing the stained preparation in a fluorescence microscope then reveals the location of the chemical of interest. The same dye-labeled secondary antibody can be used in other laboratories or at other times to reveal the location of many different chemicals because the unlabeled primary antibody determines the specificity.

The Electron Microscope

The most commonly used type of electron microscope in biology is called the **transmission electron microscope** because electrons are transmitted through the specimen to the observer. The transmission electron microscope has essentially the same design as a light microscope, but the lenses, rather than being glass, are electromagnets that bend beams of electrons (Fig. 1*3b).* An electron gun generates a beam of electrons by heating a thin, V-shaped piece of tungsten wire to 3000°C. A large voltage accelerates the beam down the microscope column, which is under vacuum because the electrons would be slowed and scattered if they collided with air molecules. The magnified image can be viewed on a fluorescent screen that emits light when struck by electrons. While the electron microscope offers great improvements in resolution, electron beams are potentially highly destructive, and biological material must be subjected to a complex processing schedule before it can be examined. The preparation of cells for electron microscopy is summarized

(Fig. 4).
A small piece of tissue (~1 mm^3) is immersed in glutaraldehyde and osmium tetroxide. These chemicals bind all the component parts of the cells together; the tissue is said to be **fixed.** It is then washed thoroughly.
The tissue is **dehydrated** by soaking in acetone or ethanol.
The tissue is **embedded** in resin which is then baked hard.
Sections (thin slices less than 100 nm thick) are cut with a machine called an ultramicrotome.
The sections are placed on a small copper grid and **stained** with uranyl acetate and lead citrate. When viewed in the electron microscope, regions that have bound lots of uranium and lead will appear dark because they are a barrier to the electron beam.

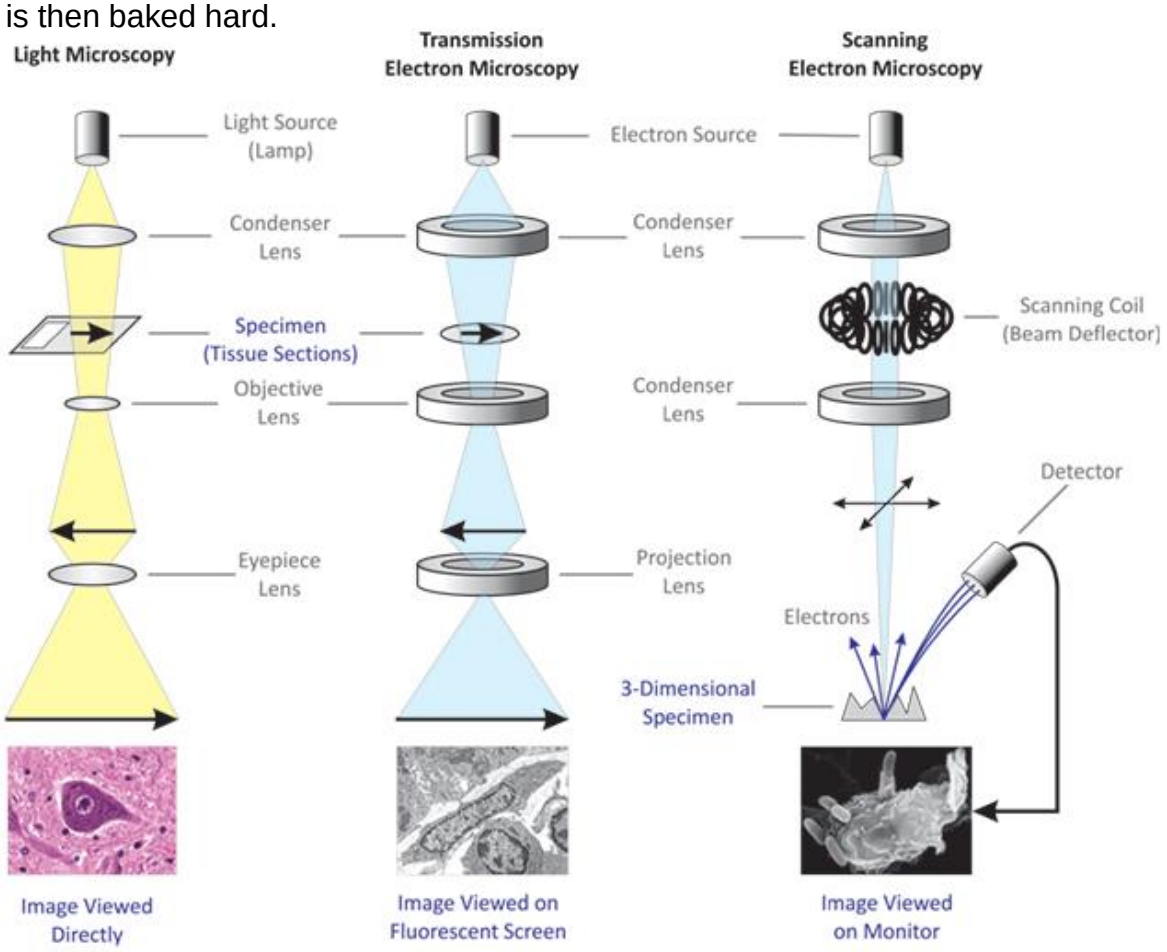


Figure 4. Comparison of light and electron microscopy.

The transmission electron microscope produces a detailed image but one that is static, two-dimensional, and highly processed. Often, only a small region of what was once a dynamic, living, three-dimensional cell is revealed. Moreover, the picture revealed is essentially a snapshot taken at the particular instant that the cell was killed. Clearly, such images must be interpreted with great care. Electron microscopes are large and require a skilled operator. Nevertheless, they are the main source of information on the structure of the cell at the nanometer scale, called the **ultrastructure.**

The Scanning Electron Microscope

Whereas the image in a transmission electron microscope is formed by electrons transmitted through the specimen, in the scanning electron microscope it is formed from electrons that are reflected back from the surface of a specimen as the electron beam scans rapidly back and forth over it (Fig. 4). These reflected electrons are processed to generate a picture on a display monitor. The scanning electron microscope operates over a wide magnification range, from 10 x to 100.000 x. Its greatest advantage, however, is a large depth of focus that gives a three-dimensional image. The scanning electron microscope is particularly useful for providing topographical information on the surfaces of cells or tissues. Modem instruments have a resolution of about 1 nm.

The Confocal Microscope

Confocal fluorescence microscopy is a microscopic technique that provides true three-dimensional (3D) optical resolution. In microscopy, 3D resolution is generally realized by designing the instrument so that it is primarily sensitive to a specimen's response coming from an in-focus plane, or by subsequently removing the contributions from out-of-focus planes. Several techniques have been developed to achieve this. For instance, 3D deconvolution [Agard and Sedat, 1983] uses both in- and out-of-focus information from a stack of images, taken at various focal planes, to reconstruct the 3D image. Another example is two- and three-photon absorption microscopy [Denk et al., 1990; Hell et al., 1996], where a nonlinear interaction with the specimen is used to confine the specimen's response to the focal plane only. In confocal fluorescence microscopy, true 3D resolution is accomplished by actively suppressing any signal coming from out-of-focus planes. This is achieved by using a pinhole in front of the detector as schematically depicted in Fig. 5. Light originating from an in-focus plane is imaged by the microscope objective such that it freely passes the pinhole, whereas light coming from out-of-focus planes is largely blocked by the pinhole. In a confocal fluorescence microscope (Fig. 5), the specimen is generally illuminated by a laser. The term "excitation" rather than "illumination" will be used in what follows, since it more explicitly refers to the contrast-generating process: the excitation of fluorophores, through absorption, causing detectable fluorescence. The light coming from the laser passes through an (excitation) pinhole, is reflected by a dichroic mirror, and focused by a microscope objective to a small spot in the specimen. The dichroic mirror reflects light of a shorter wavelength (e.g., 488 nm from an Argon-ion laser) while transmitting that of a longer wavelength (e.g., the fluorescence >510 nm from fluorescein). Specific dichroic mirrors can be made for the relevant wavelength regions of excitation and fluorescence.

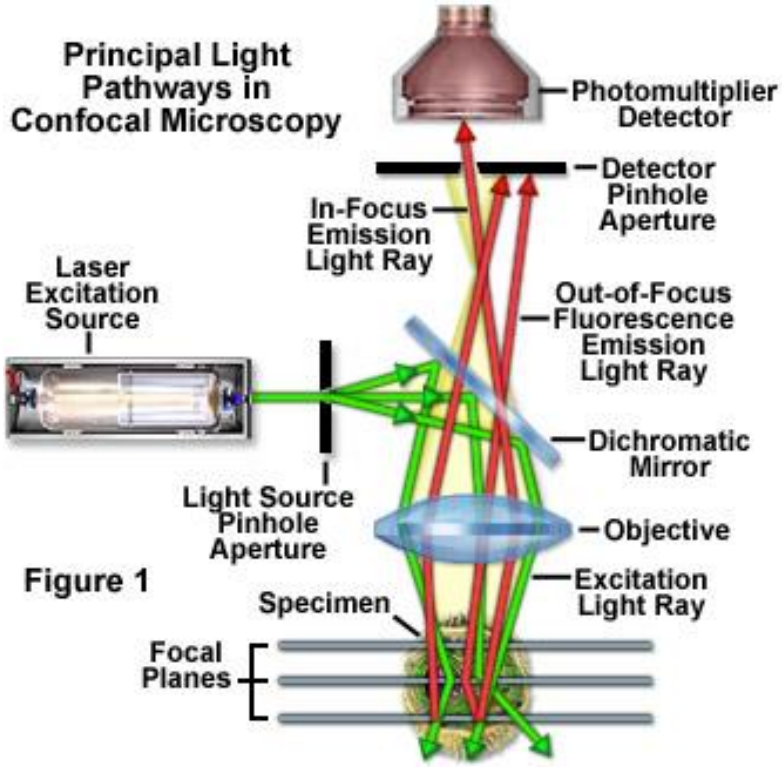
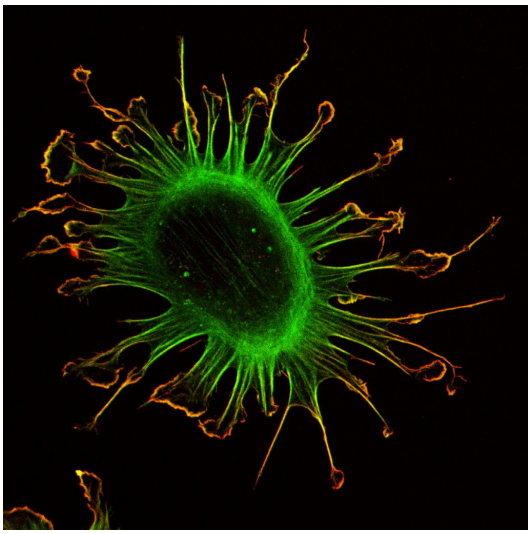
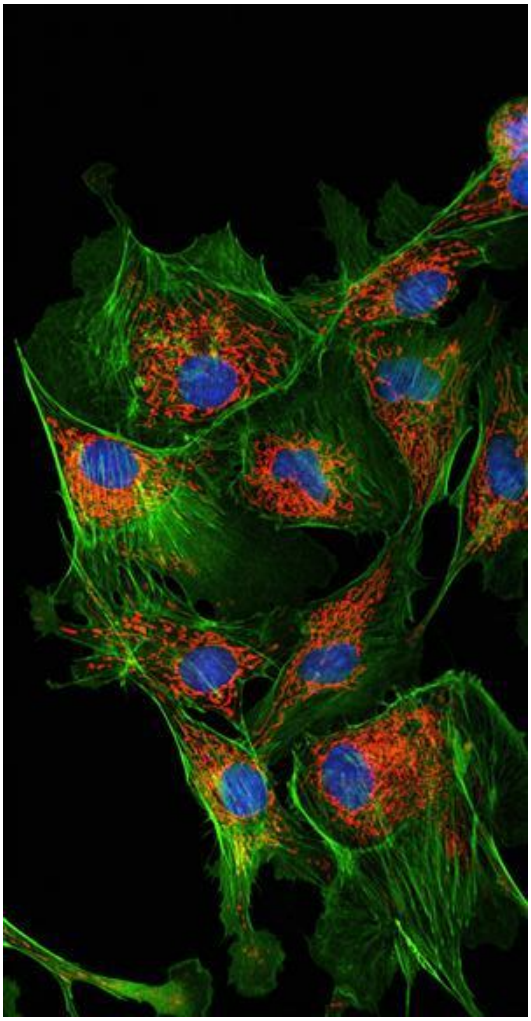


Figure 5. Confocal microscope & it optical scheme.



Digital reconstruction cells with using confocal microscopy



LESSON 2. CELLS AND TISSUES

The **cell** is the basic unit of life. Microorganisms such as bacteria, yeast, and amoebae exist as single cells. By contrast, the adult human is made up of about 30 trillion cells (1 trillion = 10^{12}) which are mostly organized into collectives called **tissues.** Cells are, with a few notable exceptions, small (Fig. 6) with lengths measured in micrometers (/xm, where 1000 /xm = 1 mm) and their discovery stemmed from the conviction of a small group of seventeenth-century microscope makers that a new and undiscovered world lay beyond the limits of the human eye. These pioneers set in motion a science and an industry that continues to the present day.

The first person to observe and record cells was Robert Hooke (1635-1703) who described the *cella* (open spaces) of plant tissues. But the colossus of this era of discovery was a Dutchman, Anton van Leeuwenhoek (1632-1723), a man with no university education but with unrivaled talents as both a microscope maker and as an observer and recorder of the microscopic living world, van Leeuwenhoek was a contemporary and friend of the Delft artist Johannes Vermeer (1632-1675) who pioneered the use of light and shade in art at the same time that van Leeuwenhoek was exploring the use of light to discover the microscopic world. Sadly, none of van Leeuwenhoek's microscopes have survived to the present day. Despite van Leeuwenhoek's Herculean efforts, it was to be another 150 years before, in 1838, the botanist Matthias Schleiden and the zoologist Theodor Schwann formally proposed that all living organisms are composed of cells. Their "cell theory," which nowadays seems so obvious, was a milestone in the development of modem biology. Nevertheless general acceptance took many years, in large part because the **plasma membrane,** the membrane surrounding the cell that divides the living inside from the nonliving **extracellular medium** (Fig. 7) is too thin to be seen using a light microscope.

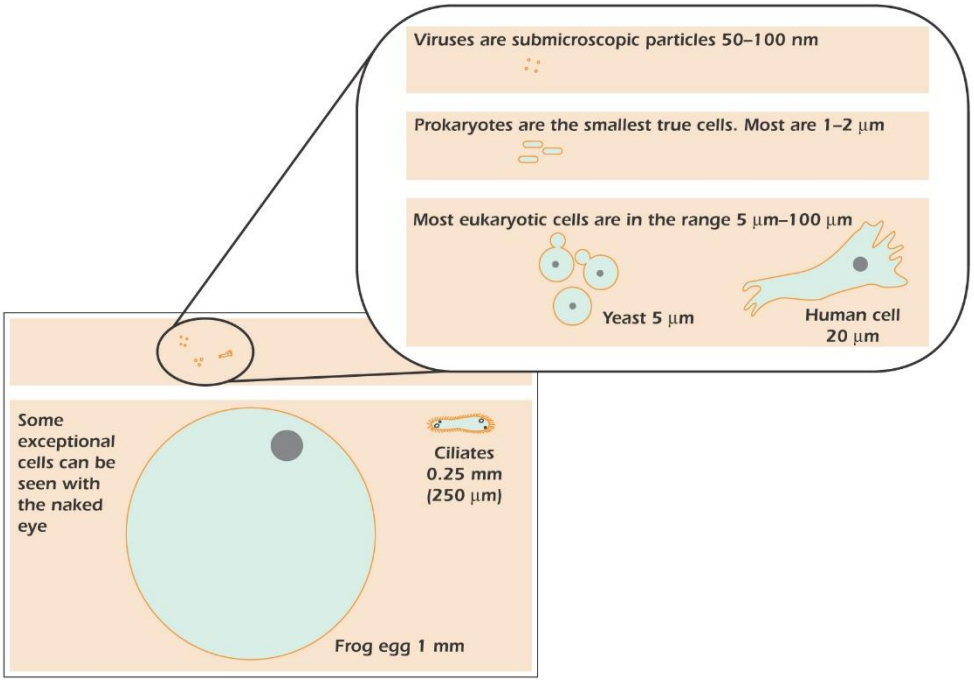


Figure 6. Dimensions of some example cells. 1 mm = 10^{-3} m; 1 μm = 10^{-6} m; 1 nm = 10^{-9} m.

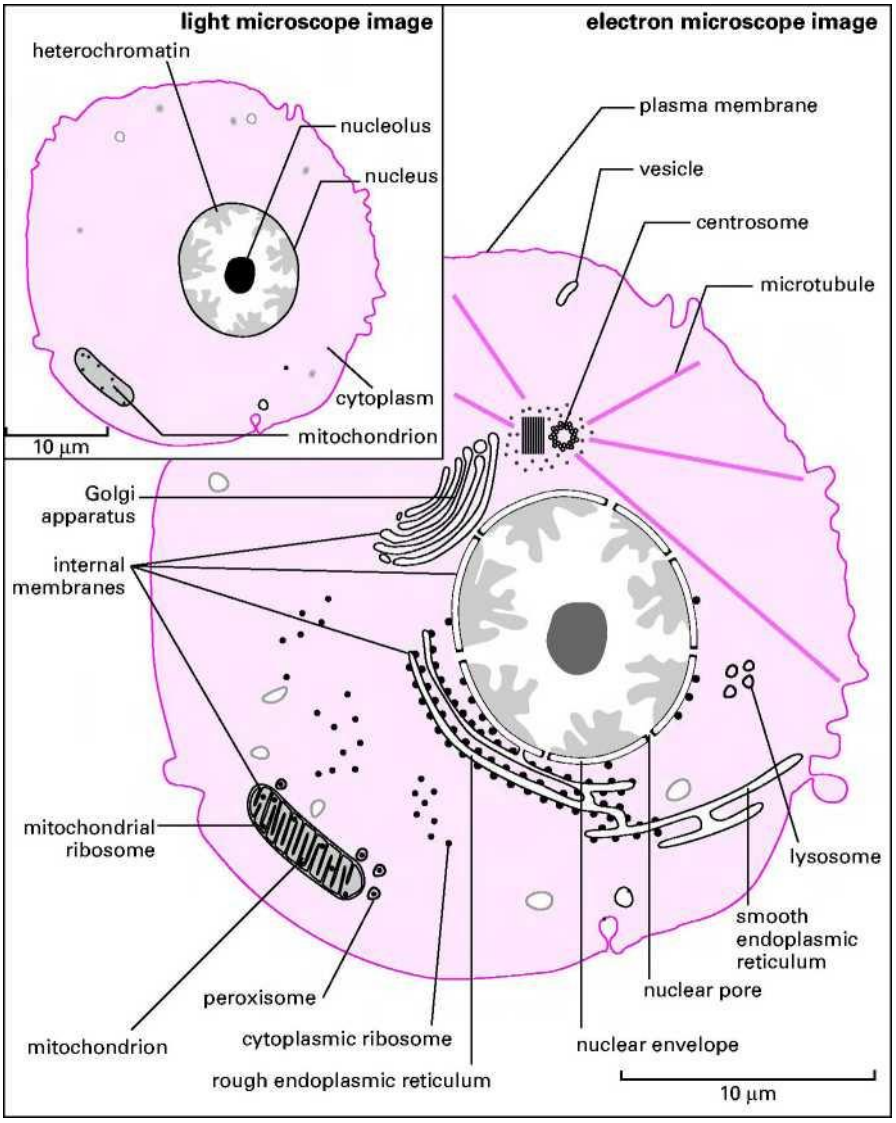


Figure 7. Cell structure as seen through the light and transmission electron microscopes.

ONLY TWO TYPES OF CELL

Superficially at least, cells exhibit a staggering diversity. Some lead a solitary existence; others live in communities; some have defined, geometric shapes; others have flexible boundaries; some swim, some crawl, and some are sedentary; many are green (some are even red, blue, or purple); others have no obvious coloration. Given these differences, it is perhaps surprising that there are only two types of cell (Fig. 8). Bacterial cells are said to be **prokaryotic** (Greek for "before nucleus") because they have very little visible internal organization so that, for instance, the genetic material is free within the cell. They are also small, the vast majority being 1-2 μm in length.

The cells of all other organisms, from protists to mammals to fungi to plants, are **eukaryotic** (Greek for "with a nucleus"). These are generally larger **(5-100** μm, although some eukaryotic cells are large enough to be seen with the naked eye; Fig. 6) and structurally more complex. Eukaryotic cells contain a variety of specialized structures known collectively as **organelles,** surrounded by a viscous substance called cytosol.

The largest organelle, the **nucleus,** contains the genetic information stored in the molecule deoxyribonucleic acid (DNA). The structure and function of organelles will be described in detail in subsequent chapters. Table provides a brief glossary of the major organelles and summarizes the differences between prokaryotic and eukaryotic cells.

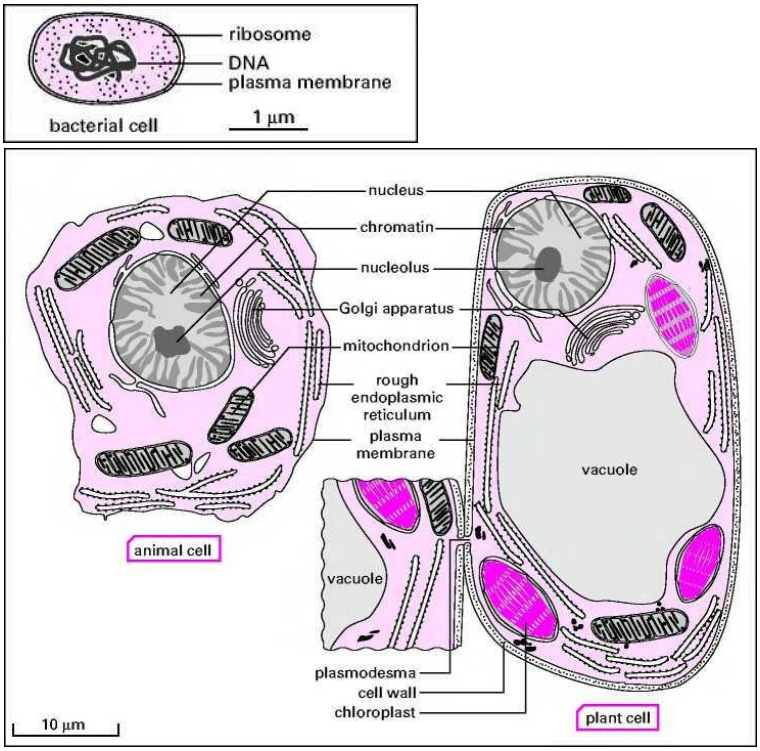


Figure 8. Organization prokaryotic and eukaryotic cells.

Table. Differences Between Prokaryotic and Eukaryotic Cells

	Prokaryotes	Eukaryotes
Size	Usually 1-2 μm	Usually 5-100 μm
Nucleus	Absent	Present, bounded by nuclear envelope
DNA	Usually a single circular molecule (=chromosome)	Multiple molecules (=chromosomes), linear, associated with protein. [a]
Cell division	Simple fission	Mitosis or meiosis
Internal membranes	Rare	Complex (nuclear envelope, Golgi apparatus, endoplasmic reticulum, etc.—Fig. 7)
Ribosomes	70S[b]	80S (70S in mitochondria and chloroplasts)
Cytoskeleton	Absent	Microtubules, microfilaments, intermediate filaments
Motility	Rotary motor (drives bacterial flagellum)	Dynein (drives cilia and eukaryote flagellum); kinesin, myosin
First appeared	3.5 x 10^9 years ago	1.5 x 10^9 years ago

[a] The tiny chromosomes of mitochondria and chloroplasts are exceptions; like prokaryotic chromosomes they are often circular.

[b]The S value, or Svedberg unit, is a sedimentation rate. It is a measure of how fast a molecule moves in a gravitational field, and therefore in an ultracentrifuge.

Sterilization by Filtration

Because even the smallest cells are larger than 1 μm, harmful bacteria and protists can be removed from drinking water by passing through a filter with 200-nm-diameter holes. Filters can vary in size from huge, such as those used in various commercial processes, to small enough to be easily transportable by backpackers. Filtering drinking water greatly reduces the chances of bringing back an unwanted souvenir from your camping trip!

Special Properties of Plant Cells

Among eukaryotic cells the most striking difference is between those of animals and plants (Fig. **1.7).** Plants have evolved a sedentary lifestyle and a mode of nutrition that means they must support a leaf canopy. Their cells are enclosed within a rigid cell wall that gives shape to the cell and structural rigidity to the organism (page **53).** This is in contrast to the flexible boundaries of animal cells. Plant cells frequently contain one or more **vacuoles** that can occupy up to **75%** of the cell volume. Vacuoles accumulate a high concentration of sugars and other soluble compounds. Water enters the vacuole to dilute these sugars, generating hydrostatic pressure that is counterbalanced by the rigid wall. In this way the cells of the plant become stiff or turgid, in the same way that when an inner tube is inflated inside a bicycle tire the combination becomes stiff. Vacuoles are often pigmented, and the spectacular colors of petals and fruit reflect the presence of compounds such as the purple anthocyanins in the vacuole. Cells of photosynthetic plant tissues contain a special organelle, the **chloroplast,** that houses the light-harvesting and carbohydrate-generating systems of **photosynthesis.** Plant cells lack **centrosomes** (Fig. **1.2)** although these are found in many algae.

VIRUSES

Viruses occupy a unique space between the living and nonliving worlds. On one hand they are made of the same molecules as living cells. On the other hand they are incapable of independent existence, being completely dependent on a host cell to reproduce. Almost all living organisms have viruses that infect them. Human viruses include polio, influenza, herpes, rabies, ebola, smallpox, chickenpox, and the AIDS (acquired immunodeficiency syndrome) vims HIV (human immunodeficiency vims). Viruses are submicroscopic particles consisting of a core of genetic material enclosed within a protein coat called the capsid. Some viruses have an extra membrane layer called the envelope. Viruses are metabolically inert until they enter a host cell, whereupon the viral genetic material directs the host cell machinery to produce viral protein and viral genetic material. Viruses often insert their genome into that of the host, an ability that is widely made use of in molecular genetics (Chapter 7). Bacterial viruses, called bacteriophages, are used by scientists to transfer genes between bacterial strains. Human viruses are used as vehicles for gene therapy. By exploiting the natural infection cycle of a vims such as adenovirus, it is possible to introduce a functional copy of a human gene into a patient suffering from a genetic disease such as cystic fibrosis.

ORIGIN OF EUKARYOTIC CELLS

Prokaryotic cells are simpler and more primitive in their organization than eukaryotic cells. According to the fossil record, prokaryotic organisms antedate, by at least 2 billion years, the first eukaryotes that appeared some 1.5 billion years ago. It seems highly likely that eukaryotes evolved from prokaryotes, and the most likely explanation of this process is the **endosymbiotic theory.** The basis of this hypothesis is that some eukaryotic organelles originated as free-living prokaryotes that were engulfed by larger cells in which they established a mutually beneficial relationship. For example, **mitochondria** would have originated as free-living aerobic bacteria and **chloroplasts** as cyanobacteria, photosynthetic prokaryotes formerly known as blue-green algae.

The endosymbiotic theory provides an attractive explanation for the fact that both mitochondria and chloroplasts contain DNA and ribosomes of the prokaryotic type (Table 1.1). The case for the origin of other eukaryotic organelles is less persuasive. While it is clearly not perfect, most biologists are now prepared to accept that the endosymbiotic theory provides at least a partial explanation for the evolution of the eukaryotic cell from a prokaryotic ancestor. Unfortunately, living forms having a cellular organization intermediate between prokaryotes and eukaryotes are rare. Some primitive protists possess a nucleus but lack mitochondria and other typical eukaryotic organelles. They also have the prokaryotic type of ribosomes. These organisms are all intracellular parasites and they include *Microspora,* an organism that infects AIDS patients.

CELL SPECIALIZATION

All the body cells that comprise a single organism share the same set of genetic instructions in their nuclei. Nevertheless, the cells are not all identical. Rather, plants and animals are composed of different **tissues,** groups of cells that are specialized to carry out a common function. This specialization occurs because different cell types read out different parts of the DNA blueprint and therefore make different proteins, as we will see in Chapter 6. In animals there are four major tissue types: epithelium, connective tissue, nervous tissue, and muscle.

Epithelia

Epithelia are sheets of cells that cover the surface of the body and line its internal cavities such as the lungs and intestine. The cells may be **columnar,** taller than they are broad, or **squamous,** meaning flat. In the intestine, the single layer of columnar cells lining the inside, or **lumen,** has an absorptive function that is increased by the folding of the surface into **villi** (Fig. 1.8). The luminal surfaces of these cells have **microvilli** that increase the surface area even further. The basal surface sits on a supporting layer of extracellular fibers called the **basement membrane.** Many of the epithelial cells of the airways, for instance, those lining the trachea and bronchioles, have **cilia** on their surfaces. These are hairlike appendages that actively beat back and forth, moving a layer of mucus away from the lungs (Chapter 18). Particles and bacteria are trapped in the mucus layer, preventing

them from reaching the delicate air exchange membranes in the lung. In the case of the skin, the epithelium is said to be **stratified** because it is composed of several layers.

Connective Tissue

Connective tissues provide essential support for the other tissues of the body. They include bone, cartilage, and adipose (fat) tissue. Unlike other tissues, connective tissue contains relatively few cells within a large volume of **extracellular matrix** that consists of different types of fiber embedded in **amorphous** ground substance (Fig. 1.8). The most abundant of the fibers is **collagen,** a protein with the tensile properties of steel that accounts for about a third of the protein of the human body. Other fibers have elastic properties that permit the supported tissues to be displaced and then to return to their original position. The amorphous ground substance absorbs large quantities of water, facilitating the diffusion of metabolites, oxygen, and carbon dioxide to and from the cells in other tissues and organs. Of the many cell types found in connective tissue, two of the most important are **fibroblasts,** which secrete the ground substance and fibers, and **macrophages,** which remove foreign, dead, and defective material from it. A number of inherited diseases are associated with defects in connective tissue. Marfan's syndrome, for example, is characterized by long arms, legs, and torso and by a weakness of the cardiovascular system and eyes. These characteristics result from a defect in the organization of the collagen fibers.

Nervous Tissue

Nervous tissue is a highly modified epithelium that is composed of several cell types. Principal among these are the **nerve cells,** also called **neurons,** along with a variety of supporting cells that help maintain them. Neurons extend processes called **axons,** which can be over a meter in length. Neurons constantly monitor what is occurring inside and outside the body. They integrate and summarize this information and mount appropriate responses to it (Chapters 15-17). Another type of cell called **glia** has other roles in nervous tissue including forming the electrical insulation around axons.

Muscle

Muscle tissue can be of two types, **smooth** or **striated.** Smooth muscle cells are long and slender and are usually found in the walls of tubular organs such as the intestine and many blood vessels. In general, smooth muscle cells contract slowly and can maintain the contracted state for a long period of time. There are two classes of striated muscle: **cardiac** and **skeletal.** Cardiac muscle cells make up the walls of the heart chambers. These upper surface of leaf cuticle epidermis — parenchyma cells are branched cells that are connected electrically by gap junctions (page 55), and then- automatic rhythmical contraction powers the beating of the heart. Each skeletal muscle is a bundle of hundreds to thousands of fibers, each fiber being a giant single cell with many nuclei. This rather unusual situation is the result of an event that occurs in the

embryo when the cells that give rise to the fibers fuse together, pooling their nuclei in a common cytoplasm**.**

Plants

Plant cells are also organized into tissues (Fig. 1.9). The basic organization of a shoot or root is into an outer protective layer, or **epidermis,** a **vascular tissue** that provides support and transport, and a **cortex** that fills the space between the two. The epidermis consists of one or more layers of closely packed cells. Above the ground these cells secrete a waxy layer, the cuticle, which helps the plant retain water. The cuticle is perforated by pores called stomata that allow gas exchange between the air and the photosynthetic cells and also constitute the major route for water loss by a process called **transpiration.** Below ground, the epidermal cells give rise to root hairs that are important in the absorption of water and minerals. The vascular tissue is composed of **xylem,** which transports water and its dissolved solutes from the roots, and **phloem,** which conveys the products of photosynthesis, predominantly sugars, to their site of use or storage. The cortex consists primarily of **parenchyma cells,** unspecialized cells whose cell walls are usually thin and bendable. They are the major site of metabolic activity and photosynthesis in leaves and green shoots.

SUMMARY

1. All living organisms are made of cells.
2. Our understanding of cell structure and function has gone hand in hand with developments in microscopy and its associated techniques.
3. Light microscopy revealed the diversity of cell types and the existence of the major organelles: nucleus, mitochondrion and, in plants, the vacuole and chloroplast.
4. The electron microscope revealed the detailed structure of the larger organelles and resolved the cell ultrastructure, the fine detail at the nanometer scale.
5. There are two types of cells, prokaryotes and eukaryotes.
6. Prokaryotic cells have very little visible internal organization. They usually measure 1-2 /xm across.
7. Eukaryotic cells usually measure 5-100 /xm across. They contain a variety of specialized internal organelles, the largest of which, the nucleus, contains the genetic material.
8. The endosymbiotic theory proposes that some eukaryotic organelles, such as mitochondria and chloroplasts, originated as free-living prokaryotes.
9. The cells of plants and animals are organized into tissues. In animals there are four tissue types: epithelium, connective tissue, nervous tissue, and muscle. Plants are formed of epidermis, cortex, and vascular tissues.

Modul N 2. **PARASITOLOGY**

LESSON 5. PARASITOLOGY KEY TERMS & SUBKINGDOM PROTOZOA

INTRODUCTION TO PARASITOLOGY

Parasitology, the study of parasites and their relationships to their hosts, is one of the most fascinating areas of the biology. While it is entirely proper to classify many bacteria and fungi and all viruses as parasites, parasitology has traditionally been limited to parasitic protozoa, helminthes, and arthropods, as well as those species of arthropods that serve as vectors for parasites. It follows, then, that parasitology encompasses elements of protozoology, helminthology, and medical arthropodology.
Human parasitology, an important part of parasitology, study the medical parasites including their morphology, life cycle, the relationship with host and environment. The objectives are to study the way or the measurement of parasitic diseases control.

GENERAL CONSIDERATION

It is necessary to remember

Symbiosis means “living together of both members of species. Any organism that spends a portion or all its life intimately associated with another living organism of a different species is known as a symbiont or symbiote), and the relationship is designated as *symbiosis*. The term *symbiosis,* as used here, does not imply mutual or unilateral physiologic dependency; rather, it is used in its original sense without any reference to “benefit” or “damage” to the symbionts. There are at least three categories of symbiosis whose are commonly recognized: commensalisms, mutualism and parasitism.
Commensalism it was from Latin for “eating at same table”, denotes an association which is beneficial to one partner and at least not disadvantageous to the other. The two partners can survive independently.
Mutualism is an association in which the mutualist and the host depend on each other physiologically. It is seen where such associations are beneficial to both organisms.
Parasitism is another type of symbiotic relationship between two organisms: a parasite, usually the smaller of the two, and upon which the parasite is physiologically dependent. The relationship may be permanent, as in the case of tapeworms found in the vertebrate intestine, or temporary, as with female mosquitoes, some leeches, and ticks, which feed intermittently on host blood. In other words, it is a symbiotic relationship in which one animal, the host, is to some degree injured through the activities of the other animal, the parasite.
Parasite Its biological definition is an animal or plant which lives in or upon another organism (technically called its host) and draws its nutriment directly from it. By this definition all infectious agents, viruses, bacteria, fungi, protozoa, and helminths are parasites, but traditionally protozoa, helnimths and medical arthropod, so called parasites, are studied in medical or human Parasitology. Therefore, the textbooks of parasitology today deal only with protozoa, helminthes and some arthropod.
The parasites broadly are of two types: **Endoparasite and Ectoparasite**. The parasite which lives within the host is called the endoparasite. Invasion

by the parasite is called infection. Usually, the endoparasites cause most human diseases. The endoparasites include three types, such as obligate parasite, facultative parasite and accidental parasite:

1. Obligate parasites are physiologically dependent upon their hosts and usually cannot survive if kept isolated from them.

2. Facultative parasites on the other hand, are essentially free-living organisms that are capable of becoming parasitic if placed in a situation conductive to such a mode.

3. Accidental parasites, the parasite that attacks an unusual host. Ectoparasite, the parasite that lives on the outer surface or in the superficial tissues of the host. The infection by these parasites is called infestation.

Host is defined as an organism which harbor's the parasite and provides the nourishment and shelter. These hosts, in comparison to their parasites are relatively larger in size. The hosts may be of the following types: definitive host, intermediate host, reservoir host and paratenic host etc.

1) ***Definitive host.*** The hosts which harbour the adult parasites, most highly developed form of the parasite or where the parasite replicates sexually are called the definitive hosts. The definitive hosts may be human or non-human living things.

2) ***Intermediate host.*** The hosts which harbour the larval stages of parasite development or the asexual forms of the parasite are called intermediate host. Sometimes two different hosts may be required to complete different larval stages. These are known as the first and second intermediate hosts respectively (e.g., snails are the first intermediate hosts and fresh water fish are the second intermediate hosts for *Clonorchis sinensis*).

3) ***Reservoir host.*** The animal which harbours the parasites and serves as an important source of infection to other susceptible hosts are known as reservoir host (e.g., water buffalo is the reservoir host for schistosomiasis.

4) ***Paratenic host or transport host.*** The larva of some parasites can invade a non-normal host, but cannot develop, and only keep the larva stage. If the larva enters a normal definitive host, it can continue to develop into adult worm. The non-normal host is called paratenic host or transport host. It functions as a transport or carrier host.

TAXONOMY

It is necessary to remember

According to the binomial nomenclature as suggested by Linnaeus ("Sistema Nature" 1758), each parasite has two names: a Genus and a Species name. These names are derived either from:

1. Greek or Latin words
2. Names of their discoverers
3. Geographical area where found
4. Hosts in which parasites are found, or
5. Habitat of the parasite

The correct scientific name of the parasite consists of the genus and species to which it belongs, the name of the designator and the year in which it was discovered (e.g., *Angiostrongylus cantonensis* Dougherty, 1946).

The animal parasites of human and most vertebrates are contained in five or more major subdivisions or phyla.

Phylum Sarcomastigophora. This phylum is divided into two subphyla: The Mastigophora or flagellates, and the Sarcodina or amebae.
Phylum Apicomplexa Members of this phylum are tissue parasites. Apicomplexa have a complex life cycle with alternating sexual and asexual generations.
Phylum Microspora Members of the Microspora are minute intracellular parasites of many kinds of vertebrates and invertebrates, and they differ significantly in structure from the Apicomplexa. Microsporidia rarely cause diseases in immunocompetent persons, but many do so with greater frequency in immunosuppressed persons.
Phylum Ciliophora The ciliates include a variety of free-living and symbiotic species. The only ciliate parasite of human is Balantidium coli, found in the intestinal tract. Although rare, it is important, as it may produce severe intestinal symptoms.
Phylum Platyhelminthes The Platyhelminthes, or flatworms, are multicellular animals characterized by a flat, bilaterally symmetric body. Most flatworms are hermaphroditic, having both male and female reproductive organs in the same individual. The sexes are separate in the schistosomes. The classes Trematoda and Cestoda contain parasitic forms only.
Phylum Aschelminthes The nematodes, or roundworms, are elongate, cylindrical worms, frequently attenuated at both ends. The sexes are separate, the male frequently being considerably smaller than the female. A well-developed digestive tract is present. While most nematodes are free-living (e.g., Caenorhabditis elegans), a large number of species parasitize humans, animals, and plants. Intermediate hosts are necessary for the larval development of some forms. Parasites of humans include intestinal and tissue-inhabiting species.
Phylum Acanthocephala The thorny-headed worms are all endoparasite organisms. While thorny-headed worms are widely distributed between wild and domestic animal, only three genera have been reported in human beings including *Macracanthorhynchus hirudinaceus*.
Phylum Arthropoda The phylum is subdivided into a number if classes, many of which are of medical importance. The classes main include the Class Arachnida and Class Insecta. The Arachnida, or spiderlike animals, possess a body divided into two parts, the cephalothorax and the abdomen. Adults have four pairs of legs. Included in this class are the scorpions, the spiders, and the ticks and mites. Certain ticks and mites many transmit diseases. Insects have three pairs of legs and a body divided into three distinct parts: Insects head, thorax, and abdomen. Included in this class are mosquitoes, flies, lice, and bugs etc.

MORPHOLOGY

The protozoa are small, unicellular organisms, which are morphologically and functionally complete. A single cell carries out all the functions such as digestion, respiration, excretion, reproduction, etc. The helminths are larger organisms. A group of special cells performs a particular function such as reproduction, digestion or excretion. Arthropods are segmented and bilaterally symmetrical animals with a body enclosed in a stiff, chitinous covering or exoskeleton and bearing paired jointed appendages. The digestive system is well developed. Sexes are separate.

LIFE CYCLE

The life cycle of a parasite may be simple or complex. In a **simple life cycle** all the developmental stage of the parasite are completed in a single host such as man. Change of host is required only to propagate the parasite in the community. Some of the parasites require two different hosts to complete their various stage of development. In a **complex life cycle** many parasites require two different hosts, one definitive host and one intermediate host to complete their life cycle. Few of the parasite require two different intermediate hosts apart from a single definitive host.

TRANSMISSION OF PARASITES

It is necessary to remember

It depends upon Source or reservoir of infection, and Mode of transmission.

Source of infection

1. Humans is the source or reservoir in a majority of parasitic infections (e.g., taeniasis, amoebiasis, etc). The condition in which the infection is transmitted from one infected man to another man is called anthroponoses.

2. Animal in many of the parasitic diseases, animals act as the source of infection. The condition where infection is transmitted from animals to humans is called zoonoses (e.g., hydatid disease).

Mode of transmission *Transmission of infection from one host to another, cause by a certain form of the parasite is known as the* infective stage*. The infective stage of various parasites many be transmitted from one host to another in the following ways.*

1. *Oral route* Ingestion of food, water and vegetable: The infection is transmitted orally by ingestion of food, water or vegetables contaminated by the faeces that contain the infective stages of the parasite. This mode of transmission is referred to as faecal-oral route (e.g., *cysts of Giardia intestinalis and Entamoeba histolytica ova of Ascaris lumbricoides, Trichuris trichura and Enterobius vermicularis*). Ingestion of raw or undercooked meat: The infection is transmitted orally also by ingestion of raw or undercooked meat harbouring the infective stage of the parasite (e.g., pork containing *cysticercus cellulosae*, the larval stage of *Taenia solium*). Ingestion of raw or uncooked fish and crab: Infection is transmitted by ingestion of raw or under cooked fish and crab containing the infective stage of the parasite (e.g., crab containing the infective stage of the parasite (e.g., crab or cray fish containing the metacercariae of *Paragonimus westerman*, fish harbouring the metacercariae of *Clonorchis sinensis*, etc). Ingestion of raw or under cooked water plants: Infection can be transmitted bt eating raw or under cooked water plants harbouring the infective form of the parasite (e.g., water chest nuts, etc., containing metacercariae of *Fasciolopsis buski* and *Fasciola hepatica*).

1) *Penetration of the skin and mucousmembrane.* The infection is transmitted by:

A) Penetration of the intact skin by filariform larvae of hookworm, *Sreongyloides stercoralis* on coming in contact with faecally polluted soil, and

B) Piercing the skin by cercariae of *Schistosoma japonicum*, *S. mansoni* and *S. haematobium* on coming in contact with infected water.

2) *Inoculation by an arthropod vector* The infection also can be transmitted by:

A) Inoculation into the blood by Anopheles (vector for *Plasmodium*).

B) Inoculation into the skin by mosquitoes (vectors for *Wuchereria bancrofti* etc).

3) ***Sexual contact*** Trichomonas is transmitted by sexual contact. Frequently, Entamoeba also is transmitted by sexual contact among homosexuals.

HOST-PARASITE EXISTENCE

Establishment of the parasite in its host is referred to as an *infection*. The outcome of the infection is highly variable. It may be subclinical latent infection, clinical disease or carrier. The *disease* is the clinical manifestation of the infection which shows the active presence and replication of the parasite causing damage in the host. It may be mild, severe, fulminant, and in some cases may even cause death of the host. The person who is infected with the parasite but without any clinical or sub clinical diseases is referred to as a *carrier.*

PARASITIC ZOONOSES

These are the infections which are naturally transmitted between the vertebrate animals and man. The condition usually includes those infections in which the proof of strong circumstantial evidence of transmission between the man and animals are documented.

PATHOGENESIS AND PATHOLOGY

It is necessary to remember

Pathogenesis of the parasitic diseases is a dynamic process and depends on the complex interaction of a variety of host and parasitic factors.

Host factors The host factor include:

1. Nutritional status of the host, whether malnutrition or under nutrition.
2. Immune response to parasitic infection
3. Immune status of the host whether there is immuno-suppression or not.
4. The presence or absence of the co-existing disease or other physiological conditions such as pregnancy, and
5. The age and level of the immunity at the time of infection.

Parasitic factors The parasitic factors include:

1. Site of the attachment of the parasite and the size of the parasite.
2. Number of invading parasites, and
3. Parasite strain (pathogenic or non-pathogenic) and the growth, development and multiplication of parasites inside the human body and their metabolic products.

The parasites can cause disease in man in various ways as follows:

1. **Trauma** by adult worm, larva, and egg (e.g., hookworm cause oozing of the blood at the site of attachment).
2. **Invasion** and destruction of host cell (*Plasmodium* and *Toxoplasma* are obligate intracellular parasites of man; they produce several enzymes which cause digestion and necrosis of host cells).
3. **Inflammatory** reaction (many of the parasite induce inflammatory reactions in the host leading to the formation of various pathological lesions).
4. **Toxin** (parasites like bacteria also produce toxins but they appear to have a minimal role in the pathogenesis of the disease processes.
5. **Allergic** manifestation (many of the metabolic and excretory products of the parasites absorbed in the circulation, produce a variety of immunological and allergic manifestations in the sensitized hosts).

LESSON 6. PARASITOLOGY. PROTOZOA

Introduction

Protozoa are usually defined as singly celled animals, they belonging to the animal kingdom, subkingdom Protozoa. Structurally, protozoa resemble single metazoan cells; a protozoan cell has a full complement of **cellular organelles,** i.e. **nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus** etc. together with endoplasm and ectoplasm. Functionally, each protozoan cell is equivalent to a whole metazoan animal; the single cell relatively has complex metabolic activities such as digestion, reproduction, respiration, excretion, etc.

At least 45,000 species of protozoan have been described to date, many of which are parasitic. Parasitic protozoa still kill, mutilate, and debilitate more people in the word than any other group of disease organisms. Because of this, studies on protozoa occupy a prominent place in parasitology.

Morphology

Protozoa, that is, the whole body consists of a singular cell. Like a cell in the tissue of metazoa, a protozoan cell is composed of **plasma membrane**, cytoplasm and nucleus.

Plasma membrane: The membrane appears three layered in electron micrographs because the central lipid portion looks light or clear and is enclosed by the darker protein layer. The trilaminar unit membrane support by a sheet of contractile fibrils, which enable the cell to change its shape and help in locomotion protection & nutrition.

Cytoplasm: The cytoplasm matrix consists of very small granules and filaments suspended in al low-density medium with physical properties of a colloid. In some species, the cytoplasm is divisible into two portions: **ectoplasm** and **endoplasm:**

1) *ectoplasm*: The ectoplasm is the outer transparent layer with function of protection, locomotion and sensation. It is often in the gel state.

2) *endoplasm*: The endoplasm is the inner granular layer containing vacuoles and organelles. It is in the sol state of the colloid, and it bears the nucleus, mitochondria, Golgi bodies, and so on. These can only be visualized by electron microscopy. The endoplasm helps in nutrition and reproduction.

3) *organelles*: There are several membrane organelles characteristic of eukaryotes, such as endoplasmic reticulum, mitochondria, various membrane-bound vesicles, and Golgi bodies, are usually found in protozoa. Mitochondria that bear the enzymes of oxidative phosphorylation and tricarboxylic acid cycle often have tubular rather than lamellar cristae in protozoa, although they may be absent.

Protozoa have several **locomotory organelles: flagella**, **cilia**, and **pseudopodia.** Flagella are long delicate thread like filaments; flagella composed a central axoneme and an out sheath that is a continuation of the cell membrane. A flagellum is capable of a variety of movements, which may be fast or slow, forward, backward, lateral, or spiral. Pseudopodia are temporary organelles found in Sarciodina that cause the organism to move and aid it in capturing food. They do not occur in all sarciodines. Cilla are structurally similar to flagella; they are fine needle like filaments covering the entire surface of the body. Some species of protozoa have rudimentary digestive organs

such as cytostome and cytopharynx.
Nuclei: it is the vital structure of a cell. It is present in the endoplasm. A membrane known as nuclear membrane surrounds nucleus externally. In all protozoa excepting *Balantidium coli*, the nucleus is vesicular. Nucleus contains a **karyosome** and chromatin granules. The karyosome is found inside the nucleus either at the centre or at the periphery. The protozoan karyosomes belonging to the **Phylum Apicomplex** (e.g.: malaria parasites) contain DNA; in contrast, the karyosomes of **trypanosomes** and parasitic amoebae do not contain any DNA. Chromatin granules giving the appearance of condensation on linin threads line the nucleus membrane internally. Only a single nucleus is present in most of the protozoa but the ciliates have two nuclei, a small nucleus and a large nucleus. They are homogeneous in composition. In certain protozoa such as trypanosomes, a non-nuclear DNA-containing body called **kinetoplast** is also present in addition to the nucleus.
Trophozoite: It is the reproductive stage of the most protozoa (e.g., intestinal flagellate, amoebae, and ciliates). It is active feeding stage of the parasite and this stage is associated with the pathogenesis of the disease.
Cyst: It is the resistant form of the protozoa with a protective membrane or thickened wall. It is produced during unfavorable circumstances. The protective wall of cyst enables the parasite to survive outside the host in an environment under adverse circumstance for a variable period ranging from a few days to years. The cyst is resting stage of the parasite. Replication usually does not occur in this stage. However, multiplication may occur in the cysts of some species (e.g., *Entamoeba histolytica*), where the nucleus divides to produce asexually. The cystsformed sexually are called oocysts.

Pathologic characterisics of protozoa
Protozoan infections often are chronic lasting months or years. These typically are associated with tissue damage leading to various clinical manifestations of the disease. Various mechanisms are suggested to be responsible for producing tissue damage in the host in many protozoan diseases.
1) ***Multiplication:*** protozoa reproduce in their host, when the number is enough, they may destroy the infected cells, or they may invade other tissue of host, and produce pathological change on host.
2) ***Opportunistic pathogen:*** Some symbiotic protozoa are nonpathogenic or cause only limited clinical symptoms in immunocompetent host, but produce serious symptoms in immunodeficient persons. Protozoan infections produce a variety of clinical manifestations depending upon the tissue affected, the host's immunity state and factors in microenvironment.

Classification of protozoa
The Protozoa are classified into six Phyla by a Committee on Systematica and Evolution of the Society of Protozoolog ists. This classification is based on the morphology of the protozoa as demonstrated by light and electron scanning microscopy. The Sarcomastigophora, the Apicomplexaand Ciliophora, are three important phyla which contain species of medical importance causing disease in man. According their locomotion organelles, protozoa can be divided into four groups: Amoebae, flagellates, ciliates and sporozoan.

ENTAMOEBA HISTOLYTICA

Kingdom: Protista
Subkingdom: Protozoa
Phylum: Sarcomastigophora
Class: Lobosea
Order: Amoebida
Family Entamoebidae
Genus+Species: *Entamoeba Histolytica*

It is necessary to remember

Species in the **Endamoebidae** are parasites or **commensals** of the digestive of **arthropods** and **vertebrates.** The gerera and species are differentiated on the basis of nuclear structure. Species of ***Entamoeba*** are found in both vertebrate and **invertebrate** host. Several species of the protozoan parasite genus *Entamoeba* infect humans, ***Entamoeba coli, Entamoeba hartmanni, Entamoeba dispar*** and *Entamoeba histolytica. E. histolytica* is the only species known to cause disease. The other species are important because they may be confused with *E. histolytica* in diagnostic investigations.
Entamoeba histolytica causes **amoebiasis.** The infection is worldwide in distribution. The parasite is the third leading parasitic cause of death in the developing countries. It remains as an important cause of diarrhoea in homosexual men suffering from the AIDs in the developed countries.
Through the years, it became obvious that *E. histolytica* occurs in two sizes. The **smaller-sized amebas** (**form minuta**) have trophozoites 12 to 15 μm in diameter and cysts 5 to 9 μm wide. This form is encountered in about a third of those who harbor amebas and is not associated with discase. The larger form (**form magna**) has trophozoites 20 to 30 μm in diameter and cysts 10 to 20 μm wide. The larger form sometimes pathogenic.
The small, nonpathogenic type is considered here as a separate specie called *E. dispar*. Ever since *E. histolytica* was first described in association with dysenteric disease by Lesh in 1875, there has been an ongoing discussion as to whether the same species of amoeba, which causes the notable pathological and clinical symptoms of amoebiasis, was also the same one associated with asymptomatic carrier cases. Observations, particularly in more temperate climates, that only a small percentage of people infected with *Entamoeba* exhibited disease symptoms. Only about 10% of people infected with this species present with invasive amoebiasis.

Morphology

The parasite occurs in 3 stage: trophozoite, precyst and cyst.
Trophozoite: it is the invasive form of the parasite and is present in the lumen and in the wall of the large intestine (Fig. 1). The diameter of most trophozoites falls into the range of 20 to 30μm, occasional specimens are small as 10μm or as large as 60μm. In the intestine and in freshly passed, uniformed stools, the parasites actively crawl about, their short, blunt pseudopodia rapidly extending and with drawing. The clear ectoplasm is rather thin but is clearly differentiated from the granular endoplasm. The nucleus is difficult to discern in living specimens, but nuclear morphology may be distinguished after fixing and staining with iron-hematoxylin. The nucleus is spherical and

is about one sixth to one fifth the diameter of the cell. A karyosome is located in the center of the nucleus, and delicate, achromatic fibrils radiate from it to the inner surface of the nuclear membrane. Chromatin is absent from a wide area surrounding the karyosome but is concentrated in granules or plaques on the inner surface of the nuclear membrane. This gives the appearance of a dark circle with a bull's-eye in the center. The nuclear membrane itself is quite thin **Food vacuoles** are common in the cytoplasm of active trophozoites and many contain host erythrocytes in sample from diarrheic stools. Red blood cells may be ingested but do not often appear in chronic infections. The haematophagous trophozoites are the characteristic features of the invasive amoebae.

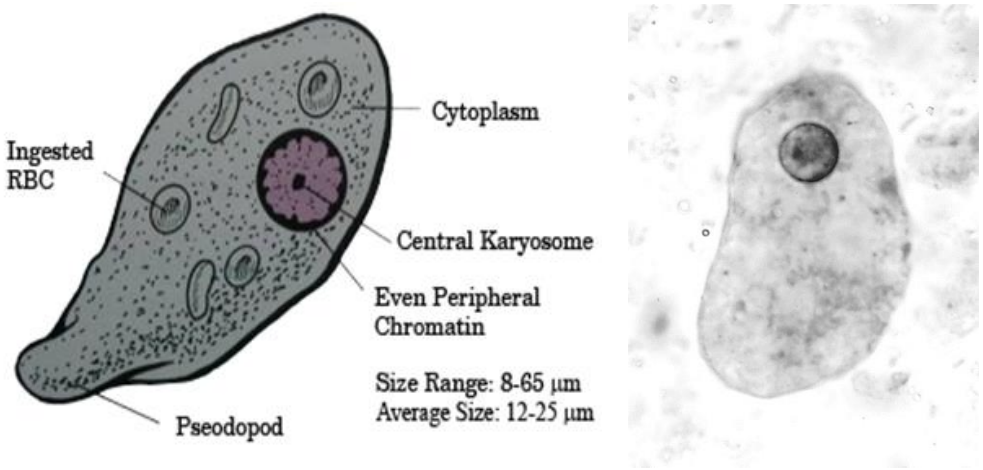


Figure 1. Trophozoite of Entamoeba histolytica.

***Cyst*:** In a normal asymptomatic infection, the amoebae are carried out in formed stools. As the fecal matter passes posteriad and becomes dehygrated, the ameba is stimulated to encyst. Cysts are neither found in the stools of patients with dysentery nor formed by the ameba when they have invaded the tissues of the host. Trophozoite passed in stools are unable to encyst. At the onset of encystment, the trophozoite disgorges any undigested food it may contain and condenses into a sphere, called the **precyst.** A precyst is so rich in glycogen that a large glycogen vacuole may occupy most of the cytoplasm in the young cyst. The **chromatoid bars** that form typically are rounded and ends. The bars may be short and thick, thin and curved, spherical or very irregular in shape, but they do not have splinter-like appearance found in *E. coli.*

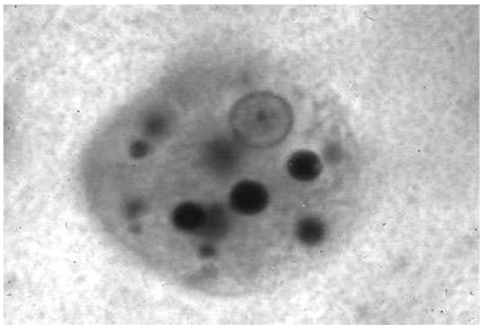


Figure 2. Cyst of *Entamoeba histolytica*

The precyst rapidly secretes a thin, tough hyaline **cyst wall** around itself to form a cyst. The cyst may be somewhat ovoid or elongate, but usually is spheroid. It is commonly 10 to 16 µm wide but may be as small as 5µm. The young cyst has only a single nucleus, but this rapidly divides twice to form two- and four-nucleus stages. As the nuclear division proceeds and the cyst matures, the glycogen vacuole and **chromatoidal bodies** disappear. In semiformed stools one can find precysts and cysts with one to four nuclei, but **quadrinucleate cysts** are most commnon in formed stools. The mature cysts can survive outside the host and can infected a new one.

Life cycle

The life cycle of *E. histolytica* is simple and is completed in a single host, the man (Fig. 3). **Man** is the main and probably the only natural host of *E. histolytica*. Man acquires infection by ingestion of water and food contaminated with mature quadrinucleate cysts. Man also can acquire the infection directly by ano-genital or oro-genital sexual contact. On ingestion, the cyst **excysts** in the small intestine. The cyst wall is lysed by intestinal trypsin liberating a single trophozoite wih four nuclei. The trophozoite quickly undergoes a series of cytoplasmic and nuclear divisions to form eight small metacystic trophozoites. These trophozoites are carried by peristalsis through the small intestine to the ileo-caecal area of the large intestine. Here they grow and multiply by binary fission. They then colonise on the mucosal surfaces and in the crypts of the large intestine. Various factors such as the intestinal motility, the transit time, the presence or absence of specific intestinal flora and the diet of the host influence the colonisation of the trophozoites. In some individuals, the multiplying trophozoites produce no or little lesion if any in tissue. They only feed on the starches and mucus secretions on the surface of mucosa. As trophozoites pass down the colon, they encyst under the stimulus of desiccation and the excreted as cysts with the stool. In other individuals infected under similar conditions, the trophozoites may invade the tissue of the large intestine. The factors those lead to invasion of the intestinal tissue are poorly understood. Trophozoites produce characteristic lesions in the colon, through the stages of gelatinous necrosis, abscess and finally ulcer. A large number of trophozoites are excreted along with blood and mucus in the stool. In a few cases, erosion of the large intestine may be so extensive that trophozoites gain entrance into the radicles of the portal vein and are carried away to the liver where they multiply. Depending upon the complex interaction of various host and parasitic factors, the trophozoites produce suppurative amoebic liver abscesspreceded by non-suppurative infection of the liver.

The **mature cysts** excreted in the feces are the infective forms. They unlike the trophozoites, which degenerate within minutes, may remain viable for weeks or months in suitable moist environment. Cysts of *E. histolytica* can remain viable and infective in a moist, cool environment for at least 12 days, and in water they can live up to 30 days. Putrefaction, desiccation, and temperatures below -5 ℃ and above 40 ℃ rapidly kill them. They can withstand passage through the intestine of flies and cockroaches. The cysts are resistant to levels of chlorine normally used for water purification. These cysts cause infection in other susceptible persons through faecal contamination of water and vegetables or direct faecal-oral contact and the cycle is repeated.

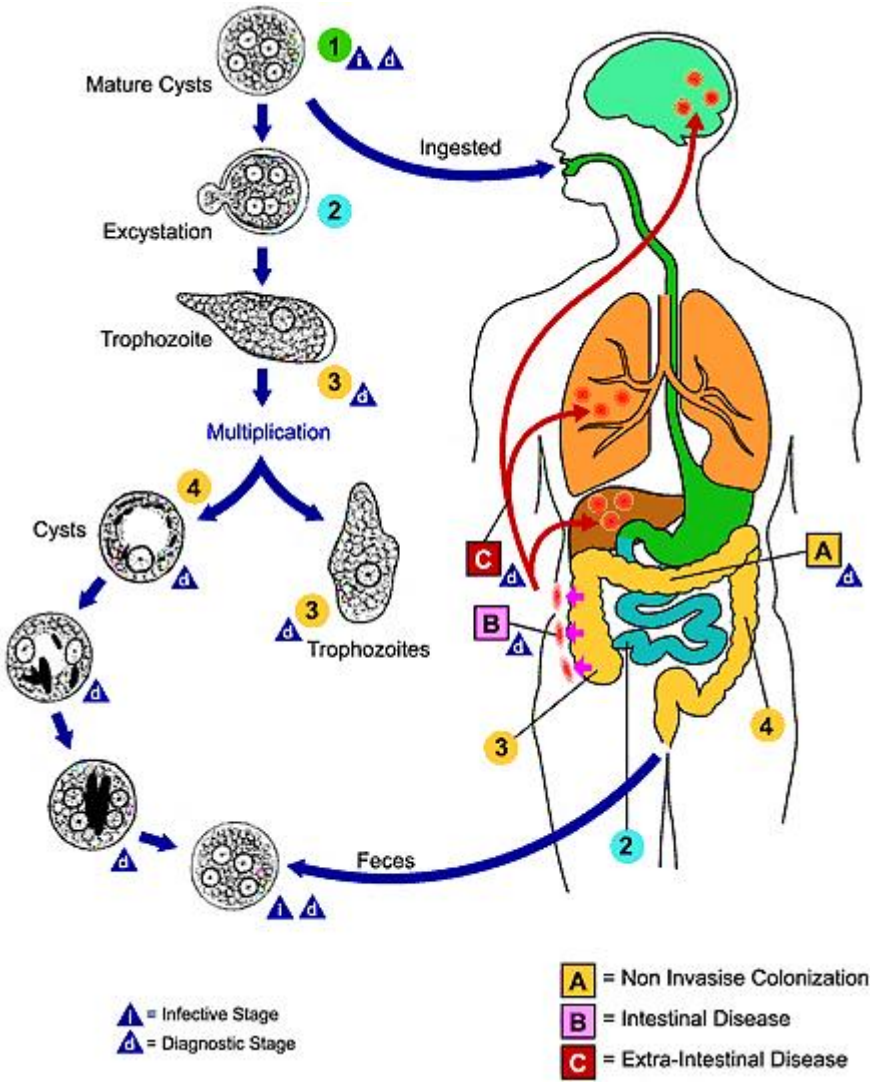


Figure 3. Life cycle of *E. histolytica,* Adapted from parasite image library of CDC, USA

Pathogenesis and clinical manifestations

***Pathogenic mechanism*:** Amoebiasis is a disease caused by potentially pathogenic strains of *E. histolytica*. These pathogenic amoebae cause invasive amoebiasis through the sequential stages of:

a) Adherence of trophozoites on the surface of the large intestine.
b) Invasion of the large intestine by the amoebae, and finally.
c) Resistance of the amoebae to variour effector mechanisms of the host.

Initially, the slow transmit of intestinal contents in the caecum and sigmoid colon helps the amoebae to invade these sites. The slow transit of intestinal contents allows amoebae to come in contact with the colonic mucosa for a longer time, thereby bringing a change in the intestine flora that may facilitate invasion. It has recently been demonstrated that the gut-associated bacterial flora affect invasiveness of the amoebae to a great extent.

Adherence of amoebae to the intestinal mucosa is mediated by a surface

lectin of the amoebae known as galactose or N-acetyl-O-galactosamine inhibitable surface lectin. After adherence, trophozoites kill target cells in the intestinal mucosa, only by direct contact and also by secreted cytotoxins. The cytolysis occurs within 20 minutes of the amoebic adherence.

E. histolytica also secretes numerous proteolytic enzymes that appear to be involved in various pathogenic processes. Cathepsin B proteinase is respondible for dissolution of extra cellular matrix containing cells and tissue components. Amoebic glycosidases such as β -glucosaminidase and a surface membrane-associated neuraminidase cause degradation of mucos membrane of the colon or alteration of membrane glycoproteins on cell surfaces of the target cell.

Resistance of the parasite to variety of host effector mechanisms, both specific and non-specific, contributes to the persistence of infection in the intestine.

Host immunity in amoebiasis may be non-immune defence mechanism, and specific immunity. Non-immune defence mechanisms play an important role in resistance against invasion amoeba. Gastric acid barrier kills amoebic trophozoites, and rapid intestinal transit reduces the time for amoebae to colonise on the intestinal mucosa. Also colonic mucin inhibits amoebic adherence to epithelial cells.

The specific immunity involves both humoral and cell mediated immunity. Humoral immunity appears to be responsible for elimination of the amoebae from the intestine and subsequent resistance to re-infection. Cell mediated immunity probably has a role in limiting invasive amoeba and resisting a recurrence after therapeutic cure. Host resistance to initial amoebic invasion of the intestinal mucosa does not appear to involve cell-mediated mechanisms as evidenced from the lack of severity of the amoebic disease in AIDs cases.

Pathogenesis changes:

E. histolytica is almost unique among the amoebae of humans in its ability to hydrolyse host tissue. Once in contact with the mucosa, the amoebae secrete proteolytic enzymes, which enable them to pentrate the epithelium and begin moving deeper. The intestinal lesion usually develops initially in the cecum, appendix, or upper colon and then spreads the length of the colon. The number of parasites builds up in the ulcer, increasing the speed of mucosal destruction. The muscularis mucosae is somewhat of a barrier to further progress, and the pockets of amoebae form, communicating with the lumen of the intestine through a slender, duct like ulcer. The lesion may stop at the basement membrane or at the musculars mucosae and then begin eroding laterally, causing broad, shallow areas of necrosis. The tissues may heal nearly as fast as they destroyed, or the entire mucosa may become pocked. These early lesions usually are not complicated by bacteria invasion, and there is little cellular response by the host. In older lesions usually the amoebae, assisted by bacteria, may break through the muscularis mucosae, infiltrate the submucosa, and even penetrate the muscle layers and serosa. This enables trophozoites to be carried by blood and lymph to ectopic sites throughout the body where secondary lesions then form. A high percentage of deaths result from perforated colons with concomitant peritonitis. Surgical repair of perforation is difficult because a heavily ulcerated colon becomes very delicate.

Sometimes a granulomatous mass called ameboma, forms in the wall of the intestine and may obstruct the bowel. It results of cellular responses to a chronic ulcer and often still contains active trophozoites. The condition is rare.

Symptoms

The symptoms of amebiasis are far from clear cut and depend in large measure on the extent of tissue invasion and on whether the infection is confined to the intestinal tract or has spread to involve other organs. According WHO Report on Amebiasis, the symptoms of amoebiasis involve:

Ⅰ Asymptomatic infections

Ⅱ Symptomatic infections

A. Intestinal amebiasis: Acute amoebic Dysenteric; Nondysenteric clitis.

B. Extraintestinal amebiasis:

1. Hepatic: a. Acute nonsuppurative; b. liver abscess.

2. Pulmonary. Other extraintestinal foci.

3. Amoeboma is a pseudo-tumoral condition.

4. Amoebic liver abscess.

5. Cerebral amoebiasis.

6. Genito-urinary amoebiasis.

7. Splenic amoebiasis.

Diagnosis

Clinically, it is difficult to establish the diagnosis of amoebiasis, either intestinal or extra-intestinal. It is always supplemented by the laboratory diagnosis. Laboratory diagnosis includes parasitic diagnosis (microscopy), serodiagnosis, biochemical diagnosis, and radio-imaging diagnosis.

Epidemiology

Geographical distribution Amoebiasis has a worldwide distribution. Amoebiasis is a major health problem in Africa, South-east Asia, Latin America, especially Mexico. More than 10 percent of the world's population is estimated to be infected by *E. histolytica*.

Source of transmission andinfection

Food and water contaminated by human faeces that contain cysts are the main sources of infection. Infected man himself, especially carriers, are the principal source of transmission infection.

Infective form Four nucleus cyst is the infective form.

Susceptible population All age of humans is susceptive for *E. histolytica.*

Prevention and control

Treating the infected persons

Treatment of amoebiasis is broadly based on: eradication of amoebae by the use of amoebicides, replacement of fluid, electrolyte and blood, and relief from the constitutional symptoms.

Tissue amoebicides: They act on tissue amoebae present ill different tissues. The tissue amoebicides which act on all the tissues include: metronidazole, tinidazole, emetine hydrochloride and 2-dehydroemetin. Amoebicides which act only on liver tissue is chloroquine. Tetracycline and erythromycin act only on the intestinal wall.

Control and prevention

The amoebic infections can be controlled and prevented by individual prophylaxis and community prophylaxis.

Individual prophylaxis consists of:

1. Avoiding faecal contamination of food and water.

2. Boiling the drinking water to kill all the amoebic cysts. The cysts also are killed by the routinely used chlorine concentration in the drinking water.

3. Treating vegetables with acetic acid and vinegar at least for 15 minutes before consumption as salad.

4. In homosexuals, by avoiding sexual practices that allow faecal-oral contact and

5. Improved personal hygiene such as washing hand before eating and after defecation.

Community prophylaxis consists of:

1. Improvement of general sanitation by proper disposal of faeces.

2. Prevention of'water supplies from faecal contamination, and

3. Better managernent of cases by an early and rapid detection and subsequent treatment of cases.

GENUS LEISHMANIA

Kingdom: Protista
Subkingdom: Protozoa
Phylum: Trypanosomatidae
Class: Leishmania
Order: Leishmania
Family: *donovani, tropica, major, meicana*

It is necessary to remember

The genus *Leishmania* was created by Ross in 1903 to include *Leishmania donovani,* the parasite causing Indian **kala-azar.** Species belonging to this genus have two stages (amastigote, promastigote) in their life cycle. They require a vertebrate and insect host to complete the life cycle.
Leishmaniasis is a **vector-borne disease** that is transmitted by sandflies and caused by obligate intracellular protozoa of the genus *Leishmania*. Human infection is caused by about 21 of 30 species that infect mammals. These include the ***L. donovani*** complex with three species (*L. donovani*, *L. infantum*, and *L. chagasi*); the ***L. mexicana*** complex with three main species *(L. mexicana*, L. amazonensis, and L. venezuelensis); *L. tropica*; *L. major*; *L. aethiopica*; and the subgenus *Viannia* with four main species (*L. braziliensis,* L. guyanensis, L. panamensis, and L. peruviana). The different species are morphologically indistinguishable, but they can be differentiated by isoenzyme analysis, molecular methods, or monoclonal antibodies.

LEISHMANIA DONOVANI

Leishmama donovani causes **visceral leishmaniasis.** The disease also known as kala-azar, Dum-Dum fever, Asian fever or infantile splenomegaly is a serious one, which can be fatal if untreated.
Both Leishman and Donovan reported the parasite simultaneously in the same year, 1903. Leishman demonstrated the parasite in the spleen smear of a soldier in England, who died of fever contracted at Dum-Dum in Calcutta. Donovan found the same in the spleen smear of a patient suffering from kala-azar in India. The sand fly, *Phlebotomus argentipes* was idenlifted as a vector of the disease by Indian Kala-azar Commission. *L. donvani* are obligate intracellular parasites of man and other mammalian hosts. They are always found as **amastigotes** in the reticuloendothelial cells of the spleen, bone marrow, liver, intestinal mucosa and mesenteric lymphnodes.

Morphology

The parasite exists in two forms: amastigote and promastigote (Fig. 4).
Amastigote: Amastigotes are round in man and other vertebrate hosts. They are found inside monocytes, polymorphonuclear leucocytes or endothelial cells. They are small, round to oval bodies measuring 2.9-5.9 µm in length. They are also known as **LD bodies.** They are stained well with Giemsa or Wright. In a stained preparation, the cytoplasm surrounded by a limiting membrane appear pale-blue. The nucleus relatively is large and staincd red. The kinetoplast is situated at right angle to the nucleus. It is slender, rod-shaped and is stained deep red. Axoneme arises from the kinetoplast arid extends to margin of the body. Vacuole, which is a clear unstained space lies alongside the axoneme.

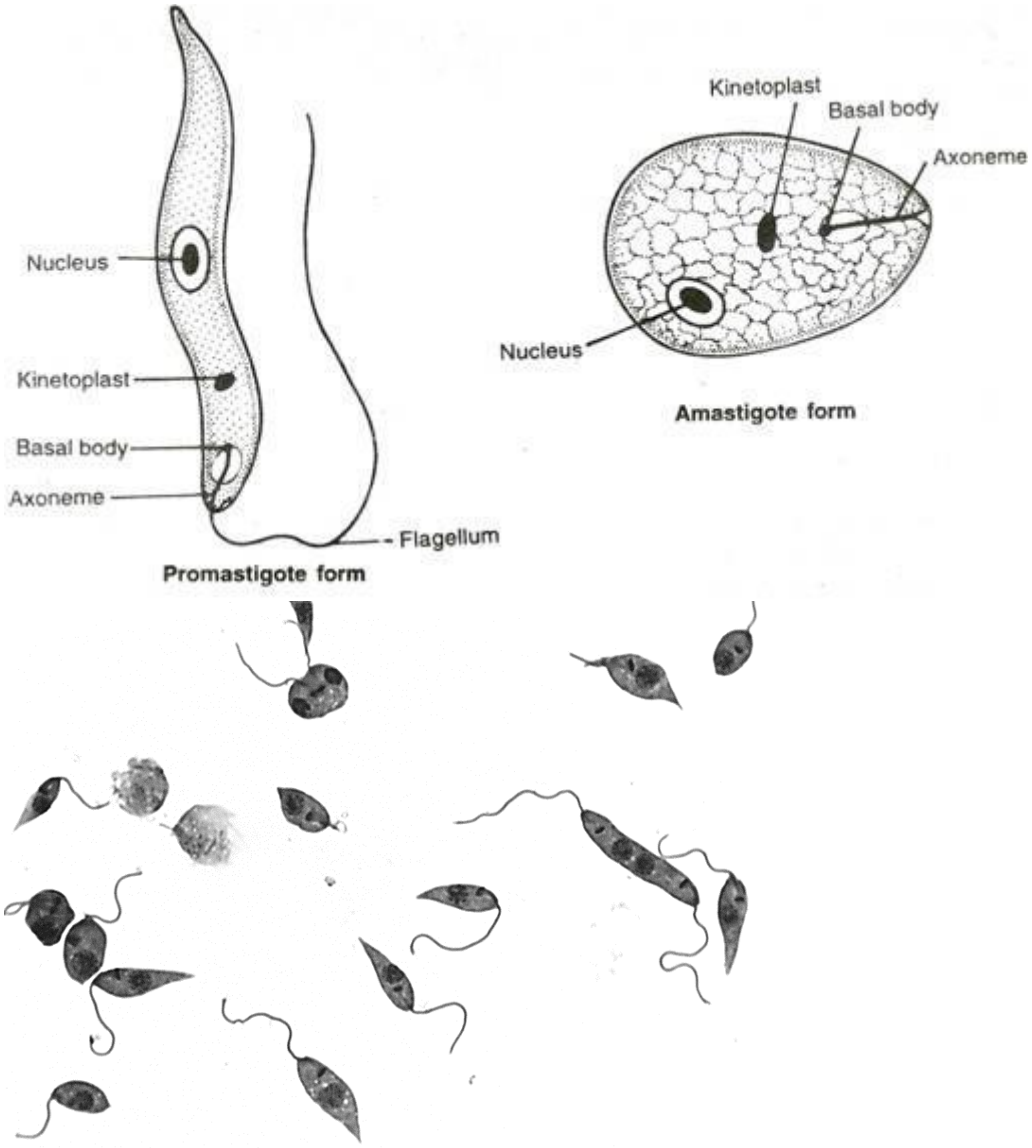


Figure 4. Leishmania donovani. Promastigote and amastigote form.

Promastigote: Promastigotes are found in the digestive tract of sand fly and in the culture media. The fully developed promastigotes are long, slender and spindle-shaped. They measure 14.3 to 20 µm in length and 1.5 to 1.8 µm in breadth. A single nucleus is situated at the center. The kinetoplast lies transversely near the anterior end. The flagellum is single, delicate and measures15-28 um. With Leishman stain, cytoplasm appears blue, the nucleus pink and the kinetoplast blight red.

Life cycle

L. donovani completes its life cycle (Fig. 5) in two different hosts Man and other mammals Sand-fly of genus *Phlebotomus* and *Lutzomyia*.

The parasite is transmitted to man and other vertebrate hosts by the bite of blood-sucking female sand-fly. The sandflies inject the infective stage, promastigotes, during blood meals. These promastigotes are immediately phagocytosed by fixed macrophages of the host, in which they are transformed

into amastigores. The amastigotes multiply by binary fission to produce a large number of amastigotes; till macrophages are filled with parasites. As many as 50 to 200 amastigotes may be present in the cytoplasm of the enlarged cell. The cell ruptures and releases a large number of amastigotes into the circulation. Free amastigotes are subsequently carried by circulation. They invade monocytes of the blood and macrophages of the spleen, liver, bone marrow, lymph nodes and other tissues of the reticuloendothelial cells. Free amastigotes in the blood as well as intracellular amastigotes in the monocytes are ingested by female sandfly during blood meal from man. In the mid gut of the sandfly, the amastigotes are transformed within 72 hours through a series of flagellated intermediate promastigote forms to flagellated promastigotes. These promastigotes multiply by binary fission and produce a large number of promastigotes completely filling the lumen of the gut. After a period of 6 to 9 days, the promastigotes migrate from the midgut to the pharynx and buccal cavity of sandfly. The sandflies, which ingest fruit or plant juice after the first blood meal, show heavy pharyngeal infection causing blockage of the pharynx. Bite of the blocked sandfly transmits infection to susceptible persons and the life cycle is repeated.

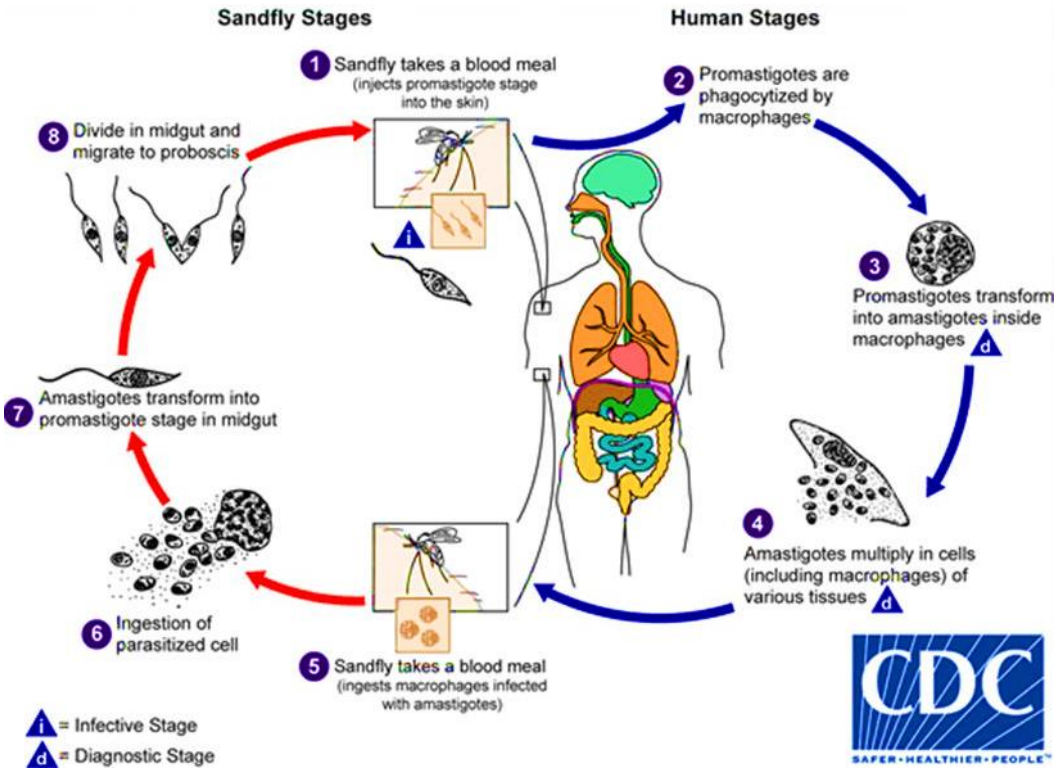


Figure 5. Life cycle of *Leishmania donovani (*Adapted from parasite image library of CDC, USA)

Pathogenesis and clinical manifestation

Bite of the female sandfly deposits promastigotes on surthce of the skin. The sandfly liberates biolog ically active substances which promote infectivity of promastigotes by partially deactivating fixed macrophages in the skin. Promastigotes phagocytosed by macrophages are transformed into amastigotes and multiply by binary fission within phagolysosomes of the macrophages.

Amastigotes subsequently invade throughout the reticuloendothelial system of the spleen, liver, bone marrow and lymph nodes and multiply in large numbers. Increased numbers of macrophages in the liver and spleen produce progressive hypertrophy of these organs. Parasitised macrophages replace lymphoid follicles in the spleen and also haematopoetic tissue in the bone marrow. They progressively replace normal hepatocytes in the liver.
Proliferation and destruction of reticuloendothelial cells of the internal organs and heavy parasitisation of external organs by parasitised cells are the characteristic pathological changes seen in visceral leishmaniasis.

Pathological changes in organs:

1) **Spleen** is grossly enlarging, surrounded by a thick capsule. It is soft and non-tender. The splenic pulp is greatly increased, congested and turns purple or brown black and becomes highly friable. Splenic cells are densely packed with amastigotes of *L. donovani.*
2) **Liver** is enlarged with a sharp edge, soft consistency and smooth surface. The Kupffer cells are largely increased in their both size and number. They are filled with amastigotes. In contrast, hepatocytes do not contain any parasites. Atrophic areas, swelling and fatty degeneration often are seen in the liver cells.
3) ***Bone marrow:*** It is dark red in colour and shows extensive proliferation of reticuloendothelial cells. Large numbers of parasitised macrophages replaces Haemopoetic tissue of the bone marrow. Plasma cells often are increased in number.
4) ***Lymph nodes*** are enlarged. In China and Mediterranean type of visceral leishmaniasis, amastigotes are demonstrated in the enlarged lymph nodes.
5) ***Kidney*** shows cloudy swelling and is invaded with macrophages parasitised by amastigotes.
6) ***Heart***: It is pale but does not show any amastigotes in the myocardium.

Haematological changes occur. Typically, **anaemia** is present in kala-azar. It is normocytic and normochromic. Anaemia is multifactoral. It is caused by increased haemolysis, haemorrhage, haemodilution, replacement of bone marrow with parasitised macrophages and splenic sequestration of red cells. **Leucopenia** is well-marked. White blood cell count falls down to as low as 1100/mm^3 of blood. Thrombocytopenia is caused by destruction of platelets.
Host Immunity: Persons with malnutrition and young people are increasingly susceptible to visceral leishmaniasis.
Amastigotes alter profoundly the immune system of man. Therefore, it is frequently referred to as the disease of the immune system. Host immunity in visceral leishmaniasis is characterised by specific inhibitions of cell-mediated immunity and profound hyperglobulinaemia. Delayed hypersensitivity reaction, as determined by leishmanin skin test and in vitro lymphocyte responses to leishmanial antigen is completely absent during the infection. The delayed hypersensitivity, however, develops again after successful treatment with **antileishmanial drugs**. The intact cell mediated immunity confers protection against the infection. Profound hyperglobulinaemia: Polyclonal lymphocyte activation causes profound hyperglobulinaemea. It is characterised by the production of a large volume of polyclonal non-specific immunoglobulins especially IgG and specific anti-leishmanial antibodies. The complement is ac-

tivated and immune complexes are produced, the circulating antibodies, however, are not protective. Persons who have recovered from kala-azar are immune from reinfection. Anorexia and wasting seen in the disease possibly is mediated by cytokines such as **tumor necrosis factor** and interleukin.

Clinical manifestation*:* Human leishmanial infections can result in two main forms of disease, cutaneous leishmaniasis and visceral leishmaniasis. The factors determining the form of disease include leishmanial species, geographic location, and immune response of the host.

Visceral leishmaniasis: also known as *kala azar*, is the most severe form of the disease, which, if untreated, has a mortality rate of almost 100%. It is characterized by irregular bouts of fever, substantial weight loss, swelling of the spleen and liver, and anaemia.

The **incubation period** of visceral leishmaniasis is generally about 3 months. It may vary from as minimum as three weeks to a maximum of 18 months.

The onset of disease may be gradual or sudden. The sudden onset occurs more frequently in persons coming from non-endemic areas to endemic areas.

Fever is the first symptom to appear. Typically, it is nocturnal or remittent with a twice-daily temperature spikes. Sweating with chills but seldom rigor, accompanies the temperature spikes, less commonly, fever is continuous. Diarrhoea and cough are frequently present.

Spleen is grossly enlarged by the third month, frequently occupying the entire left side of the abdomen. It is soft and non-tender. Liver is enlarged but less conspicuous. It is soft with a smooth surface and a sharp edge. Lymphadenopathy is seen only in some cases of African kalaazar.

Anaemia (normocytic and normochromic) is always present in kala-azar.

Leucopenia (white blood cell count as low as 1000/mm^3) is a consistent feature.

Hypergammaglobulinaemia, circulating immune complexes and rheumatoid factors are present in sera of the most patients of kala-azar. Immune complex-glomerulonephritis and interstitial nephritis have also been described.

As the disease progresses, the skin becomes dry, thin and scaly. The hairs become dull, thin and are lost. The nails become brittle. The skin on the hands, feet, abdomen and around the mouth and fore-head becomes greyish and dark coloured. This hypo pigmentation of the skill characteristically is seen in Indian patients giving tile name kala-azar, which means black fever.

Peripheral oedema, epistaxis, gingival bleeding, petechiae and echymoses are the late manifestations. Without treatment, death occurs with in 3 to 20 months in 40 to 94% of adult and in 75 to 85% cases of children. Death often is due to superinfection bacterial pneumonia, septicaemia, concurrent infections (tuberculosis, dysentery) or uncontrolled severe haemorrhage from the gastrointestinal tract and severe anaemia.

1) *Cutaneous leishmaniasis*: It is characterized by one or more cutaneous lesions on areas where sandflies have fed. Persons who have cutaneous leishmaniasis have one or more sores on their skin. The sores can change in size and appearance over time. They often end up looking somewhat like a volcano, with a raised edge and central crater. A scab covers some sores.

The sores can be painless or painful. Some people have swollen glands near the sores (for example, in the armpit if the sores are on the arm or hand).

2) *Leishmanoma*: In Africa, a primary cutaneous lesion known as Leishmanoma has been observed. This manifests as a nodule in the skin, which measures 2.5 to

4cm during 1 to 3 weeks time. This is not seen in Indian kala-azar.

3) ***Post kala-azar dermal leishmaniasis:*** It is a non-ulcerative lesion of the skin, which is seen after completion of treatment of the kala-azar. The condition is characterised by a spectrum of lesions in the skin ranging from depigmented macules to wart-like nodules over the face and exposed surfaces of limbs.

Diagnosis

In endemic areas, the persons with prolonged fever, progressive weight loss and weakness, marked splenomegaly, hepatomegaly, anemia, leucopenia, hypergammaglobulinaemia and low serum albumin are highly suggestive of visceral leishmaniasis.

Parasitic diagnosis

Demonstration of Leishmania in appropriate clinical specimens is the definitive diagnosis of the condition. The parasites are demonstrated in different clinical specimens by direct microscopy, culture or animal inoculation.

Specimen collection: In their decreasing order of sensitivity, the specimens to be examined are from spleen, bone marrow, liver, lymph node and blood.

Splenic aspiration: It is tile most sensitive (90.6 to 99.3 percent positivity) method. The major disadvantage of this method is that frequently, it is associated with the risk of life threatening haemorrhage. It is particularly seen in patients with advanced stage of the disease having an enlarged and soft spleen. The splenic aspiration should be performed only under medical supervision and preferably with a small bore needle. It is contra indicated in patients with prothrombin time more than five seconds longer than the normal or if the platelet count is below 40,000/mm^3.

Liver biopsy: It is also not a safe procedure and carries a risk of haemorrhage.

Bone marrow aspiration: Bone marrow aspiration from the sternum or lilac crest, is the safest procedure. Nevertheless, it is painful. Bone marrow aspiration and biopsy are positive in over 85 percent of cases.

Lymph node aspiration: It is positive in 60 percent of cases.

Blood: It is useful only in untreated cases.

Immunological diagnosis

Serological tests to demonstrate specifc anti-le ishmanial antibodies in the serum are especially useful in the diagnosis of early phase of visceral leishmaniasis.

Complement fixation test was the first serological test used to detect serum antibodies in visceral leishmaniasis. Now this test has been replaced with more sensitive and specific tests such as **indirect immuno-fiuorescent, indirect haemagglutination, enzyme-linked immunosorbent assay,** etc. These tests use cultured promastigotes as antigens. Drawbacks of these tests are that they often show cross-reactivity with sera from leprosy, malaria, schistosomiasis. Chagas' disease and cutaneous leishmaniasis.

A direct agglutination test, using trypsin treated Coomasie blue-stained promastigotes, has been developed recently as a simple test for use in poorly equipped laboratories.

Leishmanin skin test: It is a delayed hypersensitivity skin test. In this test, 0.2ml of Leishmania antigen (containing 100,000,000 promastigotes of L. donovani in l ml of 0.5% phenol saline) is injected intradermally. The test is read after 48 to 72 hours. A positive test shows an area of erythema and induration of 5 mm in diameter or larger, which heals in 14-25 days. Positive reaction indicates prior exposure to leishmanial parasites. In kala-azar, the skin test

becomes positive usually only 6 to 8 weeks after cure from the disease, it is negative in active cases.

Epidemiology

Kala-azar is wide spread throughout the world. Over 12 million people are infected world wide. It may occur as endemic, epidemic or sporadic.

About 30 species of sandflies can become infected when taking a blood meal from a reservoir host. Hosts are infected humans, wild animals, such as rodents, and domestic animals, such as dogs. Most leishmaniases are zoonot ic (transmitted to humans from animals), and humans become infected only when accidentally exposed to the natural transmission cycle. However, in the anthroponotic forms (those transmitted from human to human through the sandfly vector), humans are the sole reservoir host

***Human-to-human transmission*:** No animal reservoirs are present; hence animal-to-man transmission does not take place. Therefore, man is the only source and reservoir of infection. This type of transmission mainly occurs in plain areas.

Dog-to-human transmission: The infection sources is domestic dogs; it mainly occurs in hill areas of North West, North and North East of China. Most of patients are children, usually under 10 years old. **Natural focus:** Animal-to-man transmission takes place by the bite of sandflies. The infection sources are wild animals. It distributes in hungriness areas of Xingjiang and Inner Mongolia. Promastigote is the infective form. The infection is transmitted mainly by the bite of vector sandfly (*Phlebotomus argentipes*), rarely, by blood transfusion or accidental inoculation by cultured promastigores in the laboratory workers, or congenital infection, and sexual coitus.

In general, fulminant visceral leishmaniasis have been described in most of the cases of AIDS. They respond poorly to anti-le ishmanial therapy.

Prevention and control

Treatment the patient: It consists of specific therapy supplemented with treatment of secondary microbial infections, high calorie, high protein diet and blood transfusion in severe anaemia. The specific therapy includes pentavalent antimonials. These are the drugs of choice and are highly effective against Leishmania and are nontoxic. Resistant cases failing to respond to antimony compounds can be treated with pentamidine isethionate or amphotericin B. Treatment with interferon has shown promising result in a small number of cases of kala-azar. It is still at the experimental stage. Treatment of PKDL is same as for visceral leishmaniasis.

Preventive measures: The preventive measures include:

1) Reduction of sandfly population by insecticides mainly DDT, dieldrin, malathion, etc.
2) Reduction of reservoir by killing all the infected dogs in the cases of zoonot ic kala-azar and treatment of human cases, and
3) Prevention of exposure to sandfly by using thick clothes, bed nets, window mess or insect repellants.

TRYPANOSOMES

Kingdom: Protista
Subkingdom: Protozoa
Phylum: Sarcomastigophora
Subphylum: Mastigophora
Class: Kinetplastida
Family: Trypanosomatidae
Section: Salivaria
Genus: *Trypanosoma*
Species: *Brucei*
Subspecies: *gambiense, rhodesiense*

It is necessary to remember

Trypanosomes are hemoflagellate protozoa; they belong to the family Trypanosomatidae. Two distinctly forms of genus *Trypansoma* occur in humans. They cause **African trypanosomiasis** (or African sleeping sickness) and **America typanosomiasis** respectively.
The complex *Trypanosoma brucei* have two subspecies that are morphologically indistinguishable cause distinct disease patterns in humans: *T. b. gambiense* causes West African sleeping sickness and *T. b. rhodesiense* causes East African sleeping sickness. (A third member of the complex, *T. b. brucei*, under normal conditions does not infect humans). The protozoan parasite, *Trypanosoma cruzi*, causes America typansosmiasis, a zoonotic disease that can be transmitted to humans by blood-sucking reduviid bugs.

TRYPANOSOMA BRUCEI COMPLEX

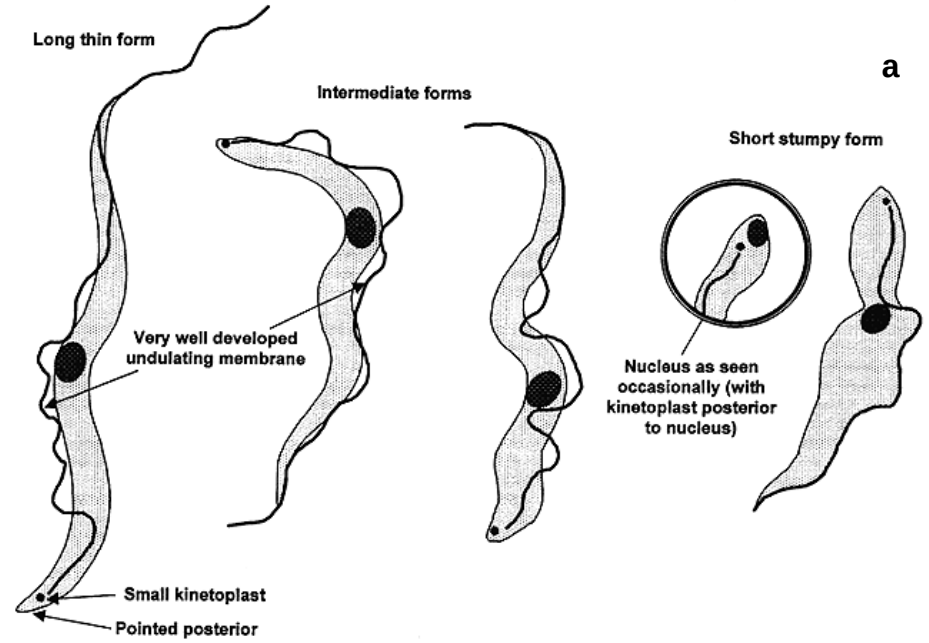
Trypansoma brucei complex causes African sleeping sickness. Originally, in the early years of 20 centuries, three African species were named: *T. brucei,* which was believed to be unable to infect man; *T. rhodesiense*, which could infect man, in whom it caused an acute disease; and *T. gambiense,* also infective to man but producing a much more chronic disease. It was later realized that the three “species” were closed related, and they were reduced to subspecies of *T. brucei*: *T. b. gambiense, T. rhodesiense* and *T. b. brucei.*
Parasites inhabit the connective tissue. In man and other vertebrate hosts, these are found in the blood stream, lymph nodes and cerebrospinal fluid.

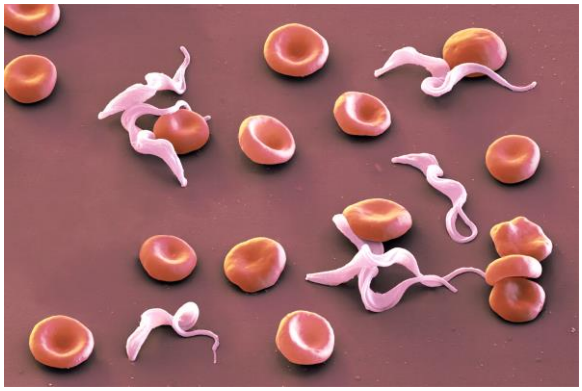
Morphology

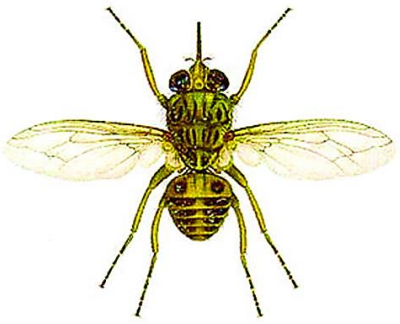
T. b. gambiense and *T. b. rhodesiense* are morphologically similar. Various forms are recognized. Basically, all these forms are flagellated.
Trypomastigote: It found in man and other vertebrate.
Trypommatigote exhibit pleomorphism. They vary greatly in their size and shape. Two distinct types are recognized – Dividing long and slender trypomastigote with a long free flagellum and non-dividing short (Fig. 6), thick and stumpy trypomastigotes:
1) ***Sender trypomastigote***: these forms are found in the blood during ascending parasitaemia. They measure 20-40μm in length and 1.5-3.5μm in breadth.

2) ***Stumpy trypomastigotes*:** they do not have any free flagella. These forms always are found in the blood during declining phase of parasitaemia. They measure 15-25μm in length and 3.5μm in breadth. Trypomastigotes are slender and fusiform organisms with pointed anterior end and blunt posterior end. They have a single and large oval nucleus situated centrally. A small kinetoplast containing blepharoplast and parabasal body is situated in the posterior end of the parasite. Cytoplasm contains volutin granules. A single flagellum arises from the kinetoplast in the posterior end, curves around the body in form of a folded undulating membrane. It continues as a free flagellum beyond the anterior end.

***Insect forms*:** It includes procyclic trypomastigotes, **epimastigotes** and **metacyclic trypomastigotes.** These form are found in the salivary glands of **tsetse fly.** Epimastigotes have a surface coat and pre-nuclear kinetoplast. They always divide by remaining attached in the lumen of the salivary glands. The metacyclic forms have variable antigen type on their surface coat. These forms do not divide and are found free in the lumen of salivary glands. They are infective to humans.







b c

Figure 6. Trypanosoma brucei: a – morphology schem, b – in blood 3d reconstruction (the discs is biconcave erythrocytes), c – *Glossina* species, vectors of trypanosomes.

Lifecycle

T. brucei complex their life cycle in vertebrate host and insect host. Vertebrate host include man and domestic animals. Insect host are **tsetse fly** of *Glossina* species (*G. palpalis, G. morsitans, G. pallidipes, etc.*). Tsetse flies are large bloodsucking Diptera. Unlike mosquitoes, both sexes of *Glossina* feed exclusively on blood, so that both can transmit trypanosomes.

Man and other vertebrate hosts acquire infection by bite of tsetse fly (Fig. 7). These flies inoculate metacyclic trypomastigotes in skin during the blood meal. These metacyclic forms are immediately transformed into long slender blood stream trypomastigotes and begin to multiply in the blood, lymphatic system or in tissue. Trypomastigotes invade heart and connective tissue, bone marrow and in later stage, invade the central nervous system. In all these sites, trypanosomes multiply as long, slender dividing forms which present in the phase of ascending parasitaemin. The infection is periodically controlled by high level of specific IgM antibodies, causing remission of the disease. The non-dividing stumpy trypomastigotes, which replace long slender forms, are found in the remission state. These short stumpy forms are infective to tsetse fly.

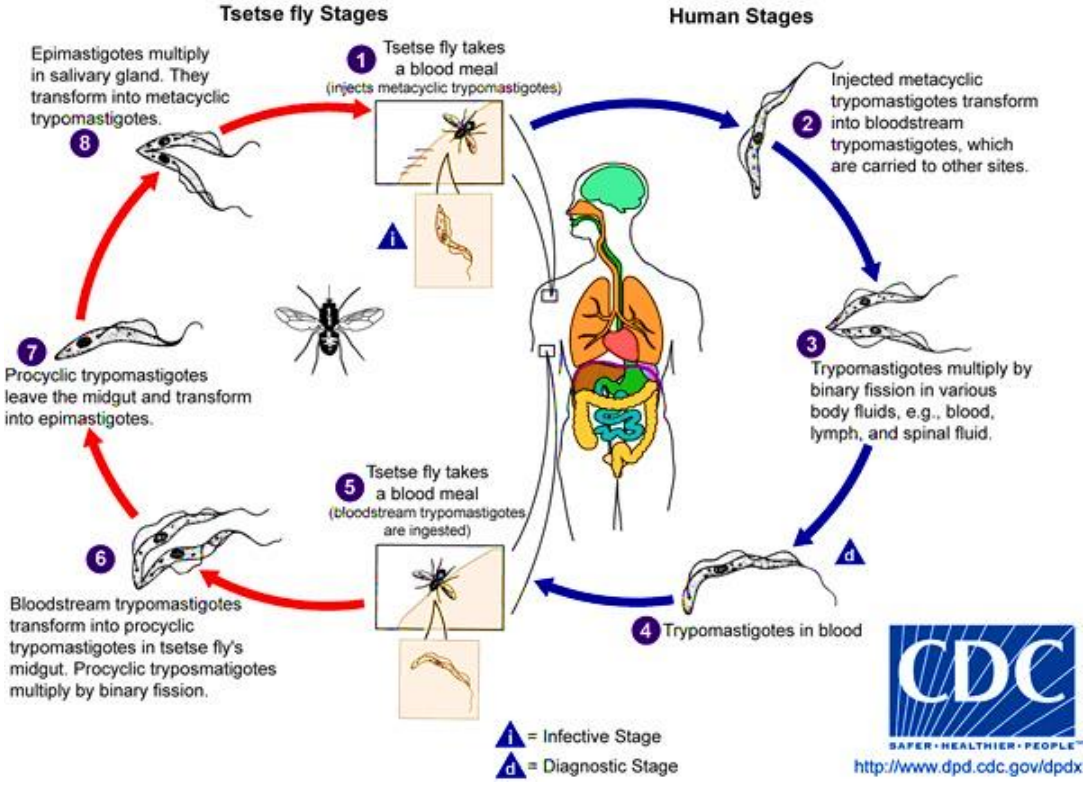


Figure 7. Life cycle of *Trypanosoma brucei* (Adapted from parasite image library of CDC, USA)

The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host. In the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission, leave the midgut, and transform into epimastigotes. The epimastigotes reach

the fly's salivary glands and continue multiplication by binary fission. After then the epimastigotes are transformed into metacyclic trypomastigotes. The cycle in the fly takes approximately 3 weeks. Humans are the main reservoir for *Trypanosoma brucei gambiense*, but this species can also be found in animals. Wild game animals are the main reservoir of *T. b. rhodesiense*

Pathogenesis and clinical manifestation

Infection occurs in 3 stages. **A trypanosomal chancre** can develop on the site of inoculation. This is followed by a **haematolymphatic stage** with symptoms that include fever, lymphadenopathy, and pruritus. In the **meningoencephalitic stage,** invasion of the central nervous system can cause headaches, somnolence, abnormal behavior, and lead to loss of consciousness and coma. The course of infection is much more acute with *T. b. rhodesiense* than *T. b. gambiense*.

Chancre: Trypansomal chancre is an acute inflammatory local response seen in a week or so after the bite of infected tsetse fly. It is large, red and rubbery. It is more frequently seen in Rhodesian trypanosomiasis. It shows an intense inflammatory infiltration, vasodilatation and interstitial oedema. The chancre tissue is filled with parasites. A painful trypansomal chancre appears within a few days at the site of bite and resolves spontaneously within several weeks. It is characterized by erythema, swelling and local tenderness.

***Haematolymphatic stage*:** In the early stage of the disease, after development of the chancre, infection of the blood and lymph system results in a more or less acute febrile illness. Infected lymph glands, especially those at back of the neck, may become very enlarged; the swollen cervical glands constitute" Winterbottom's sign", a classical diagnostic indication of *T. b. gambiense.* Oedema, hepatosplenomegaly and tachycardia are other frequent findings.

***Meningoencephalitic stage*:** More serious effects result from the penetration of the parasites into the CNS, which may occur at any time from weeks to years after initial infection. Here the parasites multiply in the blood vessels, tissue fluids and cerebrospinal fluid.

Diagnosis

The diagnosis rests upon demonstrating trypanosomes by microscopic examination of chancre fluid, lymph node aspirates, blood, bone marrow, or, in the late stages of infection, cerebrospinal fluid. A wet preparation should be examined for the motile trypanosomes, and in addition a smear should be fixed, stained with Giemsa, and examined. Concentration techniques can be used prior to microscopic examination. For blood samples, these include centrifugation followed by examination of the buffy coat; mini anion-exchange/centrifugation; and the Quantitative Buf fy Coat technique. For other samples such as spinal fluid, concentration techniques include centrifugation followed by examination of the sediment. Isolation of the parasite by inoculation of rats or mice is a sensitive method, but its use is limited to *T. b. rhodesiense*. Antigen detection assays, in a test format suitable for field use, are being developed and evaluated. Antibody detection has sensitivity and specificity that are too variable for clinical decisions. In addition, in infections with *T. b. rhodesiense*, seroconversion occurs after the onset of clinical symptoms and thus is of limited

use. However, the Card Agglutination Trypanosomiasis Test test is of value for epidemiolog ic surveys or screening of *T. b. gambiense*.

Epidemiology

T. b. gambiense is found in foci in large areas of West and Central Africa. The distribution of *T. b. rhodesiense* is much more limited, with the species found in East and Southeast Africa. African sleeping sickness is a vector-borne disease. *Glossina* is restricted to tropical Africa, which is the reason for the similar restriction of *T. brucei.* It is endemic in 36 countries of sub-Saharan Africa, in the areas where tsetse flies are found. Approximately 50 million people are at risk of acquiring the disease.

East African sleeping sickness caused by *T. b. rhodesiense* is a zoonotic disease. Wild animals, principally, antelopes (bush buck and hartbeest) and domestic animal are the important sources and reservoirs of infection. Infection in endemic areas is transmitted by bite of tsetse flies, principally *Glossina pallidipes* and *G. morsitans*. The infection is an occupational hazard amongst hunters, honey collectors and firewood collectors.

West African sleeping sickness caused by *T. b. gambiense* is not a zoonotic disease. Infected man is only source and reservoir of infection. Infection is transmitted by bite of tsetse flies of *palpalis* group, mainly *Glossina palpalis, G. tachinoides* and *G. fuscipes.* This infection is primarily seen in rural areas.

Prevention and control

Treatment should be started as soon as possible and is based on the infected person's symptoms and laboratory results. The drug regimen depends on the infecting species and the stage of infection. Pentamidine isethionate and suramin (under an investigational New Drug Protocol from the CDC Drug Service) are the drugs of choice to treat the hemolymphatic stage of West and East African Trypanosomiasis, respectively. Melarsoprol is the drug of choice for late disease with central nervous system involvement (infections by *T.b. gambiense* or *T. b. rhodiense*).

Control of tsetse fly population is the mainstay of preventive measures to control sleeping sickness. Insecticides are widely used to reduce tsetse fly population. The use of traps and baits impregnated with insecticides are the various methods to control tsetse fly population.

TRYPANOSOMA CRUZI

Kingdom: Protista
Subkingdom: Protozoa
Phylum: Sarcomastigophora
Subphylum: Mastigophora
Class: Zoomastigophora
Order: Kinetplastida
Family: Trypanosomatidae
Section: Stercoraria
Genus: *Trypanosoma*
Species: *cruzi*

It is necessary to remember

Trypanosoma cruzi (Fig. 8), causes **Chagas disease,** a zoonot ic disease that can be transmitted to humans by blood-sucking reduviid bugs. Chagas disease (South American trypansomiasis) is commonly seen in the countries of South America.

Morphology and life cycle

There are three forms exist in *T. cruzi* life cycle. In man and other vertebrate host, T*. cruzi* exists amastigotes and non-multiplying trypomastigotes; the insect form includes epimastigotes and multiplying trypomastigotes.

***Amastigote*:** it is the non-flagellated, intracellular parasite found in man and other vertebrate host. Amastigote is a round or oval body measuring 2 to 4µm in diameter. It has a nucleus, kinetoplast and an axoneme. Morphological it resembles the amastigote of *Leishmania* species, hence it is frequently called as leishmanial form. It multiplies in man in this stage only.

***Trypanomastigote*:** it is the flagellated form and is of two types. The multiplying forms are found in the stomach of reduviid bug and in the culture, and non-multiplying forms are found in the blood in man and other mammalian hosts. Trypanomastigotes are usually C-shaped and slender, measuring 11.7-30.4µm in lengths and 0.7-5.9µm in breadth. The posterior end is wedge-shaped. At the anterior end, a free flagellum originates and traverses on surface of the parasite as a narrow undulating membrane. They have a centrally placed prominent nucleus and a large round to oval kinetoplast at the posterior end.

T. cruzi need two hosts to complete its **life cycle** (Fig. 9). The vertebrate hosts are man and other reservoir hosts, the insect host Reduviid bug (kissing bug, so named because they often feed around the lips of sleeping people).

When an infected triatomine insect vector takes a blood meal and releases trypomastigotes in its feces near the site of the bite wound. Trypomastigotes enter the host through the wound or through intact mucosal membranes, such as the conjunctiva. Common triatomine vector species for trypanosomiasis belong to the genera *Triatoma*, *Rhodinius*, and *Panstrongylus*. Inside the host, the trypomastigotes invade cells, where they differentiate into intracellular amastigotes. The amastigotes multiply by binary fission and differentiate into trypomastigotes, and then are released into the circulation as bloodstream trypomastigotes.

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. Feeding on human or animal blood that contains circulating parasites infects the “kissing” bug. The ingested trypomastigotes transform into epimastigotes in the vector’s midgut. The parasites multiply and differentiate in the midgut and differentiate into infective metacyclic trypomastigotes in the hindgut. Within 8-10 days, these trypanomastigotes are excreted in the faeces of the bug, as the bug takes the blood meal from a host and the cycle is continued Trypanosoma *cruzi* can also be transmitted through blood transfusions, organ transplantation, transplacentally, and in laboratory accidents.

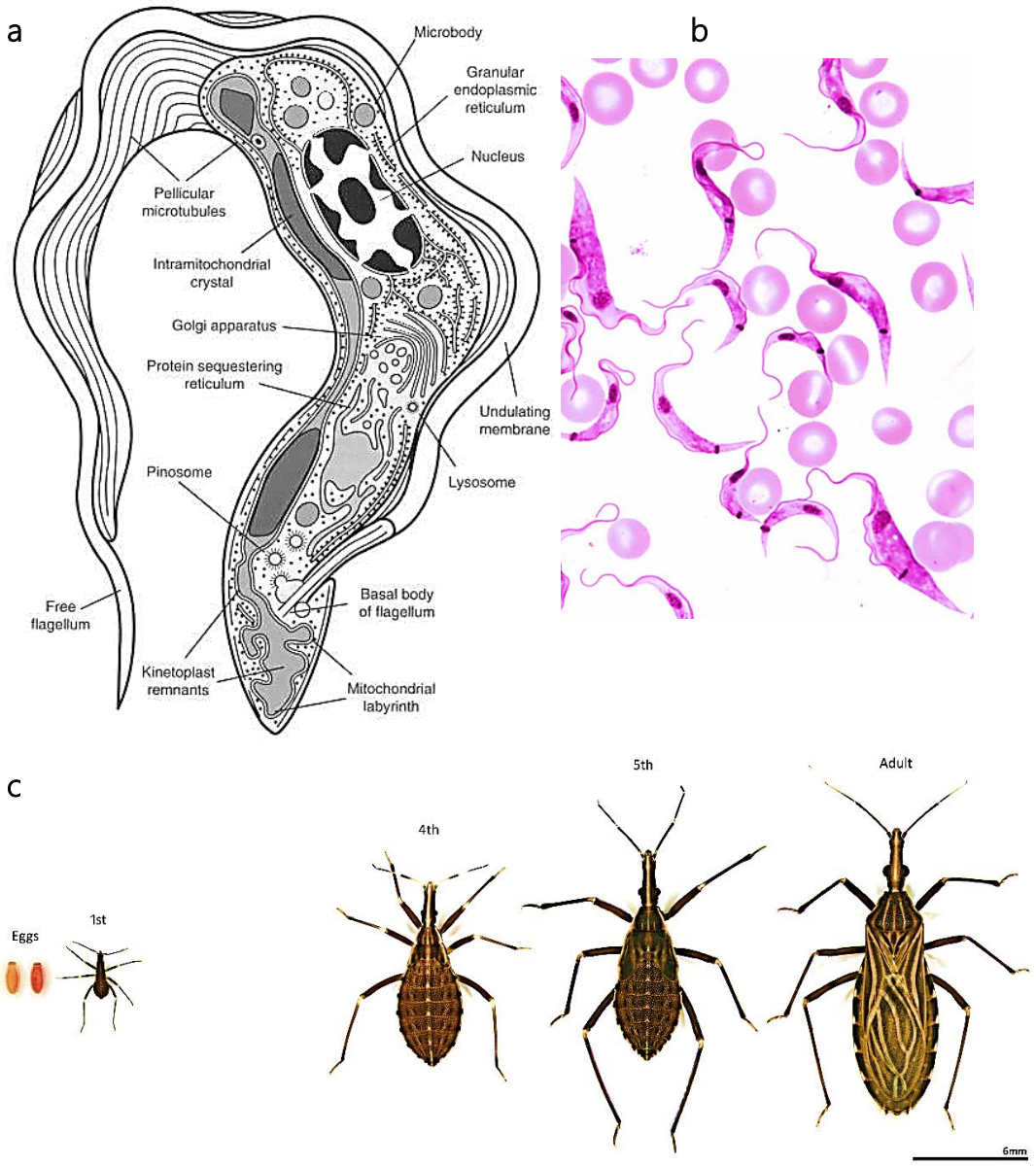


Figure 8. *Trypanosoma cruzi* morphology – a; blood histologic slices – b; and here vectors – bug *Rhodnius prolixus (*kissing bug) – *c*.

Pathogenesis and symptoms

The pathogenesis of acute Chagas' disease depends upon the destruction of parasitised and non-parasitised host cells. Destruction of host cells is responsible for the clinical symptoms of the disease at the early stage. Chagas' disease is a chronic condition. Infected persons may show few, if any, signs of disease and may survive for decades, even though still infected.

Acute Chagas' disease

It occurs most commonly in infants and children. The first sign of illness occurs at least 1 week after invasion by the parasites. A local lesion (chagoma, palpebral edema) can appear at the site of inoculation. Chagoma is localized swelling of the skin and contains intracellular amastigotes in leucocytes and subcutaneous. When the parasite is inoculated in the conjunctiva, a unilateral painless oedema of the palpebral and perioccular tissue develops in the eye. It is called Romana's sign and is the classical finding in the acute Chagas' disease. The acute phase is usually asymptomatic, but can present with manifestations that include fever, anorexia, lymphadenopathy, and mild hepatosplenomegaly; in severe infection, myocarditis may have developed. Most deaths in acute Chagas' disease are due to heart failure or meningoencephalitis. The acute stage lasts for 20-30 days. Symptoms resolve in most of the patients who then enter into asymptomatic or indeterminate stage of *T. cruzi*.

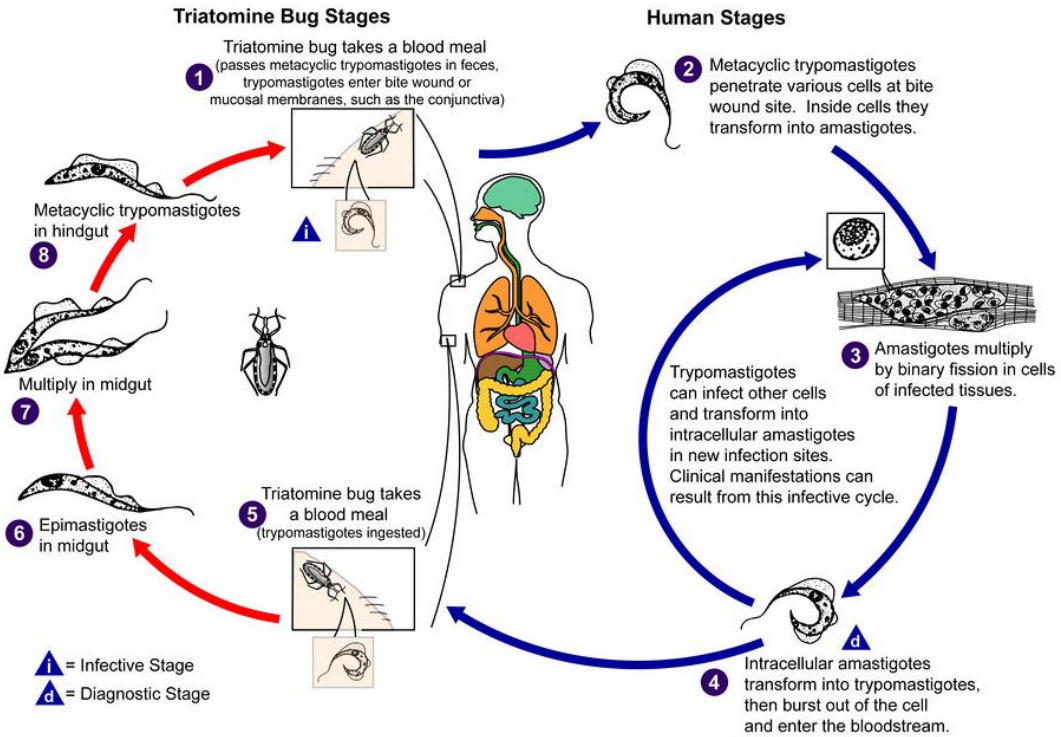


Figure 9. Life cycle of *Trypanosoma cruzi* (Adapted from parasite image library of CDC, USA).

Chronic Chagas' disease

It is seen in older children and adults between 20-40 years of age. The symptomatic chronic stage may not occur for years or even decades after initial infection; it may also be seen in persons without any previous episode of acute disease. Its manifestations include cardiomyopathy (the most serious manifestation); pathologies of the digestive tract such as megaesophagus and megacolon; and weight loss. Chronic Chagas' disease and its complications can be fatal. During the chronic phase, although signs may not be apparent, the repeated cycle of intracellular multiplication are continually destroying cells, not only those in which the amastigotes multiply, but also neighbouring cells. An autoimmune mechanism is probably involved. Neurons are particularly vulnerable to destruction. If the intracellular groups of parasite are concentrated in parts of gastrointestinal tract, especially in oesophagus or colon, peristalsis may be interfered with and the organ may become hugely distended. This condition is indicated by the prefix mega; for example, megaoesophagus or megacolon. The unfortunate patient may be unable to swallow and die of starvation. Megacolon may become so gross as to lead to rupture of colon and death.

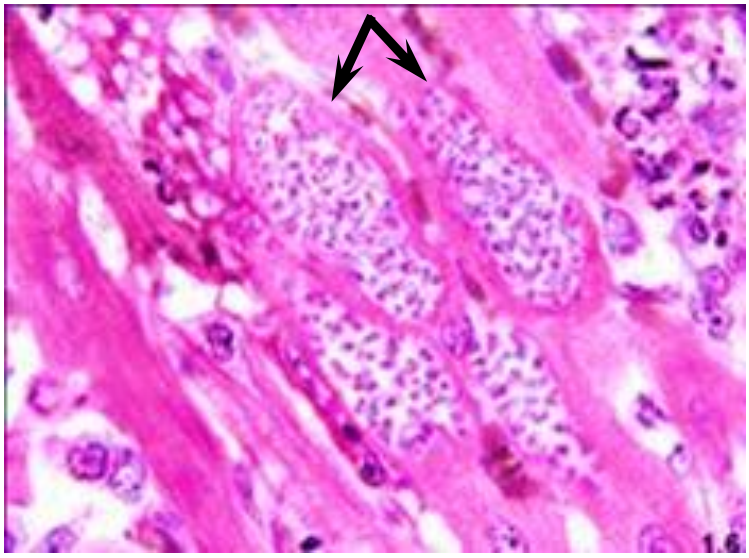


Figure 10. Amastigotes of *T. cruzi* lying in a pseudocyst in human cardiac muscle.

If the pseudocysts congregate in the heart muscle, and some strains are more prone to do this than others, the ensuing neuronal and muscle destruction may gravely weaken the heart wall, causing irreversible damage and leading to an early death from heart attack.

Diagnosis

Demonstration of the causal agent is the diagnostic procedure in acute Chagas' disease. It can be achieved by:

Microscopic examinations*:* a) of fresh anticoagulated blood, or its buffy coat, for motile parasites; and b) of thin and thick blood smears stained with Giemsa, for visualization of parasites.

Isolation of the agent by*:* a) inoculation into mice; b) culture in specialized media; and c) **xenodiagnosis,** where uninfected reduviid bugs are fed on the

patient's blood, and their gut contents examined for parasites 4 weeks later.
Immunological diagnosis: During the chronic stage of infection, parasites are rare or absent from the circulation; immunodiagnosis is the method of choice for determining whether the patient is infected. Although IFA is very sensitive, cross-reactivity occurs with sera from patients with leishmaniasis, a protozoan disease that occurs in the same geographical areas as *T. cruzi*. Sensitivity and specificity of EIA tests that use crude antigens are similar to those of the IFA test. Although differentiating between acute and chronic infection is very important in determining therapy, serology cannot be used to do so. A positive titer indicates only infection at some unknown time, and not acute infection.

Epidemiology
Chagas' disease is a zoonoses. The infection is transmitted from animals to man. It distributes in the Americas from the southern United States to southern Argentina, mostly in poor, rural areas of Central and South America. Chronic Chagas' disease is a major health problem in many Latin American countries. With increased population movements, the possibility of transmission by blood transfusion has become more substantial in the United States. Two major cycles of transmission of infection take place: domestic cycle and sylvatic cycle. In domestic cycle, the infection is transmitted between man and domestic animals by the bite of blood sucking reduviid bugs. Naturally infected dog, bug and rabbit are the reservoir hosts. They are the sources of infection of man. This type of infection is common in rural areas with low socio-economic condition and poor sanitation.
In sylvatic cycle, the infection is transmitted between sylvatic reduviid bugs and small mammals including rodents and marsupials. These are the reservoirs and source of infection for man. Chagas' disease is transmitted commonly by kissing bugs. Less frequently, the disease may be transmitted by blood transfusion or congenital infection, and laboratory infection.

Preventation and control
1) ***Treatment***: Medication for Chagas' disease is usually effective when given during the acute stage of infection. The drugs of choice are benznidazole or nifurtimox (under an investigational New Drug Protocol from the CDC Drug Service). Once the disease has progressed to later stages, no medication has been proven to be effective. In the chronic stage, treatment involves managing symptoms associated with the disease.
Acute Chagas disease must be treated early. The decision for initiating therapy must not be swayed by negative findings or delayed while waiting for results of isolation attempts, if the clinical and epidemiolog ic suspicion of the disease is strong.
2) The ***preventive measures*** include: a) application of insecticides to kill the vector bugs in human dwellings and improvement of rural housing environment to eliminate the breeding places of kissing bug. b) Personal protection by using mosquito nets and insect repellants. c) Serological screening of blood donors for *T. cruzi* to prevent transmission by blood transfusion.

GIARDIA LAMBLIA

Kingdom: Protista
Subkingdom: Protozoa
Phylum: Sarcomastigophora
Subphylum: Mastigophora
Class: Zoomastigophora
Order: Diplomonadida
Family: Hexamitidae
Genus: *Giardia*
Species: *lamblia*

It is necessary to remember

Giardia lamblia, a protozoan flagellate, inhabits the small intestine of man. This protozoan is the only intestinal flagellate known to endemic and epidemic diarrhea in man. The parasite was initially named *Cercomonas intestinalis* by Lambl in 1859 and renamed *Giardia lamblia* by Stiles in 1915, in honor of Professor A. Giard of Paris and Dr. F. Lambl of Prague.

Morphology
Giardia lamblia exists in two stages: trophozoite and cyst.
Trophozooite: It is pear-shaped with broad rounded anterior end and a tapering posterior end (Fig. 11). It measures 9-21µm in length and 5-15µm in breath and 2-4µm in thick. Dorsal surface is convex while ventral surface is concave. A sucking disc, the organ of attachment, occupies one-third to one-half of the ventral surface. Trophozoite is bilaterally symmetrical and has two nuclei, two **axostyle** and four pairs of flagella. Two **median bodies** are present on the axostyle at its origin. Cytoplasm is uniform and finely granular. The trophozoites are motile due to the presence of four pairs of flagella.
Cyst: the oval cyst measuring 8-12µm in length and 7-10µm in breath. A thick wall surrounds it. The cyst consists of cytoplasm, which is finely granular and is separated from the cyst wall by a clear space. This gives an appearance of the cyst being surrounded by a halo.
The mature cyst consists four nuclei, which may remain clustered at one end or are present in pairs at two opposite ends. Also it consists of an axostyle and margins of the sucking disc. The axostyle which is the remains of flagellum is placed diagona lly in the cyst. The four nuclei cyst is the infective stage of *G. lamblia.*
Lifecycle
The life cycle of *G. lamblia* is simple and is completed in a single host, the man (Fig. 12). Cysts are resistant forms and are responsible for transmission of giardiasis. The cysts are hardy, can survive several months in cold water. Infection occurs by the ingestion of cysts in contaminated water, food, or by the fecal-oral route. Cysts passthrough the stomach and excyst to trophozoites in the duodenum within 30 minutes of ingestion, each cyst produces two tetranucleate trophozoites. Acidity of gastric juice favours the process of excystation. In duodenum and jejunum, the tetranucleate trophozoite multiply asexually by binary fission thereby producing a large numbers of daughter trophozoites. Trophozoites browse on the mucosal surface, to which they are attached by an oval sucker. When the intestinal contents leave the jejunum

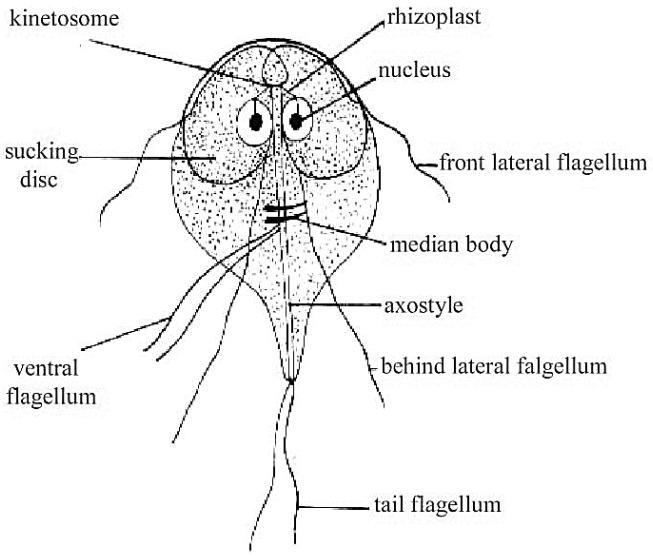


Figure 11. *Giardia lamblia* Trophozoite

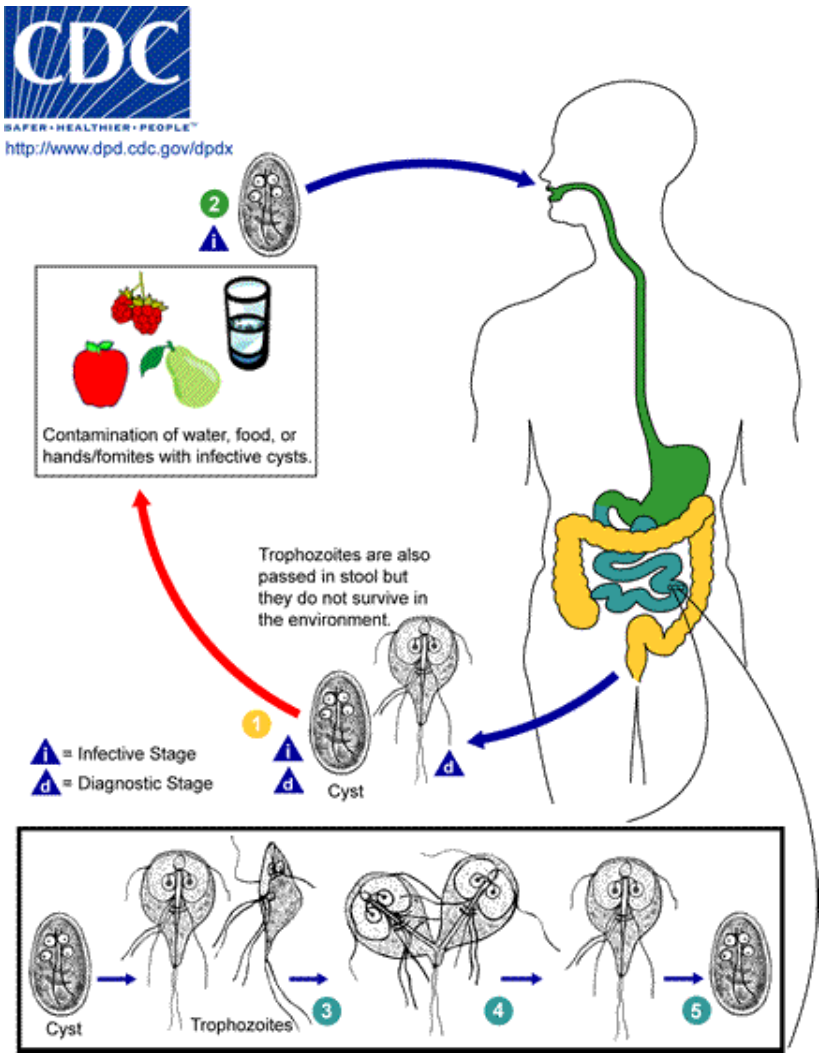


Figure 12. Life cycle of *Giardia lamblia* (Adapted from parasite image library of CDC, USA)

and begin to lose moisture, the trophozoites retract their flagella, cover themselves with a thick wall and encyst. These encysted trophozoites undergo another phase of nuclear division and produce four- nucleated mature cysts. The four nucleated mature cysts are the infective forms of the parasites, they are excreted in faeces and the cycle is repeated.

Pathogenesis and symptoms

Giardia lamblia inhabits the duodenum and upper ileum. The trophozoites may remain attached to the intestinal mucosa and rarely invades the submucosa.

As few as 10-25 cysts can cause giardiasis. Malabsorption of fat and carbohydrates in children and diarrhoea, are important clinical manifestation. The precise mechanism for these changes is not clear. The pathogenic mechanisms may be mechanical blockage of the intestinal mucosa, or competition for nutrients, or inflammation of the intestinal mucosa, or bacterial induced deconjugation of bile salts, and altered jejunal motility with or without overgrowth of intestinal bacteria and yeast.

In giardiasis the histopathology of duodenum and jejunum are highly variable and may range nearly from normal to markedly abnormal. Most commonly, there is shortening of microvilli and elongation of crypts. The brush border of the absorptive cells is damaged *Giardia mostly* are found attached to the lining of the epithelial brush border.

The clinical features vary from asymptomatic carriage to severe diarrhea and malabsorption. Majority of infected persons in the endemic area, are asymptomatic. Acute giardiasis develops after an incubation period of 5 to 6 days and usually lasts 1 to 3 weeks. Symptoms include diarrhea, abdominal pain, bloating, nausea, and vomiting.

In some patients, the infection progress to a chronic disease. In chronic giardiasis the symptoms are recurrent and malabsorption and debilitation may occur. The condition frequently is associated with malnutrition and stunted growth in pre-school children.

Diagnosis

Laboratory diagnosis is based on parasitological methods and to a less extent on serological methods.

Pathogenicdiagnosis

1) ***Fecal examination***: Giardiasis is diagnosed by the identification of cysts or trophozoites in the feces, using direct mounts as well as concentration procedures.

2) ***Duodenal contents or bile examination***: microscope examination of duodenal contents or bile is carried out, when the repeated stool examination is negative but giardiasis is still suspected. Three method are used in collecting duodenal contents:

Immunological methods:

Alternate methods for detection include antigen detection tests by enzyme immunoassays, and detection of parasites by immunofluorescence. Enzyme-linked immunosorbent assay and indirect fluorescent antibody are useful in seroepidemiolog ical studies. These methods detect anti-*Giardia* antibodies in serum, which remain elevated for a longer period.

Epidemiology

Giardiasis is worldwide in distribution, more prevalent in warm climates, and in children. *G. lamblia* infection also widely distribute in China, with an incidence varying from 0.48 to 10 percent.

Giardiasis shows two distinct epidemiolog ical patterns: endemic and epidemic. It is endemic in the developing countries like India. Mainly children are affected. In the United States and other developed countries, the condition occurs in epidemics. It affects all the age groups equally. Man who passed cysts in stool is the main reservoir of infection. Food and water contaminated by human and animal feces that contain Giardia cystsare the primary sourcesof infection.

Giardiasis is transmitted mainly by drinking fecally contaminated water and less frequently by eating contaminated food. It also can have transmitted by direct person to person spread, it occurs most commonly in persons with poor sanitation and poor faceal oral hygiene. Ocassionally, giardiasis may be transmitted by sex among male homesexual practicing anilingus.

Patients with variable immunodeficiency such as the AIDS, protein-calorie malnutrition are increasingly susceptible to infection with *Giardia.*

Prevention and control

Several prescription drugs are available to treat giardiasis; metronidazole is the drug of choice. Metronidazole, tinidazole or other 5-nitroimidazole compounds usually kill parasites in the intestine, but any in the gall bladder or bile duct may evade destruction and subsequently reinvade the intestine to produce clinical relapse. If this occurs, repeated course of therapy at higher dose may be required.

Giardiasis can be prevented and controlled by improved water supply, proper disposal of human faeces, maintenace of food and personal hygiene, and health education.

TRICHOMONAS VAGINALIS

Kingdom: Protista
Phylum: Metamonada
Class: Parabasilia
Order: Trichomonadida
Genus: *Trichomonas*
Species: *Trichomonas vaginalis*

It is necessary to remember

Trichomonas vaginalis, a flagellate, is the most common pathogenic protozoan of humans in industrialized and developing countries. It causes trichomoniasis. The infection is transmitted sexually.

Morphology

Trichomonas vaginalis only exists in trophozoite stage (Fig. 13). Cystic stage is absent. Trophozoite inhabit the vagina in female, the prostate and seminal vesicles in male and urethra in both sexes.
The trophozoites of *Trichomonas,* measuring 14-17µm×5-15µm have a single nucleus, four anterior flagella and a single lateral flagellum attached to pellicle to form an **undulating membrane.** They are actively motile, pear-shaped. The inner margin of this membrane is supported by a filament. There is also a central skeletal rod or axostyle. The cytoplasm contains a large number of hydrogenosomes and sometimes viral particles.

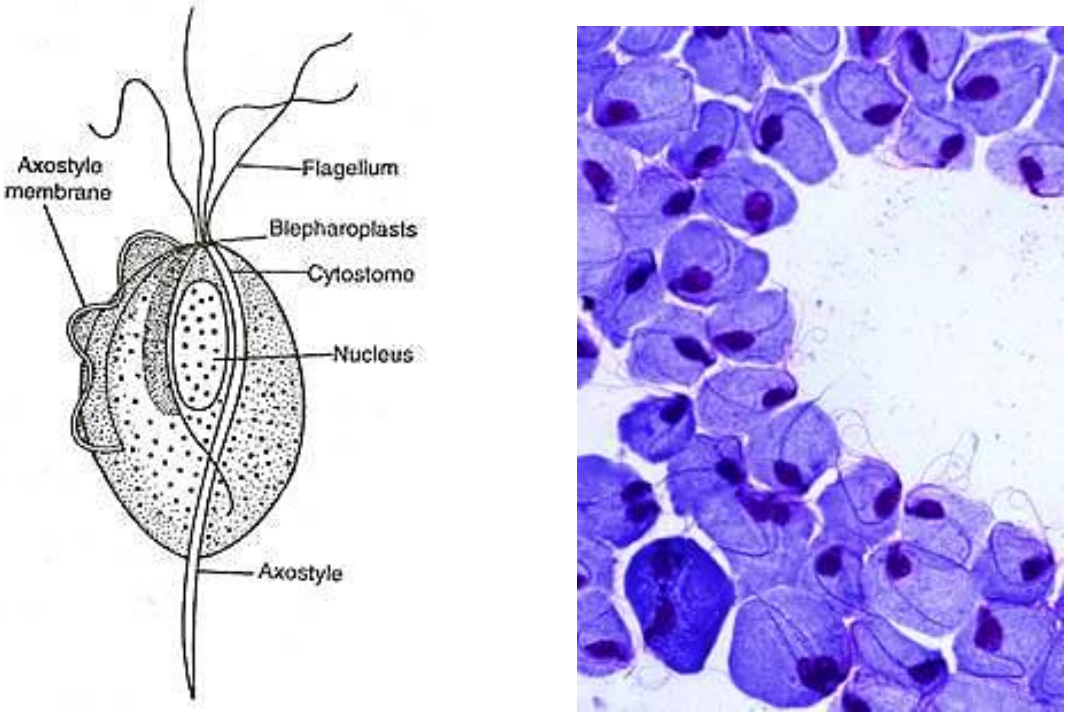


Figure 13. *Trichomonas vaginalis morphology and* obtained from in vitro culture.

Trophozoite of *Trichomonas vaginalis* is facultative anaerobic. It is identified by its characteristic twitching motility. Trophozoite is the infective form of the parasite.

Life cycle

Life cycle of *Trichomonas vaginalis* is simple (Fig. 14). It is completed in a single host either male or female. *Trichomonas vaginalis* resides in the female lower genital tract and the male urethra and prostate, where it replicates by binary fission. The parasite does not appear to have a cyst form, and does not survive well in the external environment. *Trichomonas vaginalis* is transmitted among humans, its only known host, primarily by sexual intercourse.

Pathogenesis and symptoms

Trichomonas vaginalis is an obligate parasite which cannot live without close association of the vaginal, urethral or prostatic tissues. It causes degeneration and desquamation of the vaginal mucusa. Sometimes, it is associated with small blisters or granules. The mucosa and superficial submucosa are infiltrated by lymphocytes, plasma cells and polymorphonuclear leucocytes.

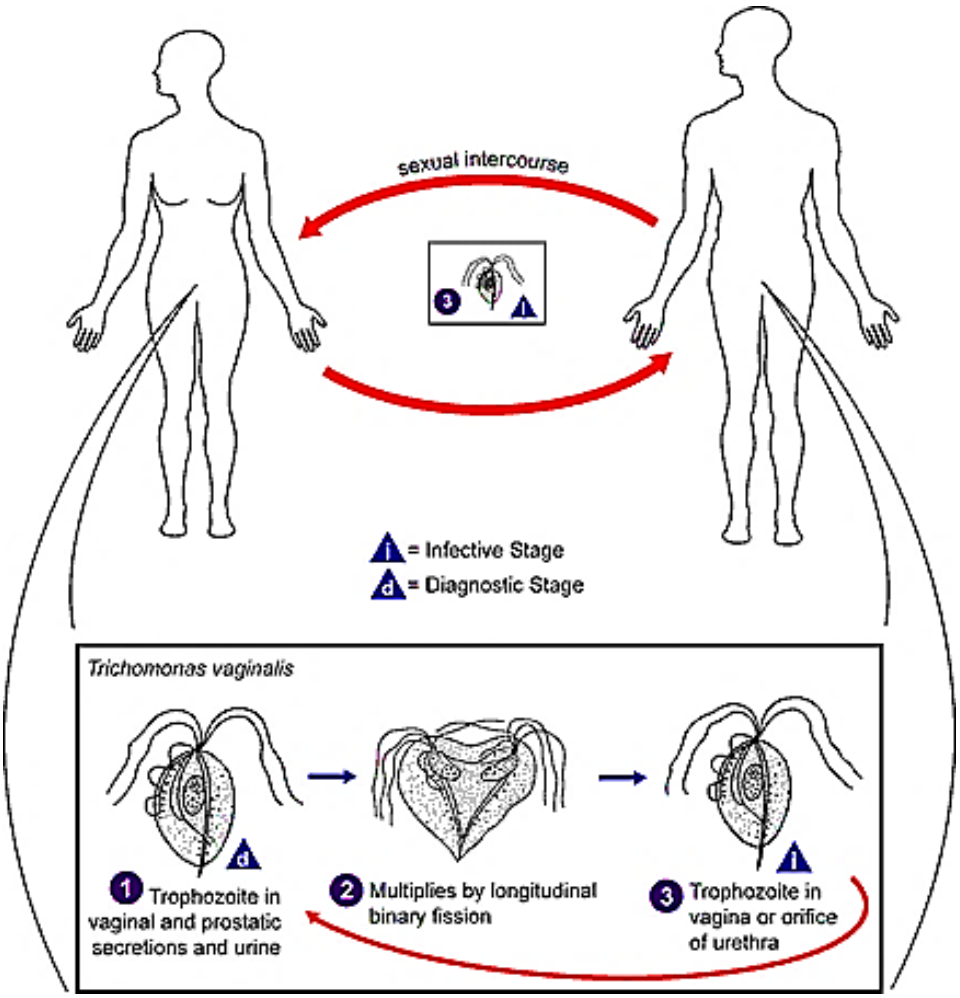


Figure 14. Life cycle of *Trichomonas vaginalis* (Adapted from parasite image library of CDC, USA)

Trichomonas vaginalis infection in women is frequently symptomatic. In symptomatic acute infection, after an incubation period, vaginal discharges is nearly in two-thirds of cases. It is frequently accompanied by vulvovaginal

soreness or irritation, dyspareunia, disagreeable odour and dysuria. The acute stage may last for a week or month and often varies in intensity. It may become severe following menstruation. Vaginitis with a purulent discharge is the prominent symptom, and can be accompanied by vulvar and cervical lesions, abdominal pain, dysuria and dyspareunia. The vaginal secretions are liquid, greenish or yellow and are present on the urethral orifice, vestibular glands and clitoris. It contains large numbers of *Trichomonas* and leucocyte. The incubation period is 5 to 28 days. In men, the infection is frequently asymptomatic; occasionally, urethritis, epididymitis, and prostatitis can occur. Persistent or reccuring nonspecific urethritis is the main clinical presentation in symptomatic cases. Infection appears to be self-limiting in many of the male possible due to trichomonicidal action of the prostatic fluid or flushing out of the flagellate mechanically from urethra during micturition.

Diagnosis

The specific diagnosis of trichomoniasis is made by demonstration of organisms in the genital specimens and also in the urine by microscopy, culture and non parasitic methods. Microscopic examination of wet mounts may establish the diagnosis by detecting actively motile organisms. This is the most practical and rapid method of diagnosis (allowing immediate treatment), but it is relatively insensitive. Direct immunofluorescent antibody staining is more sensitive than wet mounts, but technically more complex. Culture of the parasite is the most sensitive method, but results are not available for 3 to 7 days. In women, examination should be performed on vaginal and urethral secretions. In men, anterior urethral or prostatic secretions should be examined.

Epidemiology

Trichomoniasis probably is the most common sexually transmit disease worldwide. Higher prevalence among persons with multiple sexual partners or other venereal diseases. Up to 40% of women have been reported in some random surveys to be infected, and the organism has been found in up to 70% of women with vaginitis. Infected women harbouring *T. vaginalis* in the genital tract and infected men are the chief reservoir of infection. Trophozoite is the infective stage the infection may be transmitted venereally by sexual contact with infected person, also to babies during passage through an infected birth canal, and occasionally non-venereally through fomites such as towels, toilet seats, etc., and also through mud and water bath as well.

Prevention and control

Treatment should be implemented under medical supervision, and should include all sexual partners of the infected persons. The drug of choice for treatment is metronidazole; therapy is usually highly successful. Tinidazole, which is a better-tolerated alternative drug, is not available in the United States. Strains of Trichomonas vaginalis resistant to both drugs have been reported.

Preventive measures:

1) Detection and treatment of cases either male or female.
2) Avoidance of sexual contact with infected partners, and
3) Use of condoms.

LESSON 7. PARASITOLOGY. APICOMPLEXA

Kingdom: Protista
Subkingdom: Protozoa
Phylum: Apicomplexa
Class: Sporozoasida
Order: Eucoccidiorida
Family: Plasmodiidae
Genus: *Plasmodium*
Species: *falciparum, malariae, ovale, vivax*

It is necessary to remember

The causal agents of malaria are blood parasites of the genus *Plasmodium,* family Plasmodiidae in the suborder Haemosporina. There are approximately 156 named species of *Plasmodium, which* infect various species of vertebrates. Four are known to infect humans: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. In China mainly are *P. falciparum* and *P. vivax*; *P. ovale* and *P. malariae* infection are rare seen.

Morphology and life cycle

The malaria parasite life cycle involves two hosts: **human** and female ***Anopheles* mosquito.** Human is the intermediate host for asexual reproductions occur in liver and RBCs; Mosquito is the definitive host; the sexual reproduction takes place in the stomach of mosquito. Four species of human malarial parasites are more or less similar in their cycle and morphology (Fig. 15-19), with some minor differences between.

Human Cycle

Malaria parasites develop in human body includes two stages: exo-erythrocytic stage (sporozoites develop in liver) and erythrocytic stage (merozoites develop in RBCs)

***Exo-erythrocytic stage*:** During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates **sporozoites** into the human host.

The **sporozoite** a small, spindle-shaped cell with a single nucleus, which developed in mosquito, it is introduced into man by the bite of an infected mosquito. When sporozoites are injected into a susceptible host, they rapidly enter liver parenchyma cells. They then (unless hypnozoites, see below) begin a process of multiple divisions known as merogony. **Pre-erythrocytic schizont** in liver mature in 6-14 days' time, it need about 8 days to complete the exo-erythrocytic cycle in *P. vivax*, about 6 days in *P. falciparum,* 11-12 days in *P. malariae* and 9 days in *P. ovale*. Merogony in liver cells results in the production of thousands of **merozoites** per meront.

After the infected liver parenchyma cell is broken, the merozoites release from the liver cells, some of merozoites are phagyocytize by host's macrophage in the liver, which may be an important host defense mechanism; and some of them penetrate into erythrocytes in the blood, initiating the erythrocytic cycle. The sporozoites that establish in the liver cells are proved of two genetic forms: **tachysporozoite** and **bradysporozoite** or hypnozoites. The tachysporozoites develop into trophozoites and undergoes **EE schizogony** immediately after they enter the liver. Hypnozoites will remain in the liver without further development in a latent period. The latent period of hypnozoite is

more than 3 months to 2 years when the primary attack has subsided.
In *P. vivax,* hypnozoites are found inside the liver parenchyma. These are single-nucleated parasites measuring 4-6µm in diameter and are the dormant stages of the parasite. Relapse in vivax malaria is caused by these hypnozoites, which after a period of time become active and develop into pre-erythrocytic schizonts, there by causing malaria. In *P. falciparum,* only a single generation of exo-erythrocytic stage take place, secondary EE stage is absent; recent evidences indicate that hypnozoites are found in the live phase of *P. malariae.* The single nucleated intra-hepatocellular hypnozoites of *P. ovale* resemble those of *P. vivax.*

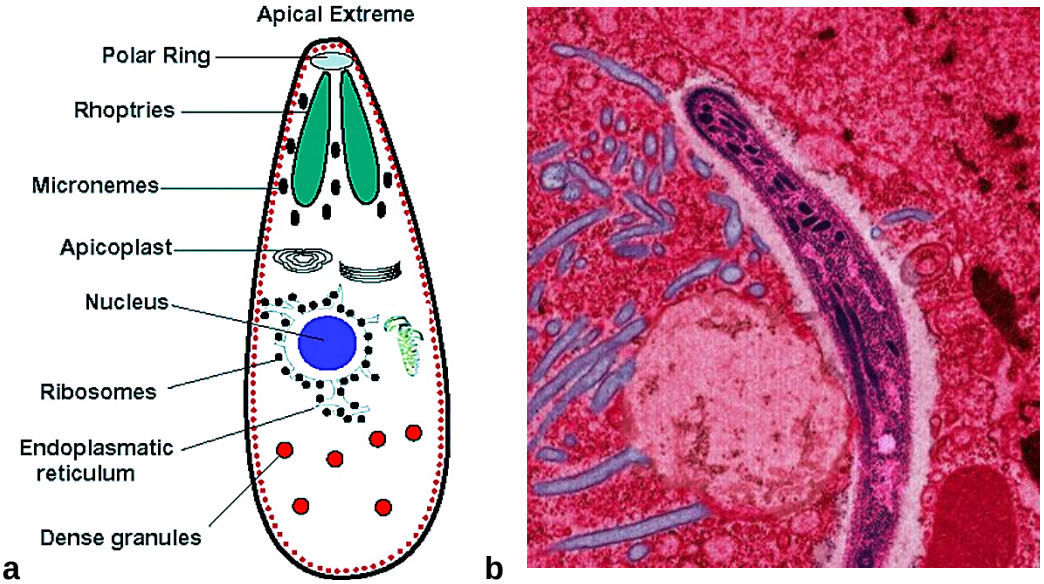


Figure 15. Diagram of a sporozoite's internal structure – a, sporozoite in histological slices – b.

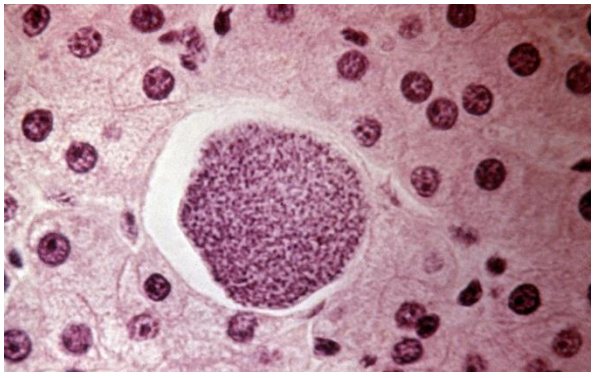
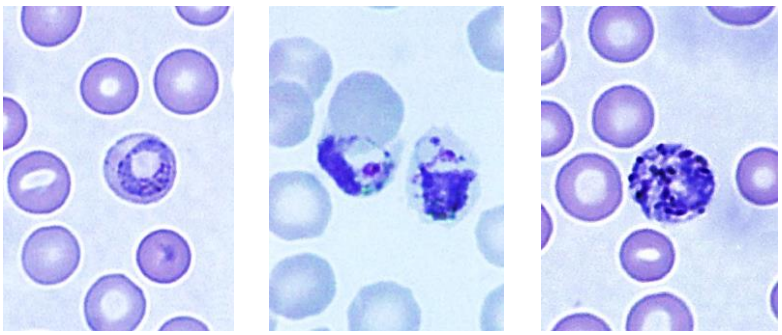


Figure 16. Pre-erythrocytic schizont in liver.

Erythrocytic stages: The merozoites invade red blood cells; these are then transformed into trophozoites and finally, develop into erythrocytic schizonts. *P. vivax* and *P. ovale* prefer invading young cells; *P. malariae* invade usually mature older cells, rarely reticulocytes; *P. falciparum* EE merozoites invade both the reticulocytes and erythrocytes. Erythrocytic stages such as **trophozoites**, **schizonts** and **gametocytes** are present.
Trophozoites: On entry into an erythrocyte, the merozoite again transforms into a trophozoite (Fig 17). The host cytoplasm ingested by the trophozoite forms a large food vacuole, giving the young Plasmodium the appearance of a ring of cytoplasm with the nucleus conspicuously displayed at one edge.

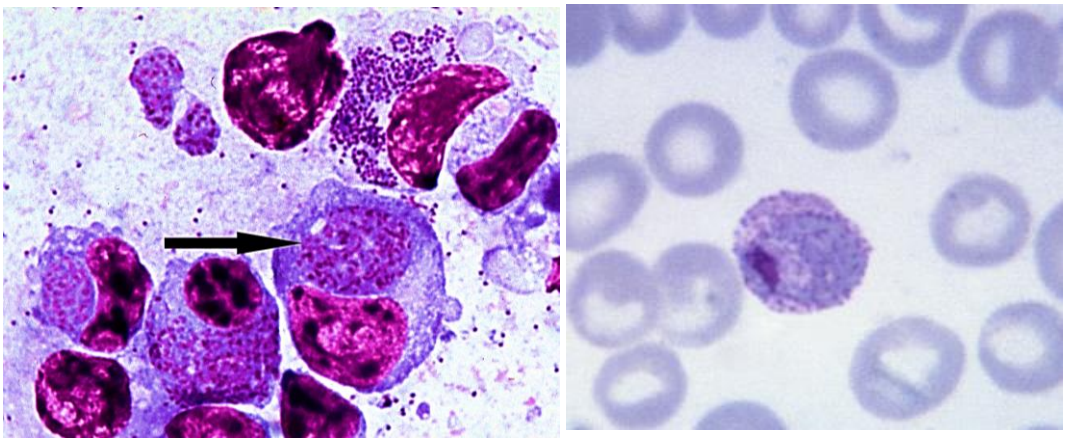
This stage of trophozoites are known as **ring forms.** The trophozoite is vacuolated, ring shaped, more or less amoeboid and uninucleate. As the trophozoite grows, its food vacuoles become less noticeable by light microscopy, but pigment granules of **hemozoin** in the vacuoles may become apparent. Hemozoin is the end product of the parasite's digestion of the host's hemoglobin but is not a partially degraded form of hemoglobin.
The ring forms of *P. falciparum* are very small, which a very thin circle of cytoplasm; some appear to have two nucle i, and some are closely pressed to the periphery of the cell, the infected host red cells are not enlarged; ring forms of *P. vivax* are larger, and as the parasite grows the infected cell becomes enlarge and deve lop red-staining **Schüffner's dots** on its surface; the growing trophozoite is actively motile and thus often appears irregular in shape; *P. malariae* trophozoite are not active and are irregular in shape, often across the cell as a bend. The infected cell is not enlarged and only rarely shows a few surface dots: Zie mann's dots. Early trophozoite or ring forms of *P. ovale* are more similar to those of *P. malariae*. *P. ovale* ring forms are relative ly compact. Late trophozoite is small and compact. It contains coarse pigments and an inconspicuous vacuole. It does not show any amoeboid movement. The host cells are round and oval, often fimbriated and invariably are enlarged; Schüffner's dots are present.



ring developing meronts mature meront

Figure 17. *P. vivax* at different erythrocytic stages of trophozoites development

***Erythrocytic Schizonts*:** The trophozoites multiply with division of nucleus by mitosis, followed by a division of cytoplasm, to become mature schizonts (Fig 18). The erythrocytic schizonts are dividing forms. The stage in the erythrocytic schizogony at which the cytoplasm is coalescing around the individual nuclei, before cytokinesis, is called the segmenter. When development of the merozoites is completed, the hosts cell ruptures, releasing parasite metabolic wastes and residual body, including **hemozoin.** The metabolic wastes thus released are one factor responsible for the characteristic symptoms of malaria, although hemozoin itself is nontoxic. A great many of the merozoites are ingested and destroyed by reticaloendothelial cells and leukocytes, but, even so, the number of parasitized host cells may become astronomical because erythrocytic schizogony takes only from 1 to 4 days, depending on the species. In *P. falciparum*, the schizonts are small, and rarely seen in peripheral blood, because infected cells adhere to the endothelium of capillaries in the internal organs. The erythrocytic is completed within 48 hours and always takes place inside the capillaries and vascular beds of internal organ.



a b

Figure 18. *P. vivax* the erythrocytic schizonts – a & gametocyte – b.

In *P. vivax,* erythrocytic schizonts are large, round and irregular in form and occupy the entire red cell, which are enlarged. All the developing stages of schizonts can be seen which contain pigment granules. A mature schizont contains usually 16 merozoites but may contain more even up to 24.
The erythrocytic cycle may be repeated or, in response to unknown stimuli, maturation into gametocytes may occur.
Gametocyte: After an indeterminate number of asexual generations, some merozoites enter erythrocytes and become macrogamonts (macrogametocytes) and microgamonts (microgametocytes). The size and shape of these cells are characteristic for each species; they also contain hemozoin. Unless they are ingested by a mosquito, gametocytes soon die and are phagocytized by the reticuloendothelial system.
The male and female gametocyte of all species can be differentiated as the male has a larger, more diffuse nucleus, in readiness for gamete production after its ingestion by the mosquito; the female has darker staining cytoplasm because it contains numerous ribosomes for protein biosynthesis following fertilization. *P. falciparum* gametocytes are crescent-shaped but those of other species are spherical.

Mosquito cycle
When an unsuitable mosquito imbibes erythrocytes containing gametocytes, they are digested along with the blood. However, if a susceptible mosquito is the diner, the gametocytes develop into gametes. Although this development would take place only in a female mosquito in nature, since only females feed on vertebrate blood, males of appropriate species can support development after experimental infection with the parasite in the laboratory. Suitable hosts for the Plasmodium spp. of humans are a wide variety of *Anopheles* spp. (Fig. 20) After release from its enclosing erythrocyte, maturation of the macrogametocyte to the macrogamete involves little obvious change other than a shift of the nucleus toward the periphery. In contrast, the microgametocyte displays a rather astonishing transformation, **exflagellation.** As the microgametocyte becomes extracellular, within 10 to 12 minutes its nucleus divides repeatedly to from six to eight daughter nuclei, each of which is associated with the elements of a developing axoneme. The doubled outer membrane

of the microgametocyte becomes interrupted; the flagellar buds with their associated nuclei move peripherally between the interruptions and then continue outward covered by the outer membrane of the gametocyte. These break free and are the **microgametes.** The stimulus for exflagellation is an increase in pH caused by escape of dissolved **carbon dioxide** from the blood. The life span of the microgametes is short, since they contain little more than the nuclear chromatin and the flagellum covered by a membrane. The microgamete swims about until it finds a macrogamete, which it penetrates and fertilizes. The resultant diploid **zygote** quickly elongates to become a motile **ookinete.** The ookinete is reminiscent of a sporozoite and merozoite in morphology. It is l0 to 12 μ m in length and has polar rings and **subpellicular microtubules** but no micronemes.

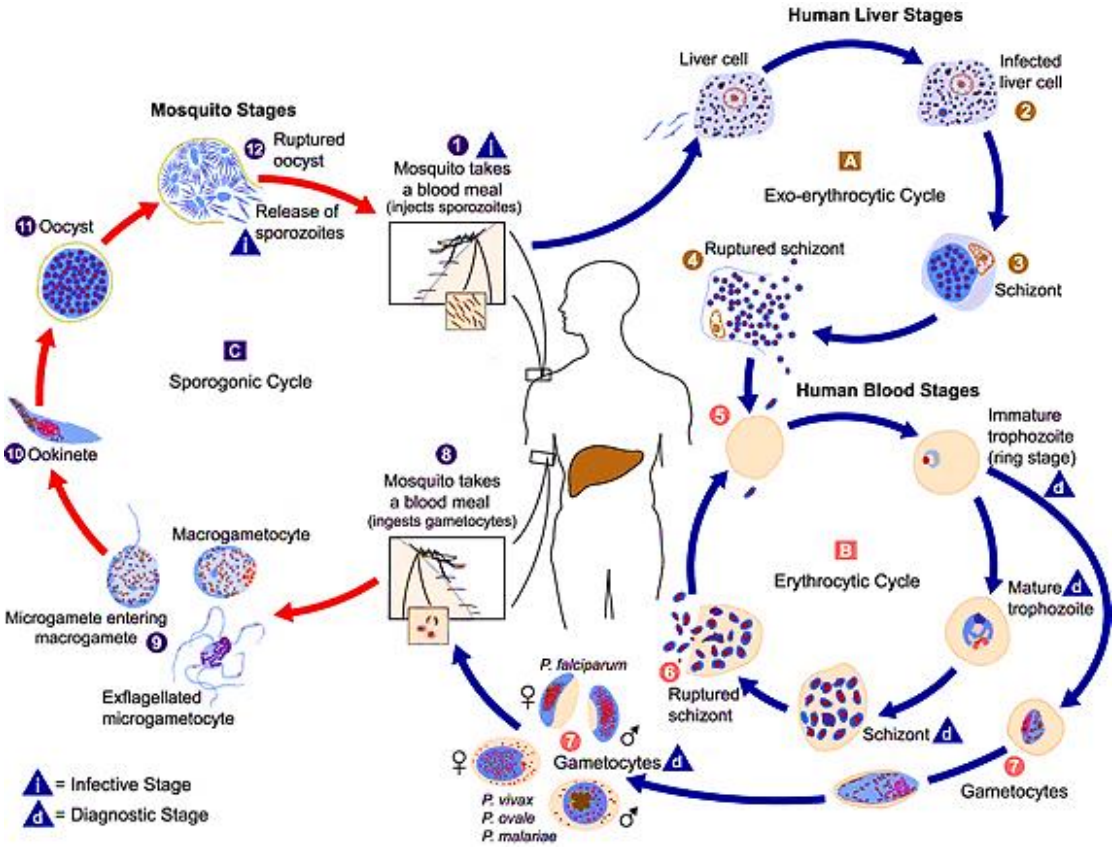
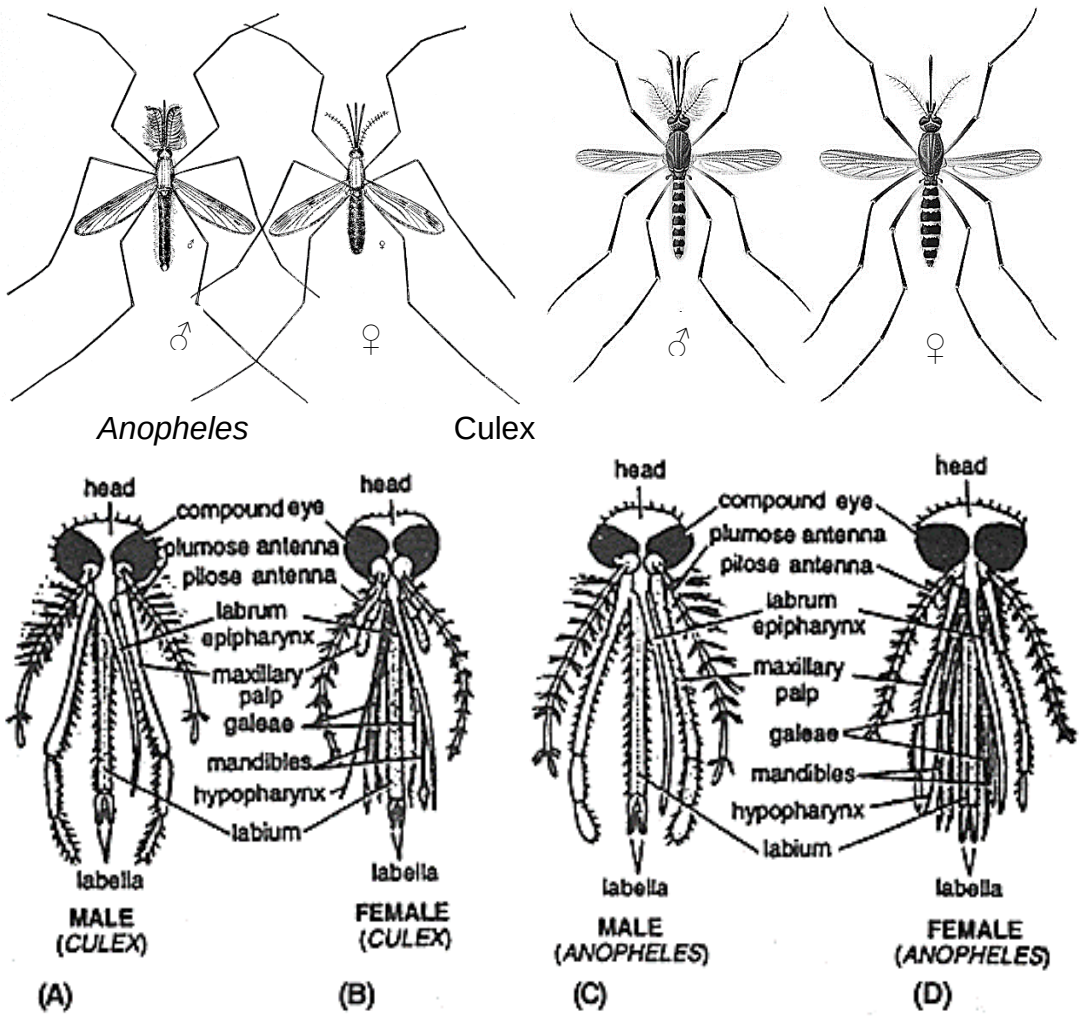


Figure 19. The life cycle of Plasmodium (Adapted from parasite image library of CDC, USA)

The ookinete penetrates the **peritrophic membrane** in the mosquito's gut. Migrates to the hemocoel side of the gut, and begins its transformation into an **oocyst.** The oocyst is covered by an electron-dense capsule and soon extends out into the insect's hemocoel.
The initial division of its nucle us is reduction al; meiosis takes place immediately after zygote formation as in other Sporozoea. The oocyst reorganizes internally into a number of haploid nucleated masses called sporoblasts, and the cytoplasm contains many ribosomes, endoplasmic reticulum, mitochondria, and other inclusions. The sporoblasts in turn divide repeatedly to form

thousands of sporozoites. These break out of the oocyst into the hemocoel and migrate throughout the mosquito's body. On contacting the **salivary gland,** sporozoites enter its channels and can be injected into a new host at the next feeding.



(A) Male Culex (B) Female Culex (C) Male Anopheles (D) Female Anopheles

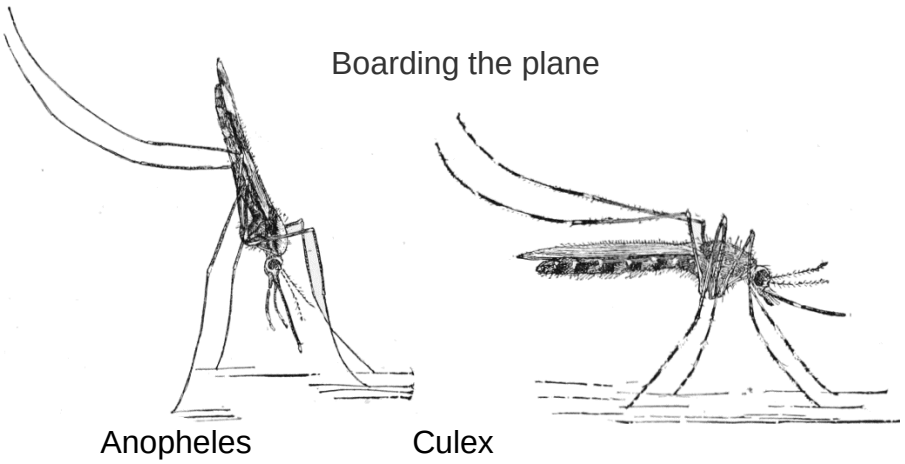


Figure 20. Male and female mosquito *Anopheles* spp. and *Culex* their differences.

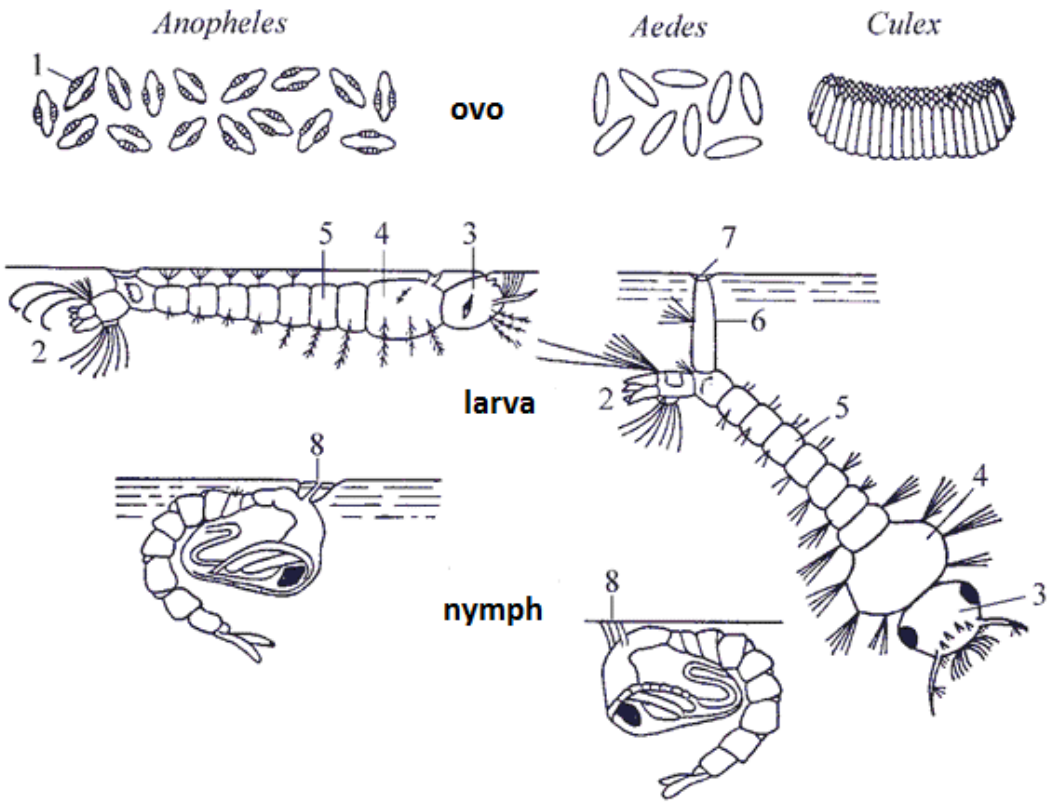


Figure 20. The differences mosquito *Anopheles* spp., *Culex pipiens* and *Aedes aegypti*.
Anopheles – separate floating eggs, *Aedes* – separate sinking eggs, *Culex* – sinking eggs laid by the cassette.
1 – floats eggs *Anopheles; 2* – spiracles of larvae; 3 – eye; 4 – chest; 5 – body segment; 6-7-8 – breathing tube

Sporozoite development takes from l0 days to 2 weeks, depending on the species of Plasmodium and the temperature. Once infected, a mosquito remains infective for life, capable of transmitting malaria to every susceptible vertebrate it bites. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle.
Plasmodium sometimes is transmitted by means other than the bite of a mosquito. The blood cycle may be initiated by blood transfusion, by malaria therapy of certain paralytic diseases, by syringe-passed infection among drug addicts, or, rarely, by congenital infection.

Pathogenesis and clinical manifestations
The major clinical manifestations of malaria may be attributed to two general factors: the host inflammatory response, which produces the characteristic chills and fever, as well as other related phenomena; and anemia, arising from the enormous destruction of red blood cells. Severity of the disease is correlated with the species producing it: falciparum malaria is most serious and vivax and ovale the least dangerous.

Incubation period

Usually after human got infection, the symptoms would not appear immediately, there is an incubation period. It represents the time interval between the infective bite of *Anopheline* mosquito and the onset of the clinical symptoms. It includes the period of the time for the sporozoite reaching liver and entering, the duration of the development in the liver, the time of development in the RBC to produce sufficient erythrocytic merozoites to cause clinical symptoms. The incubation period in *vivax* varies from 8 to 31 days; 7 to 27 days in P. falciparum; In P. malariae, the incubation period relatively is longer and varies from 18 to days; In P. ovale, it is 16 to 18 days.

Malarial paroxysm

Malarial paroxysm is preceded by a prodromal period. A few days before the first paroxysm, the patient may feel malaise, muscle pain, headache, loss of appetite, and slight fever; or the first paroxysm may occur abruptly, without any prior symptoms. The classic malarial paroxysm comprises of three successive stages: cold stage, hot stage and sweating stage. The first stage is the **cold-stage,** A typic a l attack of benign tertian or quartan malaria begins with a feeling of intense cold as the hypothalamus, the body's thermostat, is activated, and the temperature then rises rapidly to 41℃. The teeth chatter, and the bed may rattle from the victim's shivering. The skin is warm and dry, Nausea, vomiting, severe headache, back ache, and hypotension are usual. The **hot stage** begins after 1/2 to 1 hour, with intense headache and feeling of intense heat. Sweating stage is the final stage, often a mild delirium stage lasts for several hours. As copious perspiration signals the end of the hot stage, the temperature drops back to normal within 2 to 3 hours, and the entire paroxysm is over within 8 to 12 hours. The person may sleep for a while after an episode and feel fairly well until the next paroxysm. The foregoing time periods for the stages are usually somewhat shorter in quartan malaria, and the paroxysms recur every 72 hours. In vivax malaria the periodicity is often quotidian early in the infection, since two populations of merozoites usually mature on alternate days. "Double" and "triple" quartan infections also are known. Only after one or more groups drop out does the fever become tertian or quartan and the patient experiences the classical good and bad days.

Fever is a common, nonspecific reaction of the body to infection, function ingat least in part to increase the rate of metabolic reactions important in host defenses. Fever in malaria is correlated with the maturation of a generation of merozoites and the rupture of the red blood cells that contain them. It is widely believed that fever is stimulated by the excretory products of the para sites, released when the erythrocytes lyse, but the exact nature of such substances is not known. There is evidence of production of cytotoxic factors by the parasites: oxidative phosphorylation and respiration are inhibited in mitochondria from infected animals, and damage to liver cells can be observed on the ultrastructural level.

Because the synchrony in falciparum malaria is much less marked, the onset is often more gradual, and the hot stage is extended. The fever episodes may be continuous or fluctuating, but the patient does not feel well between paroxysms, as in vivax and quartan malaria. In cases in which some synchrony

develops each episode lasts 20 to 36 hours, rather than 8 to 12, and is accompanied by much nausea, vomiting, and delirium. Concurrent infections with *P. vivax* and *P. falciparum* are not uncommon.

Complications of malaria

Falciparum malaria is always serious, and sometimes severe complications are produced. The most common of these is **cerebral malaria,** which may account for 10% of falciparum malaria admitted to the hospital and 80% of such deaths. Cerebral malaria may be gradual in onset, but it is commonly sudden; a progressive headache may be followed by a coma, an uncontrollable rise in temperature to above 41℃, and psychotic symptoms or convulsions, especially in children. Death may ensue within a matter of hours. Initial stages of cerebral malaria are easily mistaken for a variety of other conditions, including acute alcoholism, usually with disastrous consequences.

Another grave and usually fatal complication of severe falciparum malaria is **pulmonary edema**, which in some cases may be a result of over administration of intravenous fluids. Difficulty in breathing increases and death may ensue in a few hours.

Meet the following complications of malaria:

Anemia,
Tropical splenomegaly syndrome,
Blackwater fever,
Hypoglycemia.

In addition, you can meet:

Congenital malaria,
Transfusion malaria.

Immunity.

Host immunity in malaria broad ly is of two types: natural or innate immunity and acquired immunity

Natural immunity

Natural immunity in malaria refers to the inherent but non-immune mechanisms of the host defence against malaria. Mainly, it is based the nature of the red cells. The nature of the red blood cells that determine the susceptibility of the cells to invasion by malaria parasites and development in the cells include

1) ***Age of red blood cell*****:** *P. falciparum* infects both young and old erythrocytes. In contrast, *P. vivax* and *P. ovale* infect only young erythrocytes and *P. malariae* only old erythrocytes.

2) ***Nature of haemoglobin***: resistance in malaria is conferred by the presence of abnormal haemog lobin molecules, seen in certain disorders. Factors that can contribute to genetic resist ance are certain heritable anemias: sickle cell, favism, and thalassemia. Although these conditions are of negative selective value in themselves, they have been selected for in certain populations because they confer resistance to falciparum malaria. The most well known of these is sickle cell anemia. In persons homozygous for this trait a glutamic acid residue in the amino acid sequence of hemoglobin is replaced by a valine, interfering with the conformation of the hemoglobin and oxygen-carrying capacity of the erythrocytes. Persons with sickle cell anemia usually die before the age of 30. In heterozygotes some of the hemoglobin is normal,

and these persons can live relatively normal lives, but the presence of the abnormal hemoglobin inhibits growth and development of *P. falciparum* in their erythrocytes. The selective pressure of malaria in Africa has led to maintenance of this otherwise undesirable gene in the population. This legacy has unfortunate consequences when the people are no longer threatened by malaria, as in the United States, where 1 in 10 Americans of African ancestry is heterozygous for the sickle cell gene, and 1 in 400 is homozygous.

3) *Enzyme content of erythrocyte*: Glucose-6 phospate dehydrogenase deficiency trait is a genetic deficiency trait believed to confer some protect ion against *P. falciparum* infection. The exact protective mechanism is not fully understood. A mechanism that probably interferes with adaptability of the parasite to the G6PD deficient condition in the red blood cells might be responsible.

4) *Presence or absence of certain factors*. Black persons are much less susceptible to vivax malaria than are whites, and falciparum malaria in blacks is somewhat less severe. The genetic basis for this phenomenon is explained by the inheritance of Duffy blood groups. In Duffy blood groups, there are two codominant alleles, Fy^a and Fy^b, recognized by their different antigen. The *Fy/Fy* genot ype is common in African and in American black people and rare in white people. It has been shown that Fy^a and Fy^b are receptors for *P. vivax* and *P. knowlest*; hence *Fy/Fy* is refractory to infection. This explains the natural resistance of people to vivax malaria. The Duffy negative genotype may represent the original, rather than the mutant, condition in tropic a l Africa.

Acquired immunity

Specific acquired immunity in malaria involves both humoral and cellular immunities. The specific immunity restricts the level of parasitemia and eventually confers protection from the disease but not from infection. The development of protection immunity in malaria is the result of a complicated interaction between the malaria parasites and immune system of the host which involve both humoral and cellular immunities.

1) *Malarial antigen* They exist in the surface and inside of the parasite; every stage of life cycle can act as antigens. Malarial antigens have species special and stage special.

2) *Humoral immunity*

Circulating antibodies against sporozoites, asexual blood stages and sexual blood stages develop in persons repeatedly exposed to malaria. Antibody response is strongest against the asexual blood forms of the parasite, which have consequently evolved various methods of **immune evasion.**

Humoral antibodies against asexual blood forms may protect against the malaria parasites by inhibiting red cell invasion, or by inhibiting growth inside the red blood cells and sequestration of parasitised red blood cells. These antibodies are responsible for the decreased susceptibility of the host to malarial infection and disease. Antibodies against sexual stages are suggested to reduce malaria transmission. Acquired antibody-mediated immunity is transferred from mother to foetus across the placenta. This passive ly transferred immunity protects the baby from severe malaria in the first few months of life. It disappears within 6 to 9 month.

3) *Cell-mediated immunity*

Recent works suggest that a variety of cellular mechanisms may play a role in conferring protect ion against malaria. The cellular mechanism is mainly of non-speciic type. In acute *P. falciparum* infection, a positive correlation has been found between natural killer and resistance to malaria.

Activated macrophages may phagocytose and induce extra-cellular killing of target cells. These reactions may be specifically amplified or induced by antibodies bound to target cell surfaces. These may also be induced non-specific by endotoxin-like substances derived from malaria parasites. The mediators released from activated macrophages are responsible for various pathogical changes found in the infected hosts during acute malarial infections. Natural acquired immunity is suppressed in pregnant women particularly primigravid, in certain serious illness and in persons receiving immunosuppressive therapy.

Immunological factors have been implicated in the pathogenesis of several complications of malaria such as glomerulonephritis, cerebral malaria, tropical splenomegaly syndrome and anaemia.

Diagnosis

The diagnosis of malaria can be based on clinical criteria and/or techniques for parasite. The condition is considered in any person who has a febrile illness and who has come from the area endemic for malaria, received blood transfusion or used intra venous drugs.

Laboratory diagnosis of malaria is established by parasitological methods by demonstration of malaria in blood. Serological methods are useful only in the epidemiology studies. Molecular diagnosis techniques can complement microscopy, especially in species identification.

Microscopy detection is the method most frequently used to demonstrate an active infection.

Quantified buffycoat technique. The detection of malaria parasites using the quantified buffy coat technique is easy to learn, has high sensitivity and specificity and is quicker to perform than standard microscopy. However, this technique requires specialized equipment and consumables, making it prohibitively expensive. It is therefore unlikely to be used by health services in the majority of endemic countries

Immunodiagnosis. In addition to microscopy and molecular methods, there are methods for detecting malaria parasites on the basis of antigens, antibodies.

Molecular diagnosisю In recent years, several specific DNA and RNA probes have been developed and tested mainly for the detection of *P. falciparum* and to a lesser extent for *P. vivax*, with the detection of all four species with specific RNA probes achieved in at least one study. The resulting methods were shown to be highly specific with minimum detection levels of 2-500 parasites of blood. The use of non-radiolabelled probes, although marginally less sensitive than radioactive labelling, allows for longer shelf life and easier storage and handling.

Polymerase-chain reaction based tests have been shown in a number of studies to detect even 1 parasite. Again, in most cases, only *P. falciparum* and *P. vivax* have been targeted but one assay has been developed that can

detect *P. malariae* and *P. ovale* with similar specificity and sensitivity. Recently, experimental assays that will allow the non-specific detection of all human *Plasmodium* species have been developed.

Epidemiology

Malaria is the most important tropical disease, remaining widespread throughout the tropics, but also occurring in many temperate regions. It exacts a heavy toll of illness and death - especially amongst children and pregnant women. It also poses a risk to travelers and immigrants, with imported cases increasing in non-endemic areas. Treatment and control have become more difficult with the spread of drug-resistant strains of parasites and insecticide-resistant strains of mosquito vectors. Health education, better case management, better control tools and concerted action are needed to limit the burden of the disease.

Geographic Distribution

Malaria generally occurs in areas where environmental conditions allow parasite multiplication in the vector. Thus, malaria is usually restricted to tropical and subtropical areas and altitudes below 1,500 m. However, this distribution might be affected by climatic changes, especially global warming, and population movements. Both *Plasmodium falciparum* and *malariae* are encountered in all shaded areas of the map (with *P. falciparum* by far the most prevalent). *Plasmodium vivax* and *P. ovale* are traditionally thought to occupy complementary niches, with *P. ovale* predominating in Sub-Saharan Africa and *P. vivax* in the other areas; however, these two species are not always distinguishable on the basis of morphologic characteristics alone; the use of molecular tools will help clarify their exact distribution.

In addition to natural or biological transmission, discussed below, malaria can be transmitted from human to human. Accidental transmission can occur by blood transfusion and by the sharing of needles by drug addicts. Although rare, infection of the newborn from an infected mother also occurs. Neurosyphilis was formerly treated by deliberate infection with malaria. (A great deal of knowledge about malaria was gained during these treatments, but we still do not understand why infection with malaria alleviated the symptoms of the terrible disease of neurosyphilis).

Prevention and control

Treatment of infected individuals. Appropriate drug treatment of persons with the disease, as well as prophylactic drug treatment of newcomers to malarious areas, is an integral part of malaria control. The most important of these was chloroquine.

Subsequently a number of valuable drugs have been deve loped, including primaquine, mefloquine, pyrimethamine, proguanil, sulf onamides such as sulf adoxine, and Antibiotics such as tetracycline. Only primaquine is effective against all stages of all species; the others vary in efficacy according to stages and species, with the erythrocytic stages being most susceptible. The drugs of choice are chloroquine and primaquine for *P. vivax* and *P. ovale* malarias and chloroquine alone for *P. malariae* infections. Chloroquine is still recommended for strains of P. falciparum sensitive to that drug.

Mosquito control

Valuable actions in mosquito control include destruction of breed ing places when possible or practical, introduction of mosquito predators such as the mosquito-eating fish *Gambusia affinis*, and jud icious use of insecticides.

Prevention of mosquitebite

These include 1) personal protect ion by proper use of mosquito nets while sleeping; 2) Wearing protective clothings that minimize contact with mosquitoes and 3) Use of mosquito repellants.

Resistance of *P. falciparum* to chloroquine has now spread through Asia, Africa, and South America, and resistance to other drugs is often present. A combination of sulfadoxine and pyrimethamine has been in use for chloroquine-resistant falciparum malaria. For multidrug resistant *P. falciparum*, mefloquine is still effective (although there are reports of mefloquine resistance), or quinine and tetracycline can be given over a period of 7 days. Resistance to qinghaosu has not been reported in the field, but resistant strains have been produced in the laboratory?

TOXOPLASMA GONDII

Kingdom: Protista
Subkingdom: Protozoa
Phylum: Apicomplexa
Class: Sporozoasida
Order: Eucoccidiorida
Family: Sarcocystidae
Genus: *Toxoplasma*
Species: *gondii*

It is necessary to remember

Toxoplasma gondii Nicolle & Manceaux, 1908 is a protozoan parasite that infects most species of **warm-blooded animals,** including humans, causing the disease toxoplasmosis. The parasite probably is the only protozoan, who's all the stages (tachyzoite, tissue cyst and oocyst) are infection for man.
Toxoplasma gondii was first described by Nicolle and Manceaux in 1908 in gundi, a small rodent of North Africa. It was named as *Toxplasma,* due to crescent shape of its tachyzoite. The parasite was subsequently demonstrated in man by Darling. It was found in congenitally infected child in 1937. The life cycle of *Toxoplasma gondii* was fully described only in 1970, when it was known that cats are the definitive hosts, man and other warm-blooded animals are the intermediate hosts.
T. gondii is an obligate intracellular parasite, which is found inside the reticuloendothelial cells and many other nucleated cells of the host. It causes the disease toxoplasmosis, especially in the immunocompetent hosts or in the immuno-compromised hosts. *T. gondii* is an important **opportunistic protozoan.**

Morphology

There are five forms in *T. gondii* life cycle: trophozoite (Fig. 21), tissue cyst, schizont, gametocyte and oocyst. Tachyzoites, tissue cysts and oocysts are important stages seen during the life cycle of the parasite, all these stages are infectious to man.
Trophozoite: it is oval to crescent-shaped with a pointed anterior end and a rounded posterior end. It measures 4-7µm in lengths and 2-4µm in breadth. An ovoid nucleus is present in the posterior end of the parasite. Tachyzoite is the active, multiplying form seen during the acute stage of the infection. It can invade any type of cell in a host and once inside a cell, it multiplies within a vacuole by a processknown as endodyogeny, or by binary fission or schizogony. Tachyzoites divide until they fill the host cell, which then liberates them, and they reinvade other macrophages, repeating the process. The cell which contains them, when it becomes merely a bag full of tachyzoites, is called a pseudocyst.
Tissue cyst: it is spherical and may vary in size from 5 to 100µm in diameter. This is the resting form and is found during chronic stage of the infection. The tissue cysts can be found in any organ of the body but are commonly found in the brain and the skeletal and heart muscels. An eosinophilic cyst wall surrounds each cyst. The cyst contain hundreds of bradyzoite or cystozoites. Bradyziotes multiply slowly.

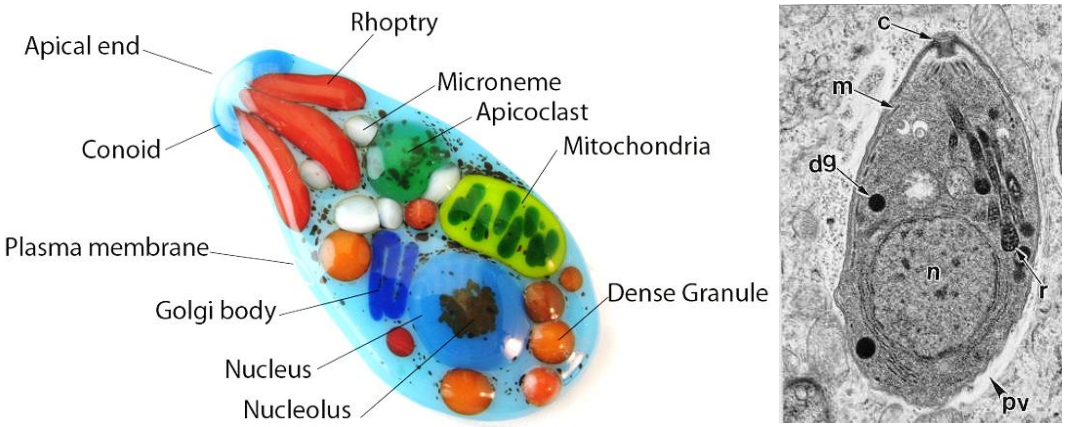


Figure 21. Ultrastructure of a *Toxoplasma gondii* tachyzoite: c – conoid, m – microneme, dg – dense granule, n – nucleus, r – rhoptries, pv – parasitophorous vacuole.

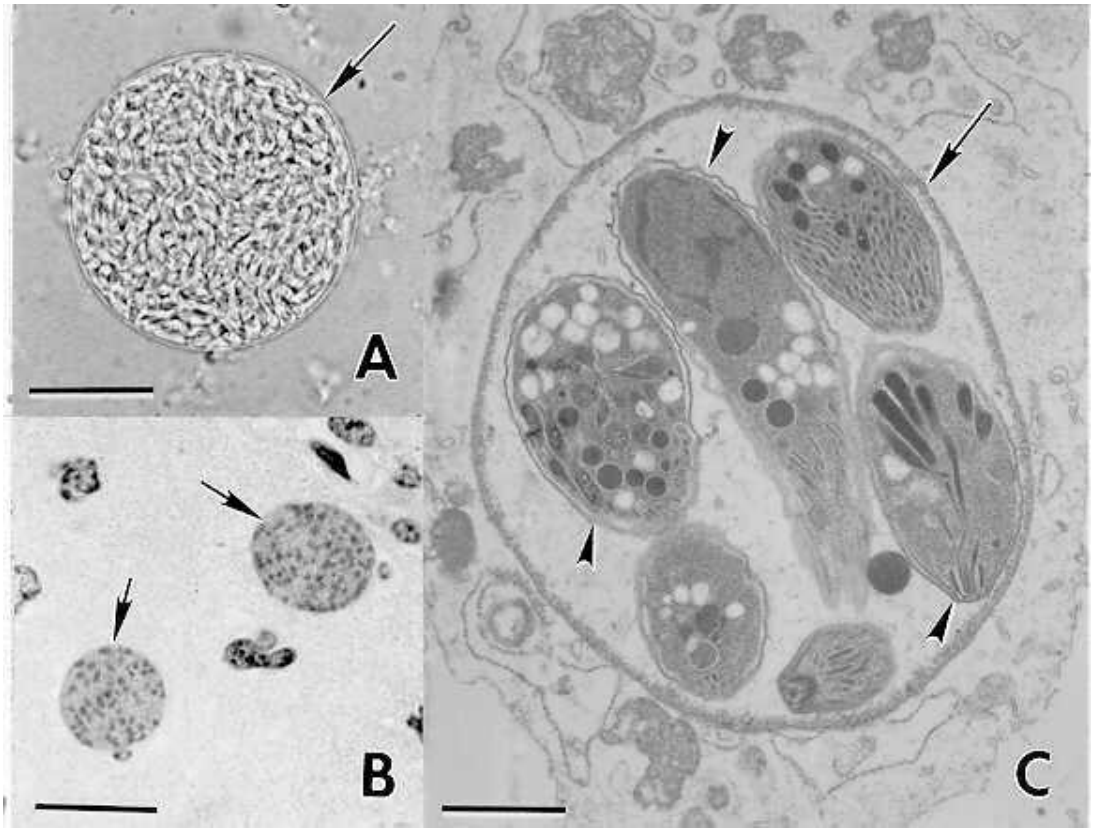


Figure 22. *Toxoplasma gondii* pseudocyst, cyst (in brain).
A. Tissue cyst freed from mouse brain. Note a thin (arrow) cyst wall enclosing hundreds of bradyzoites. Unstrained. Bar = 20 µm.
B. Two tissue cysts (arrows) in section of brain. Hematoxylin and eosin stain. Bar = 20 µm.
C. Transmission electron micrograph of a small tissue cyst in cell culture. Note thin cyst wall (arrow) enclosing 6 bradyzoites (arrowheads). Bar = 1.0 µm. (Courtesy of Dr. D.S. Lindsay, Auburn University, Auburn, AL.)
Oocyst: This stage is only present in cat and other felines but not in humans.

It is oval and measures10-12µm in diameter. Each cyst is surrounded by a thick resistant wall which encloses a spheroplast. The oocyst is liberated from the intestinal epithelial cell while still immature; it completes its development while passing down the gut and after expulsion in the faeces. Its contents divided first into two cells; these then secrete cyst walls to form two sporocysts. The contents of each sporocyst then divide once more to produce two infective sporozoites. Once mature, the oocyst may have infected any warm-blooded animal which swallows it.

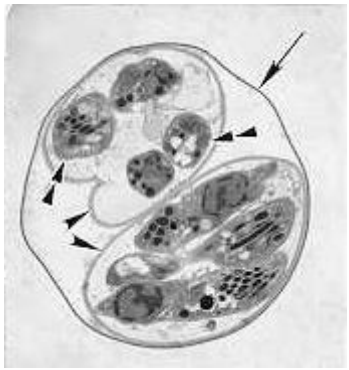


Figure 23. Transmission electron micrograph of a sporulated oocyst. Note thin oocyst wall (arrow), 2 sporocysts (arrowheads) and 4 sporozoites (double arrowheads) in sporocysts. Bar - 2.25 µm. (Courtesy of Dr. D.S. Lindsay, Auburn University, Auburn, AL.)

Lifecycle

Toxplasma gondii needs two hosts to complete its life cycle (Fig. 24). The definitive hosts are domestic cat and other members of the family Felidae such as bob cats, ocelots, Bengal tigers, mountain lion, etc. The sexual multiplication or gametogony take place in the epithelial cells of the small intestine. The oocysts are passed in the unsporulated form in the faeces.

The intermediate hosts are human and mice and other non-feline hosts (e.g., goat, sheep, pig, cattle, etc.). The asexual multiplication or sporogony (the extra-intestinal cycle) occurs in the extra-intestinal tissue.

Develop in intermediatehosts

Human infection may be acquired in several ways: a) ingestion of undercooked infected meat containing *Toxoplasma* cysts; b) ingestion of the oocyst from fecally contaminated hands or food and water; c) organ transplantation or blood transfusion; d) transplacental transmission; e) accidental inoculation of tachyzoites.

Swallowing the oocyst or tissue cyst initiate the development of extra intestinal asexual cycle. This process occurs mainly in macrophages. The sporozoites from the ingested oocyst and bradyzoites from the tissue cyst invade the mucosal epithelial cell of the small intestine in which they multiply as tachyzoites by endodyogeny. The tachyzoites divide until they fill the host cell, which then liberates them, and they reinvade other macrophages, repeating the process and form pseudocyst. The multiplying tachyzoites also spread to distant extra-intestinal organ (e.g. brain, eye, liver, spleen, heart,

skeletal muscle and placenta of pregnant mother) by invading lymphatics and blood. The multiplication of tachyzoites constitutes the acute phase of infection. If the host lives, and the infection is untreated, the host's immune system becomes effective and tachyzoites are destroyed, presumably at the vulnerable stage of passing from cell to cell. However, the parasite responds to this by entering other cells (muscle cells, neurons, and perhaps others) and secreting a thin but tough cyst wall around itself form a tissue cyst. A tissue cyst contains hundreds of bradyzoites. If another intermediate host eats uncooked meat containing these tissue cysts, the bradyzoites emerge in the duodenum and repeat the extraintestinal cycle. However, if a non-immune cat ingests tissue cysts in infected prey (or raw meat and offal fed to it), the emerging bradyzoites enter cells of the duodenal mucosa and begin the intestinal cycle of development, which occurs only in the definitive host.

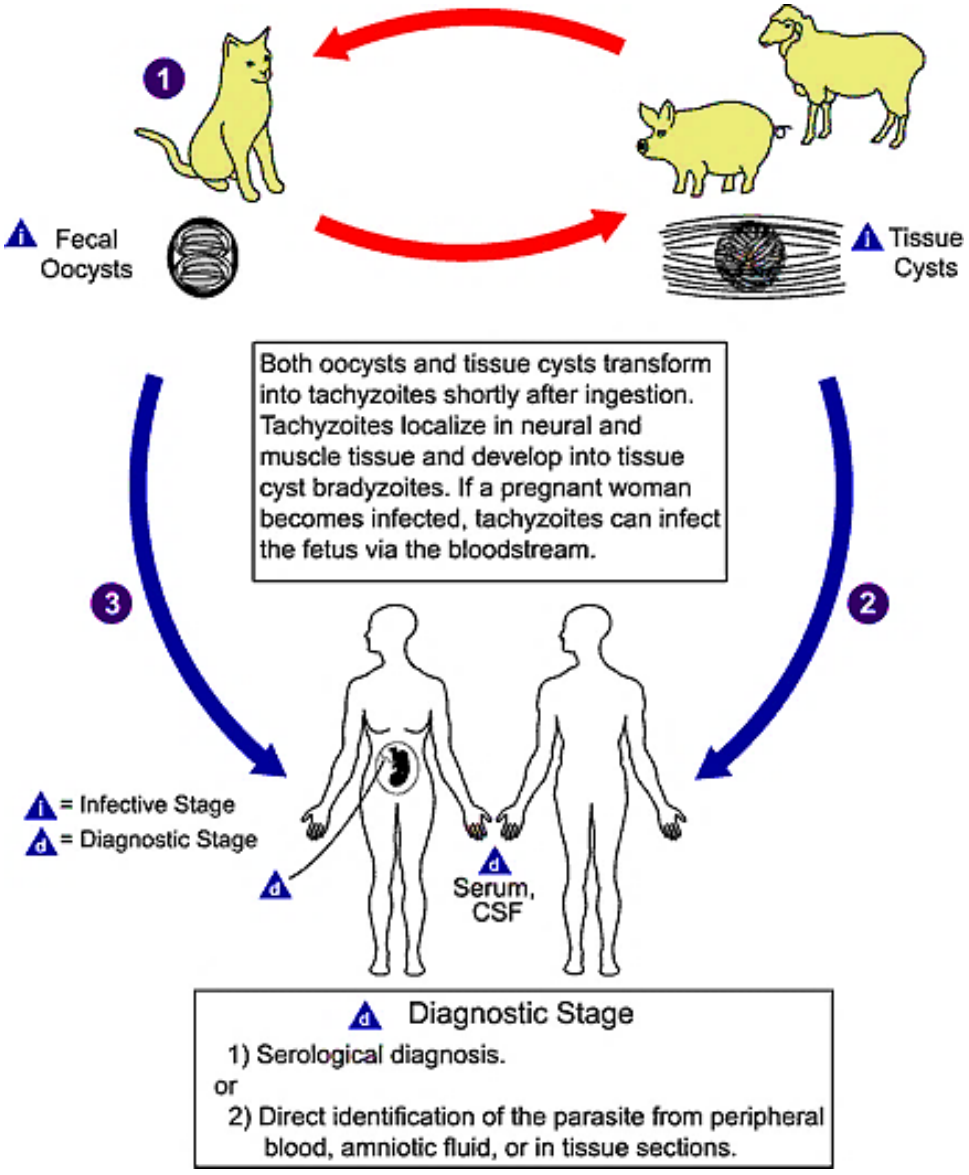


Figure 24. Life cycle of *Toxoplasma gondii* (Adapted from parasite image library of CDC, USA)

Develop in definitive hosts

Members of the cat family are the only known definitive hosts for the sexual stages of *T. gondii* and thus are the main reservoirs of infection. Cats become infected with *T. gondii* by their predatory habit of feeding on the muscles, brain and other tissues of infected mice, which harbour the tissue cysts. They also get infection by being fed raw meat of domesticated animals containing these cysts. After the cat ingests tissue cysts or pseudocysts or oocysts, viable organisms are released and invade epithelial cells of the small intestine where they undergo an asexual followed by a sexual cycle and then form oocysts, which are then excreted. The sexual cycle consists of a limited number of merogonies, producing merozoites which reinvade other mucosal cells, until the final generation of merozoites enter mucosal cells and commence the sexual cycle of gametogony, gametogony fertilization and sporogony within the developing oocyst. The oocysts are then released into the lumen of the intestine by rupture of the host cell. These oocysts, which are non-infectious, are shed in non-sporulated form up to 21days in cat's faeces.

Millions of oocysts are excreted in the faeces daily, up to 3 weeks. The oocysts sporulate outside the host with the formation of two sporocysts, each containing four sporozoites, within few days. These sporulating oocysts can survive in the environment for several months and are remarkably resistant to disinfectants, freezing, and drying, but are killed by heating to 70°C for 10 minutes. Man acquires infection by ingesting these spouulating oocysts and the cycle is repeated.

Pathogenesis and clinical features

The outcome of acute infection depends upon the immune status of host and the strain of the parasite. In acute infection, the proliferation of tachyzoites in the gastro-intestinal tract as well as in the extra-intestinal sites, cause disruption and death of cells, resulting in the foci of necrosis, surrounded by an intense mononuclear cell reaction. The development of both the humoral and cell mediated immunities in the immunocompetent hosts, resolve the acute infection. It is associated with the disappearance of tachyzoites from various tissues, especially from the extra-neural tissues and the formation of tissue cysts. The tachyzoites may persist in the central nervous system and even in the eye due to the absence of circulating antibodies in the tissue.

In the immunodeficient hosts and even some apparently normal hosts, the acute infection does not resolve but progress to cause severe necrotising lesions such as acute necrotising encephalitis, pneumonitis and myocarditis, which may prove even fatal. The presence of cysts in many organs through out the life of the host is probably the unique feature of the infection. In chronic infection, these cysts remain in a viable latent form and retain their potential for reactivation. Reactivation of chronic infection possibly results from the rupture of cysts. This causes recurrent parasitaemia frequently seen in some asymptomatic patients with chronic infection. Rupture of cyst also liberates many tachyzoites, which cause recrudescent toxoplasmosis in the immunodeficient hosts or chorioretinitis in the old children and adults suffering from congenital toxoplasmosis. The heart, liver, kidney and various other organs in the immunocompetent hosts and the pancreas in immunodeficient hosts are involved in disseminated toxoplasmosis.

Congenital toxoplasmosis

Congenital toxoplasmosis results from an acute primary infection acquired by the mother during pregnancy. Transplacental transmission from a chronic infection does not occur. Congenital toxoplasmosis occurs approximately in one-third of infants born to pregnant women, who acquire the infection during first trimester of pregnancy. In pregnancy, abortion, death in utero, or severe neurological/ocular manifestations, hydrocephalus, convulsions, intracerebral calcifications may result. Infection of the foetus, during last trimester of pregnancy, is more likely to be mild or asymptomatic at birth. Asymaptomatic infection at birth, however, may manifest as several sequalae of infection during the later life of the child.

The incidence and severity of congenital toxoplasmosis vary with the trimester during which infection was acquired. Because treatment with leucovorin of the mother may reduce the incidence of congenital infection and reduce sequelae in the infant, prompt and accurate diagnosis is important.

Acquired toxoplasmosis

Acquired infection with Toxoplasma in immunocompetent persons is generally an asymptomatic infection. However, 10% to 20% of patients with acute infection may develop cervical lymphadenopathy and/or a flu-like illness. The clinical course is benign and self-limited; symptoms usually resolve within a few months to a year. Immunodeficient patients often have central nervous system disease but may have chorioretinitis, or pneumonitis. In patients with AIDS, toxoplasmic encephalitis is the most common cause of intracerebral mass lesions and is thought to be caused by reactivation of chronic infection. Toxoplasmosis in patients being treated with immunosuppressive drugs may be due to either newly acquired or reactivated latent infection.

Occular tocoplasmosis

Acute infection of eye begins as single or multiple foci of necrosis of retina with severe inflammation and exudation into the vitreous. Granulomatous inflammation of choroids occurs secondary to necrotising retinitis. Both the tachyzoites and tissue cysts are found in the retinal lesions.

Chorioretinitis is the major manifestation of ocular toxoplasmosis and account nearly for 35 percent of case of chorioretinitis in children and adults. The majority of cases occur as a consquence of congenital infection. Bilateral central chorioretinitis and vitreous exudates are the typical ocular manifestations of congenital toxplasmosis in the new born infants. Patients are often asymptomatic until the second or third decade of life, when lesions develop in the eye. Unilateral chorioretinitis along with photophobia blurred vision and pain in the eye are the frequent clinical manifestations.

Infectionin the immunocompromised host

All types of *T. gondii* infections that occur in the immunocompetent hosts, are also seen in the immunocompromised hosts. The infection is more serious in immunosuppressed patients receiving immuno-suppressive therapy for malignancies; or persons receiving organ transplantations and AIDS.

Infection of the central nervous system especially, toxoplasmic encephalitis is one of the most commonly recognized manifestation of the infection in patients with AIDS. Unless the immune status of the host is restored, the disease progresses rapidly and death is the frequent out come of the condition.

Immunity

Development of both the antibody and cell-mediated immunities significantly appear after the course of *T. gondii* infection and its clinical manifestations. However, the relative role of humoral immunity or CMI in the pathogenesis of acute infection and in the resistance against infection still remains to be clear. The humoral immunity is characterised by the production of specific circulating antibodies both the IgM and IgG. *Toxoplasma* specific IgM antibodies are first to appear; hence their detection is suggestive of acute infection. The IgG antibodies appear late but are present in the circulation for a longer period as in chronic infction. The role of humoral antibodies as the major component in the host immunity against *Toxoplasma* infection is questionable.

The CMI through activated macrophages and monocytes, is suggested to play an important role in conferring resistance to re-infection as well as in the development of initial resistance in toxoplasmosis, possibly in co-operation with humoral antibodies.

Diagnosis

Clinically, the diagnosis of toxoplasmosis is difficult, as the signs and symptoms are the protean and mimics those of a variety of other diseases.

The laboratory diagnosis of toxoplasmosis may be documented by

Pathogenicdiagnosis:

1) Observation of parasites in patient specimens, such as bronchoalveolar lavage material from immunocompromised patients, or lymph node biopsy. Tachyzoites occasionally may be demonstrated microscopically in the smears of lymph node, bone marrow, brain and other specimen.

2) Isolation of parasites from blood or other body fluids, by intraperitoneal inoculation into mice or tissue culture.

Serological diagnosis Serologic testing is the routine method of diagnosis, a variety of serodiagnostic tests based on the demonstration of specific circulating antibodies and recently antigen in the serum are being used in the serodiagnosis of toxoplasmosis.

1) *Antibody detection*: The detection of *Toxoplasma*-specific antibodies is the primary diagnostic method to determine infection with Toxoplasma. The indirect immunofluorescence test, indirect haemagglutination and direct agglutination, latex agglutination and enzyme-linked immunosorbent assay are most frequently used tests.

2) *Antigen detection:* The development of ELISA for detection of circulating *Toxoplasma* antigen in the serum is a recent method. It has the potential for diagnosis of toxoplasmosis in the immunocompromised hosts. This also offers the possibility of detection of antigen in the amniotic fluid or acquous humour to diagnose congenital toxoplasmosis and chorioretinitis respectively, but still are at experiment stage.

Molecular diagnosis

Detection of parasite genetic material by PCR, especially in detecting congenital infections in utero.

Epidemiology

T. gondii is a very successful parasite. Serologic prevalence data indicate that toxoplasmosis is one of the most common of human infections throughout the world. Infection is more common in warm climates and at lower altitudes than in cold climates and mountainous regions. High prevalence of infection in France has been related to a preference for eating raw or undercooked meat, while high prevalence in Central America has been related to the frequency of stray cats in a climate favoring survival of oocysts. The overall seroprevalence in the United States as determined with specimens collected by the third National Health and Nutritional Assessment Survey between 1988 and 1994 was found to be 22%, with seroprevalence among women of childbearing age of 10% to 15%. In China, 509 cases of recognizable disease result from1964 to 1998. Surveys of people come from 26 provinces have indicated an average prevalence of 4.992%.

Reservoir, source of infection

Domestic cats are the key reservoir source of infection. They shed millions of oocysts in their faeces after ingesting the infected tissue. Cat faeces is the chief source of infection. Other non-feline hosts (e.g., goat, sheep, pig, cattle, etc) also are the secondary infection source. Tachyzoite, mature oocyst and tissue cyst are the infective stage.

Transmission**:** *T. gondii* infection usually is transmitted from infected rat and domestic animals to man infection. There are several transmission ways:

1) *Oral transmission*: postnatal acquired *T. gondii* infection is acquired by eating raw or undercooked meat (chicken, pork and goat meat) containing the tissue cyst and ingesting food and water contaminated with mature oocysts form cat faeces.

2) *Congenital transmission*: the infection is transmitted from the infected pregnant mother to the foctus, by the tachyzoits passing through the placenta.

3) *Other modes of transmission*: laboratory infection is caused by accidental self-inoculation of tachyzoites. It is less common. The infection may be transmitted by blood transfusion, unpasteurised milk, and egg and organ transplantation.

The immunosuppressed hosts including AIDS, homosexuals and those receiving the organ transplantation are at increased risk to the infection.

Prevention and control

The combined therapy with sulfonamide and pyrimethamine are widely used in the treatment of toxoplasmosis. They are synergistic in combination and are effective against tachyzoites but not against tissue cysts of *T. gondii.*

Treatment is not needed for a healthy person who is not pregnant. Symptoms will usually go away within a few weeks. For pregnant women or persons who have weakened immune systems, pyrimethamine plus sulfadiazine with leucovorin are the drugs of choice.

For high risk individuals such as immunodeficient patients and pregnant women, avoidance of contact with cat faeces containing oocysts and eating meat adequately cooked are important measures for prevention of acquired and congenital toxoplasmosis. Adeuqate cooking kill all the cysts in the meat. Fruits and vegetables that may be contaminated with oocysts should be washed adequately before eating.

PNEUMOCYSTIS CARINII

Kingdom: Fungi
Subkingdom: Dikarya
Phylum: Ascomycota
Class: Taphrinomycotina
Order: Pneumocystidales (Ascomycota)
Family: Pneumocystidaceae
Genus: *Pneumocystis*
Species: *carinii sp.f. hominis, carinii sp.f. rattus, carinii sp.f. oryctolagi*

It is necessary to remember

Pneumocystis carinii is a pathogen of uncertain taxonomic status. The capability to produce spores and cysts, places *Pneumocystis* within either of the two protistan groups, fungi or protozoa.
Pneumocystis carinii causes **interstitial plasma cell pneumonia,** occurring almost exclusively in infants, children and immunocompromised adults.
P. carinii was discovered in 1909 by Charles Chagas', who mistakenly described the organism as a trypanosome. Delanoe and Delanoe described the organism in rats and they were the first to identify the organism as a distinct aetiolog ical agent. They named the organism as *Pneumocystis carinii* in the honour of Dr. Carinii, another early worker in the field.
The parasite was later implicated as the aetiolog ical agent of interstitial plasma cell pneumonia by Van der Meet and Brug. Since then, there is an increased report of *P. carinii* infection, especially associated with the acquired immuno-deficiency syndrome.

Morphology

Three morphologically distinct stages are recognized: trophozoite or trophic form, pre-cyst and cyst.
Trophozoite: Trophozoites or trophic forms are always present in large numbers (Fig. 25). These are small, pleomorphic and usually occur in clusters. They are readily stained by Giemsa or acridine orange. In Giemsa stain, the nucleus is stained red and cytoplasm blue. The electron micrograph of a trophozoite shows a nucleus, mitochondria, a few other organelles and filopodia (tubular cytoplasmic extension). Trophozoites multiply asexually by binary fission.

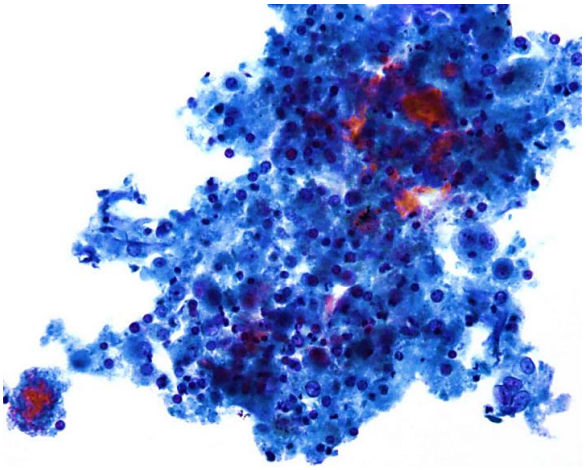


Figure 25. *Pneumocystis carinii* trophozoites in bronchoalveolar lavage material. Giemsa stain. The trophozoites are small, and only their nuclei, stained purple, are visible. AIDS patient seen in Atlanta, Georgia. (Adapted from parasite image library of CDC, USA)

***Pre-cyst*:** It is an intermediate stage between the trophozoite and cyst. It is oval in shape and measures 4-6 µm in diameter. It lacks pseudopodia. Typically, it is surrounded by a thick limiting layer or cell wall. Periodic-acid schiff and silver methenamine stain clearly the cell wall. It is difficult to demonstrate this stage in the tissue.
Cyst: It is spherical. 5-8 µm in diameter and is surrounded by a 70-140 nm thin cell wall (Fig. 26).

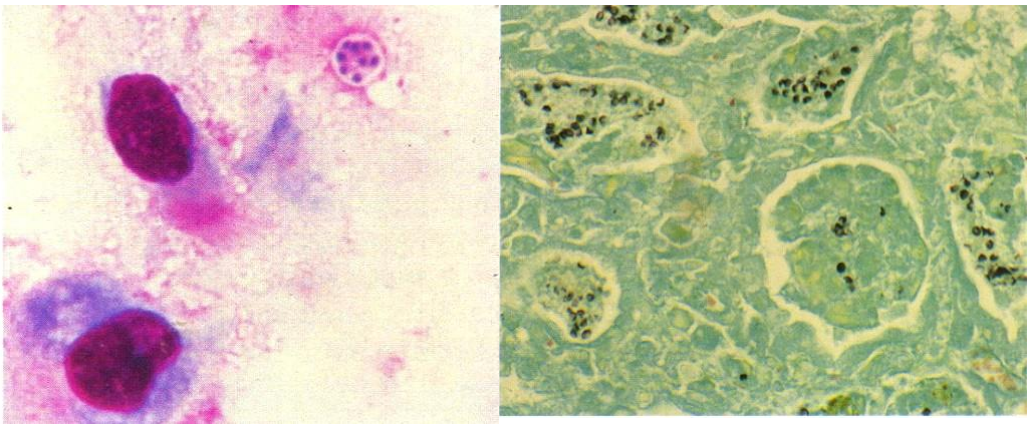


Figure 26. Cyst of *Pneumocystis carinii.*

A mature cyst consists up to eight daughter forms or extra-cystic bodies called sporozoites. The sporozoites are spherical, crescent-shaped, measure1-1.5 µm in diameter. Each sporozoite consists of a nucleus, mitochondria, ribosomes and endoplasmic reticulum. The cyst is the diagnostic form of the parasite and is easily recognised by staining with

Life cycle
P. carinii occurs as a saprophyte in the human and in a variety of mammals in nature. It is an extra-cellular parasite which inhabits the pulmonary alveoli of the lungs.
The life cycle of *P. carinii* is still incompletely understood (Fig. 27). It is based on the morphologic studies of the lung sections obtained from the rat and on the parasites grown in culture. In man, the life cycle of *Pneumocystis* is extracellular and occurs in the lung alveoli, lntra- cellular stage has not been described.
Trophozoites may develop into cysts either during sexual or asexual phase. The existence of a sexual phase is probable, and no evidence exists for an intracellular phase in the parasite life cycle. The trophozoites multiply either by binary. fission or endogeny.
Trophozoite first develops into a single-nucleated structure called pre-cyst. The latter develops into a cyst by a process similar to sporogony. In this process, a single nucleus divides by meiosis into four haploid nuclei. These nuclei undergo post-meiotic mitosis to produce eight daughter nuclei.
A membrane, surrounds each daughter nucleus in the late phase during which pre-cyst develops into a cyst. Each cyst contains eight sporozoites or daughter cells. The mature cyst on rupture releases these daughter cells.

The specific factors that cause excystation of cysts or encystation of trophozoites are not known. The mature cyst with eight intra- cystic bodies is believed to be the infective form of the parasite responsible for transmission of infection from man to man. Congenital infection may also be caused by trophozoites.

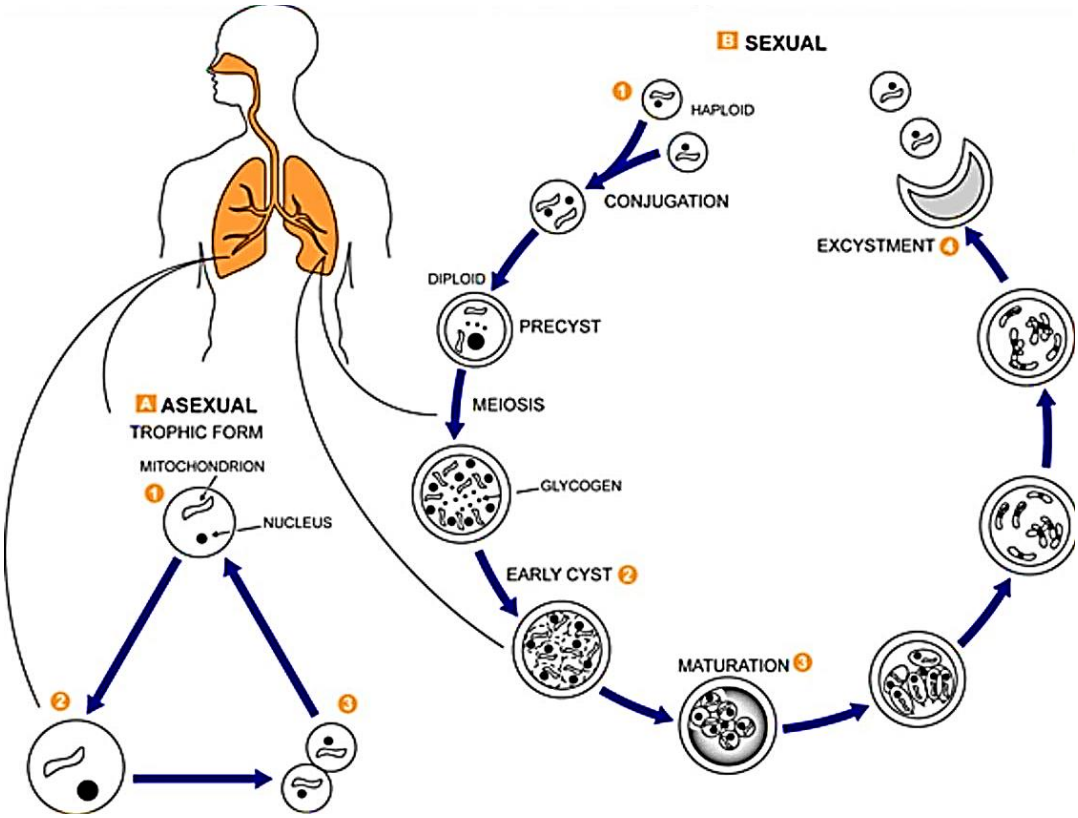


Figure 27. Life cycle of *Pneumocystis carinii.*

Pathogenesis and clinical manifestation

P. carinii inhabits the lung alveoli. In man and other animal species, it causes disease by attaching itself to Type-1 alveolar epithelial cells. The specific factors involved in the process of attachment have not been recognised. The organism lives on the lining layer of the alveoli. It has been demonstrated that surface of the organism and alveoli epithelial cells are closely apposed to each other without any fusion of the cell membrane or changes in the intramembranous particls.

The lungs, in pneumocystosis in humans are consolidated and appear reddish grey at necropsy. Histologic studies of the lung show the alveoli to be completely filled with pink frothy honey combed materials and a large number of *P. carinii* Infected infants show extensive plasma cell infiltration of the alveolar space but immuno- suppressed children and adults do not show these changes, instead they show only intestinal thickening.

P. carinii rarely causes symptomatic disease in healthy individuals. It causes diffuse pneumonia only in immunocompromised hosts. In these hosts, the organism as well as the disease always remain localised to the lungs.

The severity of clinical manifestations, to some extent, depend on the age of the host as follows:

Epidemic or infantile pneumoeystosis: This occurs in premature, malnourished and debilitated infants. Incubation period varies from 1 to 2 months. The symptoms of *P. carinii* pneumonia include dyspnea, non-productive cough, and fever. Chest radiography demonstrates bilateral infiltrates. In 1 to 4 weeks, respiratory manifestations become well marked. The condition may last 4 to 6 weeks and shows a mortality of 25 to 50 percent.

Sporadic pneumocystosis: *P. carnii* produces sporadic pneumocystosis in immunocomprornised children and adults with acquired immunodeficincy syndrome, or in persons receiving immunosuppressive therapy for the treatment of malignant conditions, organ transplantation, etc.

The clinical manifestations are similar to that of epidemic pneumocystosis except that the onset of sporadic pneumocystosis is abrupt. Course of the disease is rapid and begins with fever, tachypnoea and respiratory distress. Extrapulmonary lesions occur in a minority of patients, involving most frequently the lymph nodes, spleen, liver, and bone marrow. Typically, in untreated PCP increasing pulmonary involvement leads to death. The condition has a high mortality of 90- 100 percent.

The pathogenesis of pneumocystosis in AIDS and other immunodeficiency disease still remains unclear. It may be due to simple reactivation of latent infection or additional exposure to exogenous sources of the organism.

Diagnosis

Clinical manifestations of *P. carinii* infection are nonspecific and can be observed in many different infectious and non-infectious conditions. Hence, the diagnosis of the condition depends mainly on the laboratory diagnosis.

Pathogenicdiagnosis

The specific diagnosis is based on identification of *P. carinii* in bronchopulmonary secretions obtained as induced sputum or bronchoalveolar lavage material. In situations where these two techniques cannot be used, transbronchial biopsy or open lung biopsy may prove necessary. Microscopic identification of *P. carinii* trophozoites and cysts is performed with stains that demonstrate either the nuclei of trophozoites and intracystic stages or the cyst walls (such as the silver stains).

Specimen: The methods of collection of specimens are essentially invasive procedures. These include:

1) Open lung biopsy.
2) Percutaneous needle biopsy or needle aspiration of the lung.
3) Bronchoalveolar biopsy and bronchoalveolar lavage. Fibre optic bronchoscopy with broncho-alveolar lavage and or transbronchial biopsy is the most commonly used procedure.
4) Inhalation of a saline mist. Frequently, in AIDS patients the organisms can be demonstrated it’s the sputum induced by inhalation of a saline mist.

Serodiagnosis

The indirect fluorescent antibody, complement fixation test and enzyme-linked immunosorbent assay are being used for the demonstration of serum antibodies to *P. carinii*. These tests use whole parasites or soluble

extracts of parasites as antigens.
The counter-current immunoelectrophoresis and latex agglutination test also are frequently used for the detection of antigen in the serum to diagnose the refection.
Chest x-ray: In some cases, it shows bilateral diffuse infiltrates originating from the perihilar regions of the lungs.

Epidemiology
P. carinii is widespread in nature. It occurs in humans and many species of animals (rats, rabbits, mice. sheep, goats, dogs, guineapigs, horses, chimpanzees and monkeys).
It has been reported from India, China, Japan, Iran. Israel, South America, Congo, Malaysia, Australia. New Zealand, USA, Canada, Brazil and erstwhile USSR.
Reservoir of infection
Infected man is the main source and reservoir of infection. Mature cyst containing eight intracystic bodies appear to be the infective stage.
Transmission:
P. carinii occurs in following ways:
1) *Man-to-man transmission*: It occurs by inhalation of mature cysts. Airborne infection seems to be the major mode of transmission.
2) *Congenital transmission* occurs rare. Milk-borne transmission occurs less frequent. The populations at risk for *P. carnii* infection include:
1) Premature malnourished infants;
2) Children with primary immunodeficiency.
3) Patients receiving immuno suppressive drugs such as corticosteroids for treatment of malignancies, organ transplantations and other diseases; and
4) Protein malnutrition.

Treatment and control
Treatment of *P. carinii* infection is broadly based on the supportive therapy and specific chemotherapy. It consists of aeration by high concentration of oxygen, blood transfusion and good nursing care.
Pentamidine, trimethoprimand sulfamethoxazole are the drugs currently available for the treatment of *P. carinii* infection.
Treatment of *P. carinii* infection ill patients with AIDS relatively is complex. It requires treatment with higher dose and for a longer duration. The control measures include respiratory isolation of high-risk cases susceptible to infection, and chemoprophylaxis by trimethoprim and sulphamethoxazole.

LESSON 8. TEST

Test is conducted in the form of a written reply to the ticket.
Each ticket includes two theoretical questions and a diagnostic task.

LESSON 9. PARASITOLOGY. WORMS. TREMATODES

Trematodes are members of phylum platyhelminthes. which also includes the cestodes or tapeworms. The parasites are known as flukes. All trematodes parasitic to humans belong to Class Trematoda, subclass Digenea.
The digenetic（trematodes, or flukes, are among the most common and abundant of parasitic worms, second only to nematodes in their distribution. They are parasites of all classes of vertebrates, especially marine fish, and some species, as adults or juveniles, inhabit nearly every organ of the vertebrate body. Their development occurs in at least two hosts, the first a mollusk or, very rarely, an annelid. Many species include a second and even a third intermediate host in their life cycles. Several species cause economic losses to society through infections of domestic animals, and others are medically importance. These medical trematodes include bellow species.

Species

Digentic trematodes constitute one of the largest groups of platyhelminths, parasitizing a wide range of invertebrate and vertebrate hosts. Within human hosts, these worms are found in numerous organs, including the intestine, lungs, liver and vascular system.

FASCIOLA HEPATICA

Kingdom: Animalia
Phylum: Platyhelminthes
Class: Trematoda
Subclass: Digenea
Order: Echinostomiformes
Family: *Fasciola*
Species: *hepatica*, *gigantica, halli, californica, jacksoni, nyanzae*

It is necessary to remember

Fascioliasis is caused by two species of parasitic flatworms or trematodes that mainly affect the liver. The two species of trematodes that cause fascioliasis (*Fasciola hepatica* and *Fasciola gigantica*) are leaf-shaped worms, large enough to be visible to the naked eye (adult *F. hepatica* measure 20-30 mm x 13 mm; adult *F. gigantica* measure 25-75 mm x 12 mm). They cause similar diseases in humans.

Morphology

F. hepatica is a trematode (fluke) parasite that infests humans and many species of animals. *F. hepatica* is the usual cause of fascioliasis. It is one of the largest flukes, measuring up to 3.5 cm by 1.5 cm. The parasite lives in the liver and bile duct. Its hosts include herbivorous mammals and it is found in 46 species of domestic and wild animals as well as in man. The intermediate host is the *Lymnaea* genus of snail which lives in marshy areas and standing water. *F. gigantica* may also cause similar human disease, and several other species cause disease in animals. *Fasciola halli* and *Fasciola californica* infest sheep and cattle in the USA and may be synonymous with *Fasciola jacksoni* which infests elephants in Africa and India, *Fasciola nyanzae* whose

host is the hippopotamus, and*Fasciola magna* which infests mostly deer, but also cattle and sheep.

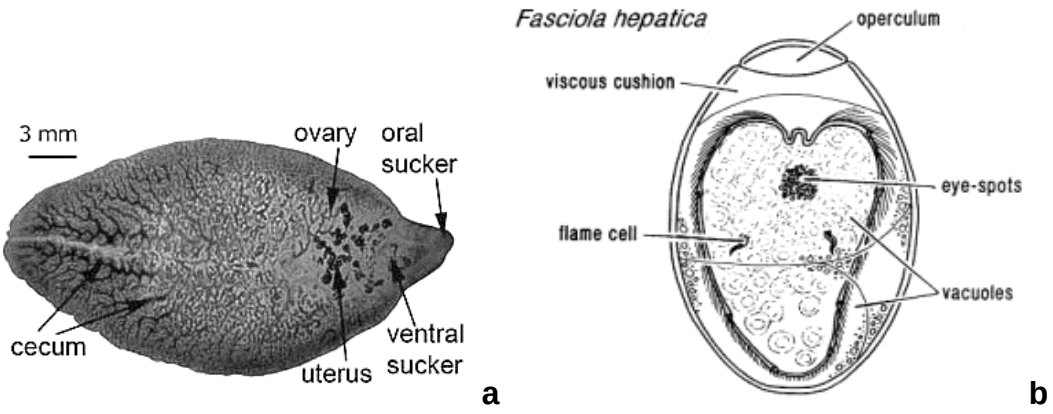


Figure 27. Morphology of *Fasciola hepatica* – *a*; and here ovo (increased) – b.

Life cycle

Immature eggs are discharged in the biliary ducts and in the stool. The eggs release miracidia, which invade a suitable snail intermediate host. In the snail the parasites develop into cercariae, which are released from the snail and encyst as metacercariae on aquatic vegetation or other surfaces. Mammals become infected by eating contaminated vegetation. Humans become infected by ingesting contaminated freshwater plants, especially watercress.

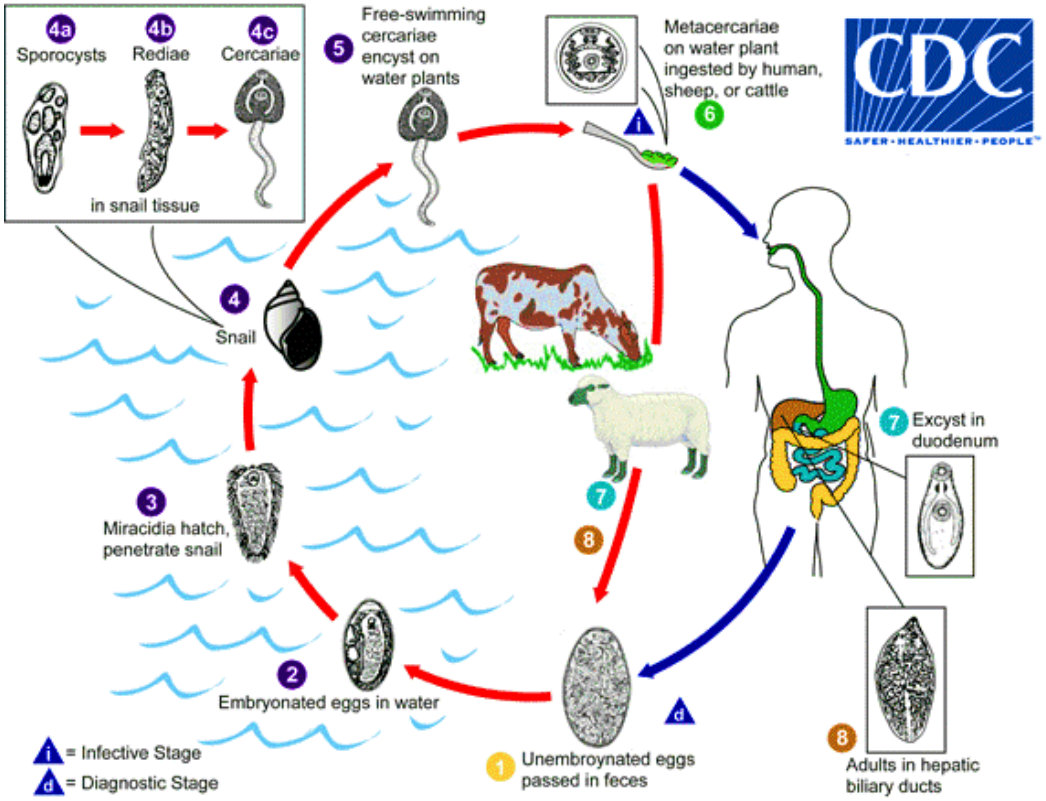


Figure 28. Life cycle of *Fasciola hepatica.*

After ingestion, the metacercariae encyst in the duodenum and migrate through the intestinal wall, the peritoneal cavity, and the liver parenchyma into the biliary ducts, where they develop into adults. The adult flukes live in the large biliary ducts of the mammalian host.
Human infection by consumption of raw liver from infected sheep, goats, and cows has also been reported.

Pathogenesis and clinical manifestation

Acute fascioliasis

In its severe form it occurs in sheep but rarely in man and requires large numbers of parasites, usually over 10,000, to be ingested. Large numbers of migrating larvae invade the liver and cause a traumatic hepatitis that is frequently fatal. Sometimes the liver capsule may rupture into the peritoneal cavity, causing death from peritonitis.
More usually the invasive phase lasts many weeks, with the most common symptoms being intermittent fever, hepatomegaly, and abdominal pain, although up to 50% of infections may be subclinical.
Abdominal pain is usually in the epigastrium or right hypochondrium. Other symptoms include malaise and wasting. Urticaria and eosinophilia are usual.

Chronic fascioliasis

After reaching the liver, there is then a latent phase lasting months or even years, when infection is asymptomatic.
However, with maturation there may be an obstructive phase causing hepatitis, cholangitis, or pancreatitis. *Fasciola* spp. are not adapted to using man as a definitive host and so the flukes may cause ectopic infections, especially in the lungs and subcutaneous tissues where they may form cysts.
Halzoun is one such type of infection following consumption of raw liver. There is severe pharyngitis, dysphagia, sensation of a foreign body in the throat, and possibly airways obstruction.

Diagnosis

FBC will usually show eosinophilia and probably anaemia. ESR may be raised. LFTs may show evidence of hepatocellular damage or evidence of obstruction. Stool microscopy may show the pathogen or the eggs.
Various immunoassays are available and enzyme-linked immunosorbant assay (ELISA) tests are sensitive and specific.
X-ray of the liver may show tract-like small abscesses and subcapsular lesions. Even with pulmonary symptoms, CXR is rarely rewarding.
Ultrasound of the gallbladder and biliary tract may show adult worms as focal areas of increased echogenicity.
Cholangiography may reveal multiple cystic dilatations of the ducts. Large cystic dilatation, small cystic ectasia, and mulberry-like dilatation are considered diagnostic of fascioliasis.

Epidemiology

F. hepatica is found in all continents except Antarctica.*F. hepatica* infects various animal species, but mostly herbivores. It affects ruminants much more than man.

Fascioliasis is one of the most economically important parasitic diseases of livestock, causing disease in sheep and other domestic animals in Latin America, Africa, Europe, and China.
Of the 750 million people who live in endemic areas, over 40 million are thought to be infected in total by food-borne trematodes.
Specific figures for *F. hepatica* are estimated at 2.4 million in 61 countries and the number at risk is more than 180 million throughout the world. It is most common in Bolivia, Ecuador, Egypt and Peru, but is also found in European countries, including France, the UK, Spain and Portugal. The incidence has apparently increased over a period of 20 years.

Treatment and control

Non-drug

Bed rest and a protein-rich diet are recommended. Iron and vitamins may be required.

Drugs

If dealing with such a case, seek expert advice. Triclabendazole is the drug of choice. Praziquantel is not effective and therefore not recommended.

Surgical

Parasite removal at endoscopic retrograde cholangiopancreatography is effective in the biliary stage.
Ascending cholangitis may require surgery.

Complications

It is often associated with anaemia, especially in children.
Pancreatitis.
Biliary fibrosis.
Rarely, cholangiocarcinoma can occur.

Prevention

Water-grown vegetables should be washed with 6% vinegar or potassium permanganate for 5-10 minutes, which kills the encysted metacercariae. This approach is more successful than attempts to halt the consumption of raw vegetables.
Cook water-grown vegetables thoroughly before eating.
Avoid sewage contamination of growing areas.
Use of molluscicides is the most frequent public health intervention, as it prevents the transmission of many other trematodes, including *Schistosoma* spp.
Treatment of animals to reduce the reservoir and reduce stock losses has been used. Until the introduction of single-dose triclabendazole, bithionol was the only available treatment, much limited by expense and treatment duration.
For the future, vaccination would seem to be a feasible option.

CLONORCHIS SINENSIS

Kingdom: Animalia
Phylum: Platyhelminthes
Class: Trematoda
Order: Opisthorchiida
Family: Opisthorchiidae
Genus: *Clonorchis*
Species: *sinensis*

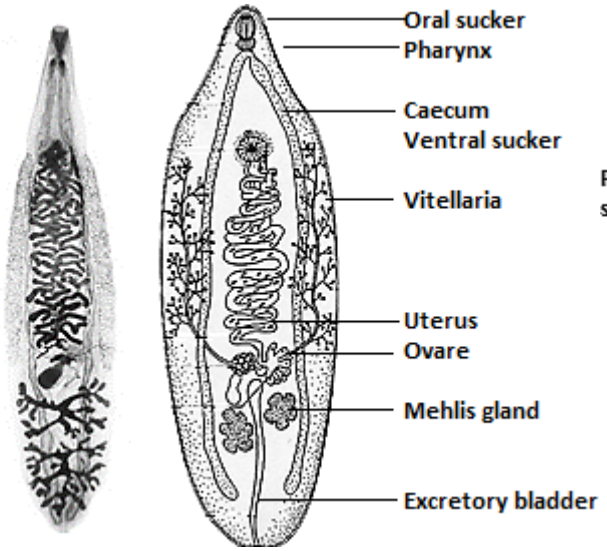
It is necessary to remember

The Chinese liver fluke, the trematode *Clonorchis sinensis* (Cobbold,1875: Looss,1907) was found from the biliary passage of a Chinese in Calcutta, India in 1874 firstly. The worm is the causal agent of clonorchiasis. In China, the earliest endemic of clonorchiasis was found in Chaozhou and Guangzhou in 1908. In 1975, *C. sinensis* eggs were found in a West Han Dynasty corpse in Jiangling, Hubei Province. Later, those eggs were found in an ancient corpse of Zhangguo Dynasty's Chu tomb. So it was said that the prevalence of clonorchiasis has continued for more 2300 years. Today it is known that the "Chinese liver fluke" is widely distributed in China (mainland, Hong Kong and Taiwan), Japan, Korea, and Vietnam.

Morpgology

Adult worm. It is flat with pointed anterior and rounded posterior end (Fig. 28), measuring about long 10～25mm, wide 3～5 mm. It is relatively a small fluke. The tegument lacks spines. Oral sucker is larger than ventral sucker. Ventral sucker is located one-fifth of the way from the anterior end. The presence of two large, deeply lobulated and branched testes with 7 branches situated in the posterior third of the body, one behind the other, and anterior uterus.

Mature egg The eggs are flask-shaped, operculated and relatively smaller in size, and measure 29 × 17mkm. They are yellow-brown, containing a well-developed miracidium, and possessing a small knob at the posterior end giving an appearance of aelectric bulb.



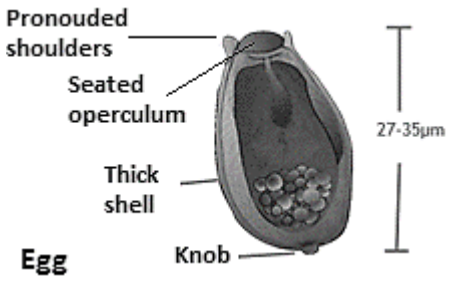


Figure 28. Morphology of *Clonorchis sinensis*

Life cycle

C. sinensis requires one definitive host and two different intermediate host for completion of its life cycle.

In definitive host adult worm mature in the bile ducts of definitive host, which include human or mammalian animals. After eating raw or under-cooked fish or crustaceans with metacercaria, definitive host will be infected. The young flukes excystin the duodenum. The route of migration to the liver is not clear; but it seems probable that juveniles migrate up the common bile duct to the liver. After about one month of infection, adult worms is develop. Mammalian, i.e. cats, dogs and rates etc are important reservoir hosts.

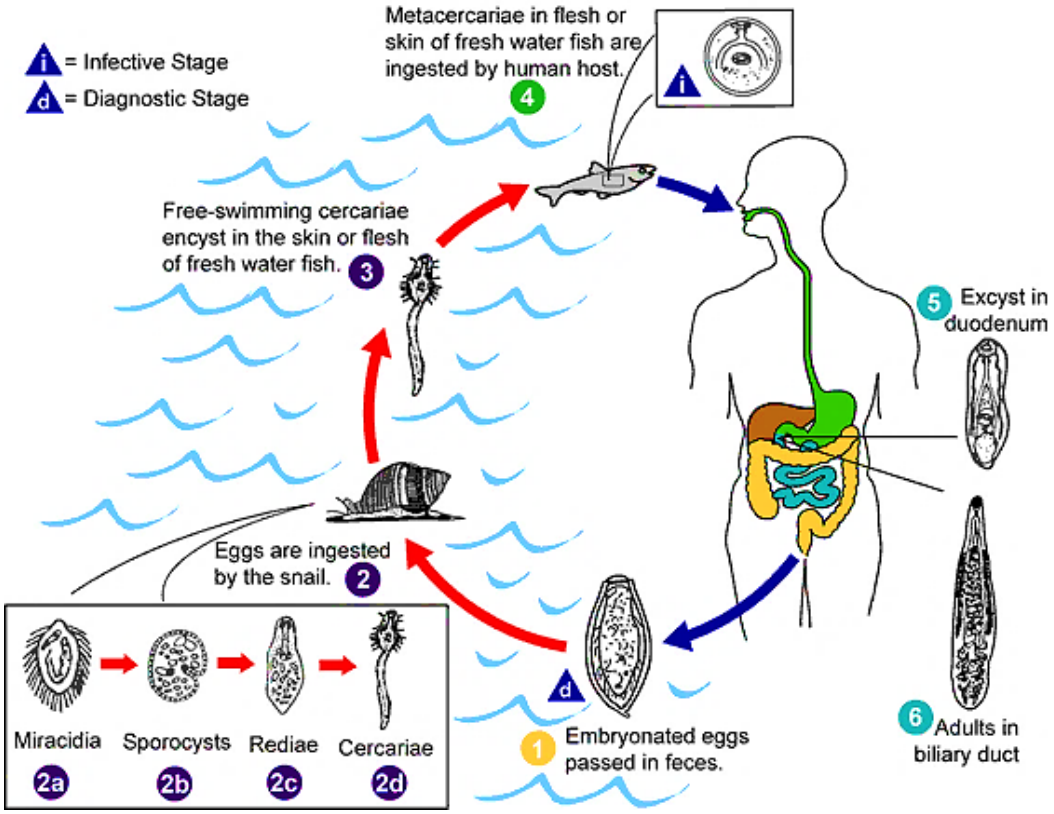


Figure 29. Life cycle of *Clonorchis sinensis* (from Parasite image library of CDC, USA)

In the first intermediate host Eggs are hatched into miracidium after being eaten by a suitable snail, then develop into a sporocys; sporocyst transforms into redia; redia produce cercariae with long tail.

In the second intermediate host: When contacting fish or crustaceans in freshwater, the cercaria will bores through the skin, coming to muscle and encysting.

First Intermediate host: fresh-water snails eg. *Parafossarulus striatulus*, *Alocinma longicornis*, *Bithynia fuchsianus*.

Second intermediate host: fresh-water fish i.e. *Ctenopharyngodon iddellus*, *Pseudorosbora parva* et al.

Pathogenesis and clinical manifestation

The basic pathogenesis of the infection is erosion of the epithelium lining the bile ducts, which results from mechanical irritation caused by flukes and toxic substances that may be produced by them. Because flukes prefer to reside in the second-order bile ducts, the pathological changes usually appear in the second-order bile ducts. But in heavy infection they are found throughout the biliary system, including the gallbladder, and sometime in the pancreatic duct. Flukes feed on secretions from the bile duct mucosa. They cause low-grade inflammatory changes of biliary tree, proliferation of the biliary epithelium, and progressive portal fibrosis. In light infection, the pathological changes are not significant; but heavy infection will lead the bile ducts to gradual enlarging and thickening. There is obstruction of the biliary tract by the worm bodies, which leads to bile retention, and fibrosis proliferation in the wall of the tract. In late stage with complication, the change of liver parenchyma is present, and liver function are interfered. Some research data showed that there was the relationship between clonorchiasis and biliary tract cancer, liver cancer.

Acute clonorchiasis occurs one to three weeks after the ingestion of encysted metacercariae. There many be fever, chills, abdominal pain, diarrhea, tender hepatomegaly, and mild jaundice. The white blood cell count is raised with marked eosinophilia, and serum alkaline phosphataes, SGOT, and SGPT are elevated. The clinical presentation is often confused with acute viral hepatitis and seldom recognized. The history of eating raw fish in the endemic area and the eosinophilia should suggest the diagnosis.

The majority of people with *C. sinensis* in their stools have no symptoms. The biliary system is blocked by numerous flukes and becomes secondarily infected. The flukes occasionally block the pancreatic ducts and induce pancreatitis. Cholangiocarcinoma is a late complication of chronic clonorchiasis. Clonorchiasis has no causal relationship with hepatocellular cancer. Some advanced cases have serious complications such as cirrhosis of the liver and ascites.

Diagnosis

Chonorchiasis or chonorchis infection can be tentatively diagnosed by a history of having stayed in or been to the endemic areas and eating raw fresh water fish, together with some clinical manifestations. Correct diagnosis must, however, be confirmed by laboratory finding of ova.

1) *Parasitological examinatio*n Finding the small operculated eggs in stool by direct smears method and stoll's egg counting methods (egg-concentration method). Sometime examination of bile that drawing from the duodenum (duodenal aspirate) is recommended also.

2) Immunological tests The intradermal test, IHA, ELISA for specific antibodies have been applied for screening in the fields. These tests are also useful to help for individual diagnosis.

Epidemiology

Geographical distribution Human clonorchiasis or chonorchis infection is endemic in Far East Asian such as China, Japan, Korean, Vietnam etc.

Epidemic characters In endemic areas, the first and second intermediate host are found and where the population is accustomed to eating raw fish.

In most areas, the fish are raised in fish ponds that are commonly fertilized with human and animal feces. This provides excellent nutrient for the growth of plan and animal life upon which the snails and fish feed, and also provides an opportunity for perpetuating the life cycle of the parasite. In free endemic areas, neither the snail nor fish intermediate hoists are indigenous. But infected fish originate from endemic areas are shipped in daily here, infected individuals are often found.

Susceptible population Humans is a susceptible host of *C. sinensis*. Those who eat raw fish or uncooked fish frequently is easy to acquire infection. For example, in Pearl river areas the prevalence of infection is higher than that in other areas.

Infective form Metacercaria is the infective form. It is found encysted in the flesh of fresh-water fish.

Theroute of acquiring infection It is through the eating raw or uncooked fresh water fish.

Infection season In September and October the number of metacerariae in fish are the highest. When temperature is below 10℃, metacerariae can not enter fish.

Prevention and control

Preventive measurement Metacerariae will withstand certain types of preparation of fish, such as salting, pickling, drying, and smoking. Because of this, people can become infected when they eat such fish. But under 90 ℃， metacerariae in 1 mm slice of fish will be killed within 1 second; 75℃ for 3 second; 70℃ for 6 second and 60℃ for 15 second. So to change eating habits of people by health education is considered to be the most important measure to control the diseases. Eating cooked fish or no eating raw fish are recommended.

Chemotherapy Praziquantel（ has been reported to be effective for clonorchiasis.

The recommended dosage is 75 mg per kilogram of body weight, divided into three doses on same day. In Guangdong, dosages of 120 ～ 150mg per kilogram of body weight for 2 days is recommended.

LESSON 10. PARASITOLOGY. WORMS. TREMATODES

PARAGONIMUS WESTERMANI

Kingdom: Animalia
Phylum: Platyhelminthes
Class: Trematoda
Subclass: Digenea
Superorder: Epitheliocystidia
Order: Plagiorchiida
Family: Troglotrematidae
Genus: *Paragonimus*
Species: *Paragonimus westermani*

It is necessary to remember

More than 30 species of trematodes of the genus *Paragonimus* have been reported which infect animals and humans. Among the more than 10 species reported to infect humans, the most common is *P. westermani*, the oriental lung fluke, which causes classical endemic haemoptysis or pulmonary paragonimiasis in man, it is also a parasite of carnivores. In 1878, *P. westermani* was first described from tigers.

Morphology
Adult worm It is thick, fleshy and when freshly passed it is redish brown, in colour (Fig. 30). It is ovoid -shaped and covered with scale-like spines. It measures 7.5-12.0 mm in length, 4.0-6.0 mm in breadth and 3.5-5.0 mm in thickness. The anterior end of the fluke is slightly broader than the posterior end. The oral and ventral suckers are equal in size, and ventral sucker at pre-equator. There is t***wo lobated testes, present side-by-side at the posterior fourth of the body.*** The ovary is with 5-6 lobes at the left of midline, uterus is tightly coiled at the right of the ventral sucker. The vitelline is follicles.
Egg The eggs are yellow-brown in color, oval-shaped and operculated. They measure 80-118 um by 48-60 um. Each egg contains a fertilized unsegmented ovum surrounded by more than 10 vitelline cells.

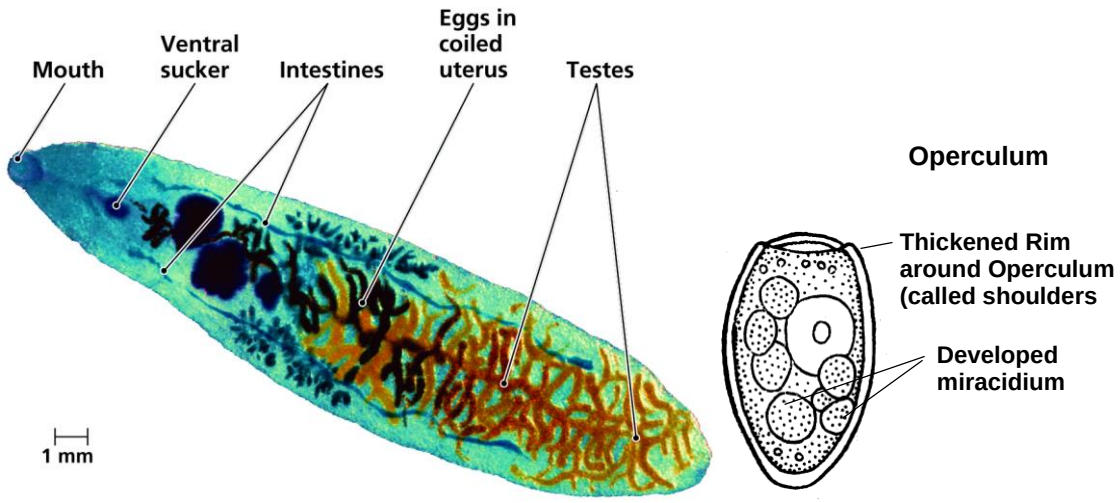


Figure 30. The morphology of *Paragonimus westermani*

Life cycle

The life cycle is completed in three different hosts, one definitive host and two intermediate host (Fig. 31). The life cycle contains **egg, miracidium, sporocyst, mother rediae and daughter rediae, cercariae, metacercariae, larvae** and **adult worm.**

The eggs are excreted unembryonated in the sputum, or alternately they are swallowed and passed with stool. In the external environment, the eggs become embryonated， and miracidia hatch and seek the first intermediate host, a snail, and penetrate its soft tissues. Miracidia go through several developmental stages inside the snail: sporocysts, rediae, with the latter giving rise to many cercariae, which emerge from the snail. The cercariae invade the second intermediate host, acrustacean such as a crab or crayfish, where they encyst and become metacercariae.

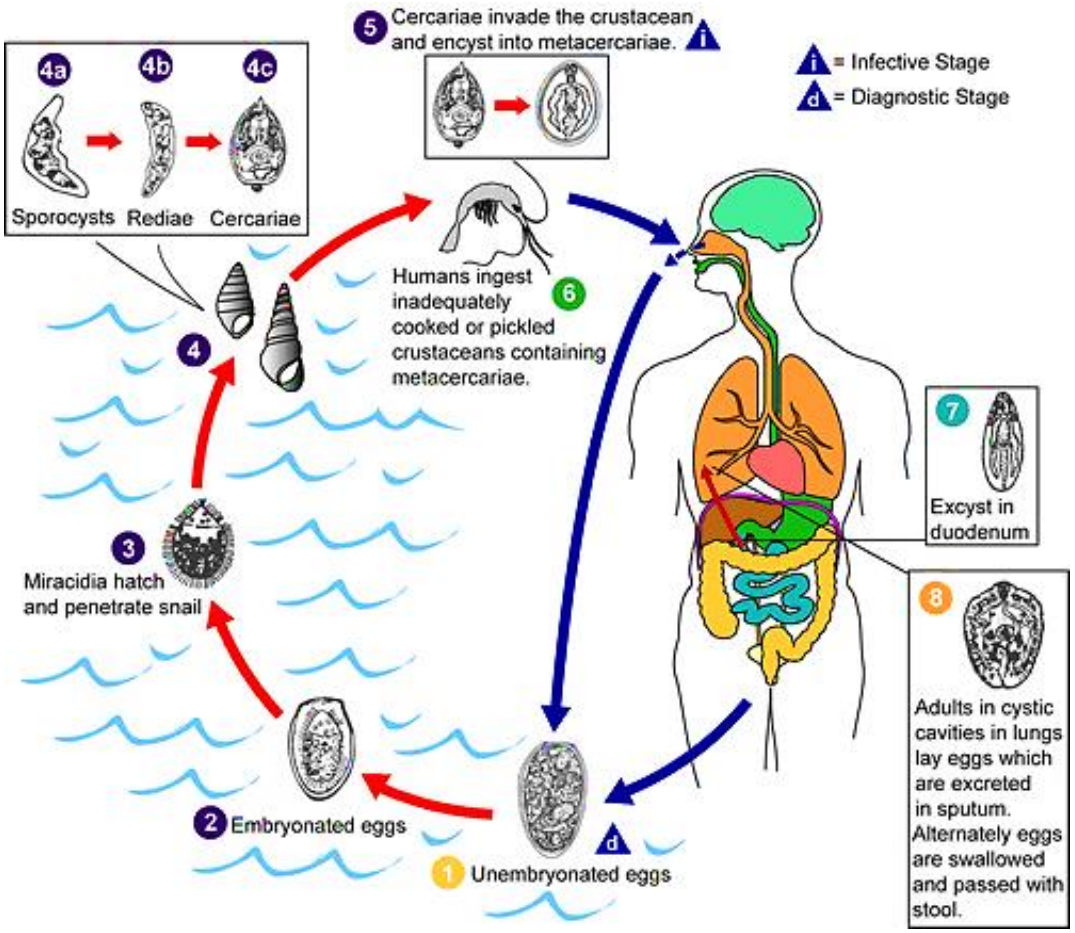


Figure 31. Life cycle of *Paragonimus westermani* (from Parasite image library of CDC, USA)

This is the infective stage for the mammalian host Human infection with *P. westermani* occurs by eating inadequately cooked or pickled crab or crayfish that harbor metacercariae of the parasite. The metacercariae excyst in the duodenum, penetrate through the intestinal wall into the peritoneal cavity, then through the abdominal wall and diaphragm into the lungs, where they

become encapsulated and develop into adults. The worms can also reach other organs and tissues, such as the brain and striated muscles, respectively. However, when this takes place completion of the life cycles is not achieved, because the eggs laid cannot exit these sites. Time from infection to oviposition is more than two months. Animals such as pigs, dogs, and a variety of feline species can harbor *P. westermani*.

Pathogenesis and clinical manifestation

Pathological changes in host caused by **physical trauma** of young flukes or adult worms' migration and lodge in the tissues, and ***chemical damages*** of flukes' toxins **The process of the pathological changes** may be divided into an **early or acute stage,** and **late or chronic stage.**

The acute stage Caused by **invasion** and **migration** of the young flukes, symptoms appear several days or one month after eating raw crab with metacercariae. Because the metacercariae excyst in the small intestine, and penetrate through its wall as well as other organs, ***hemorrhage*** in the tissue is a common pathological changes. The **symptoms have** fever, diarrhea, abdominal pain, chest pain or tight sensation, cough and eosinophilia etc.

The chronic stage *The stage is* divided into **abscess, granuloma**, and **Fibrous scar.**

1) ***Abscess*** The migration of flukes caused the destruction and **hemorrhage** of tissues; after that **neutrophilic** and **eosinophilic leukocytes infiltrate** and abscess is formed by granuloma wall gradually.
2) ***Granuloma*** Following the development of abscess, the content of the abscess will become dense fluid with reddish brown in color. The granuloma tissues are stimulated to proliferate and form "**worm cys**t".
3) ***Fibrous scar*** When the worm dies or move to other sites, the content of the cyst will be voided through the bronchioles. Then empty cyst will be filled with fibrous scar.

Young flukes or adult worm also lodge ectopic sites e.g. the intestine, mesenteric lymph nodes, liver, diaphragm, pleura, heart muscle, subcutaneous tissue, testes, uterus, brain and spinal cord. Ectopic parasitism may have caused **ectopic lesions, worm cysts,** or **abscesses i**n these other organs. The metabolic products, secretions and protein of dead worm can induce allergic or toxic reactions Paragonimiasis may be involve many organs expect for lung. Generally, the manifestations may be divided into **pulmonary** and **extrapulmonary.** Extrapulmonary Paragonimiasis are named after the organs, such as cerebral paragonimiasis etc.

***Pulmonary paragonimiasis*:** The symptoms **include** chronic cough, chest discomfort, blood-tinged sputum or rusty-brown sputum. The results of the chest X-rays show there are some significant changes in lungs, which changes are often considered to be the clinical symptoms of tuberculosis and other pulmonary diseases.

***Cerebral paragonimiasis*:** It occur commonly in young age groups. The disease may be acute or chronic. The symptoms of the acute stage are fever, headache, nausea, vomiting, visual disturbances, and paralysis.

Liver paragonimiasis: Disorder of liver function, large liver, and liver pain are common symptoms of liver paragonimiasis.

Cutaneous paragonimiasis: Some patients with lightly infection don't appear symptoms, but serological examination usually shows positive.

Diagnosis

Usually, it is important clue for diagnosis to ask the history of having stayed in or been to the endemic area and eating raw crustaceans, together with blood spitting and the characteristic sputum. Correct diagnosis must, however, be confirmed by laboratory finding of ova. The laboratory diagnosis is based on the parasitic, immunological and radiography diagnosis.

Parasitological examination Definitive diagnosis of pulmonary paragonimiasis depends on the microscopic demonstration of characteristic operculated eggs in the **sputum or faeces**. The larva or adult worm may be found from **biopsy** of nodules or cysts.

Immunodiagnostic tests **Intradermal test is available** for screening, its sensitive is more than 90%, but false positive and false negative are not low; ELISA for detecting antibodies are found to be highly sensitive, which positive is 90-100%.

X-ray examination of chest /CT In pulmonary paragonimiasis, chest x-ray shows radioopaque shadows in the middle and lower segments of the lung.

Epidemiology

Distribution Three main foci of the disease: in Asia endemic areas including China, Japan, Korea, Laos, the Philippines, and Thailand; in Africa, the Cameroon, the Congo Valley, Gambia, and Nigeria in South and Central America, Colombia, Costa Rica, Mexico, and Peru.

Epidemiological features

1) *Definitive hosts**:*** humans, and carnivores such tiger, dog, cat etc. The carnivores are also called as **reservoir host.**

2) *Paratenic host/transport host*: boar etc. It was reported that the disease possible is transmitted by ingestion of infected raw meat from the wld boars containing immature parasite.

3) *Natural focus andparasitic zoonosis.* In some forest and desert the parasitic zoonoses transmit among vertebrate, which areas is called natural endemic focus. **I**n Kuandian county of Liaoning, infected dog is major reservoir, there non-human host play more important role in transmission. So paragonimiais is a typical zoonos is.

4) *First intermediate hosts*: *Melaniidae*, fresh water snail, including *Semisulcospira libertina* (*etc.* **Second intermediate hosts**: freshwater crabs and crayfish, such as *Potamon spp, Sinop otamon spp and Cambaroides spp.*

The way of acquiring infection. The infection is transmitted to man in the following ways:

1) ingesting raw or uncooked crab or crayfish containing metacercariae
2) ingesting the meat of a paratenic host containing immature flukes
3) drinking water containing metacercariae or cercariae

Prevention and control

To interruption of the life cycle of the parasite can eliminate its spread, which measures include **chemotherapy, use of molluscacides** etc. **Bithionol and praziquantel** are the drugs of effective chemotherapy.

Specific measure to change the eating habits of the people by **health education** is considered to be the most important measure to control the disease.

SCHISTOSOMA JAPONICUM

Kingdom: Animalia
Phylum: Platyhelminthes
Class: Trematoda
Subclass: Digenea
Order: Strigeiformes
Family: Schistosomatidae
Genus: *Schistosoma*
Species: *japonicum, mansoni, haematobium*

It is necessary to remember

Schistosomiasis caused by Schistosoma species infection, is a major human health problem in many part of the developing world, which is endemic in African, Latin American and Asia including 76 endemic countries or areas. More than 600 million people in the endemic areas are at risk of schistosomiasis and the number of infected individuals worldwide is near 200 million.
Schistosomes are a group of digenetic dioecious trematods. The three main species infecting humans are *Schistosoma haematobium, S. japonicum,* and *S. manson.* Two other species, more localized geographically, are *S. mekongi* and *S. intercalatum.* S. japonicum is the most pathogenic of all the human schistosome species. It causes schistosomiasis japonica or Oriental schistosomiasis in human. Schistosomiasis japonica was first described by a physican Fujii. The eggs were demonstrated in the faeces by Fujinami. Katsurada first described the adult worm in the year 1904, which he obtained from dog and cat. He assigned the specific name *japonicum* to the parasite. Miyagawa described the details of the life cycle of the parasite. In China, there is only endemic *S. japonicum*. **In 1905**, Doctor **Logan** firstly found eggs of *S. japonicum* from human feces in Hunan province. *S. japonicum* calcified eggs were also found in Xihan dynasty mummies.
The discovery history of other human schistosomiasis are as follows:
1) *S. haematobium* eggs were found in the kidneys of twentieth-dynasty Egyptian mummies
2) The first Europeans to record contact with *S. haematobium* were surgeons with Napoleon's army in Egypt.
3) In 1858, Weinland proposed the name *Schistosoma haematobium.*
4) In 1905, Sir Patrick Manson found another species of worm, and was named *S. mansoni by Sanbon.*

Morphology

Adult Worm The adult worm of *S. japonicum* have elongate and slender bodies, and is sexual dimorphism (Fig. 32). The oral sucker is at anterior end and ventral sucker is near anterior end. There is no muscular pharynx, a single canal is formed behind the ventral sucker by the union of bifurcated caeca to form the intestinal caecum. The males measure 10-22mm in length and 0.5-mm in breadth. A total of 7 testes are present side by side in a single row in the male fluke. The males have a deep ventral groove known as the *gynecophoric canal*. The females measure 20-25 mm in length, and 0.3 mm in breadth. The ovary is at the middle of the body, and uterus is long, containing up to 300 eggs, the vitellaria in lateral fields (posterior quarter of body).

Eggs: They are 89 μ m in long and 67 μ m wide, oval and possess a lateral small rudimentary knob or delicate spine without operculum. There is a mature eggs in the feces with miracidium in the egg.
It is about 99μm in long and 35μm wide, the body like veritable spinning ball with cilia.
Cercaria Cercaria is consist of a body, a tail and a pair of furca. The body is 100-150μm long, tail 140-160μm long, and furca 50-70μm long. The oral sucker is comparatively large, and the ventral sucker is small. The body covere with minute spines. Four types of glands open through ducts at the anterior margin of the oral sucker.

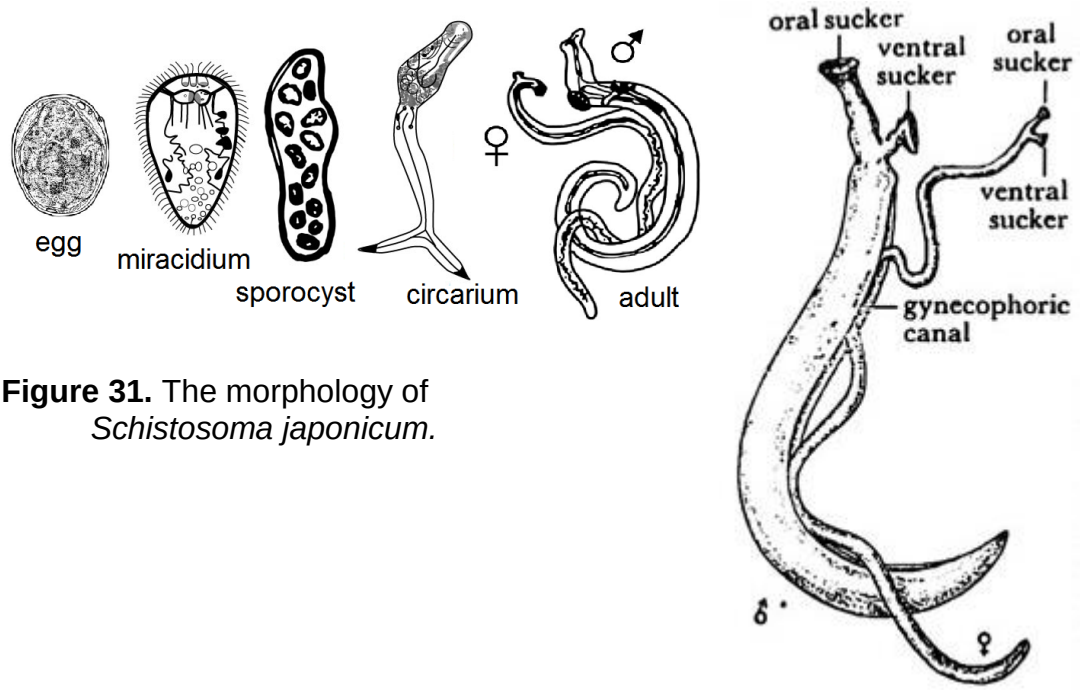


Figure 31. The morphology of *Schistosoma japonicum.*

Life cycle
Life cycle include adult worm, egg, miracidium, sporocyst (mother and daughter sporocyst), cercariae, and schistosomula etc development stage. It is completed in following hosts: a) definitive host: man, domestic animals (pig, cattle/buffalo, pig and wild animal); b) Intermediate host: *Oncomelania species* (*Oncomelania hupensis* in mainland of China).
Adult worm and deposition. Adult worms live in the veins of the small intestine (inferior mesenteric veins) of definitive host, where mature female is often found in the *gynecophoric* canal of the male worm. Sometimes worms found in ectopic site such as the lungs, testis, kidney etc. After mating, the females are laying eggs. The worm works their way" upstream" into smaller veins, the females deposit 300-3000 eggs/per female/ daily, and the eggs can live for about 22 days in the host tissue. Masses of eggs cause pressure on the thin venule walls, which are weakened by secretions from the histolytic glands of the miracidia with the eggs. The wall rupture, and the eggs penetrate the intestinal walls and thus pass the outside by feces. They are completely embryonated and hatch when exposed to freshwater. Some eggs are flowed into liver or capillary beds and calcified late.

Hatching of miracidium in freshwater. After mature eggs with miracidium enter water, the miracidium will be hatched out. The factors related to hitching include temperature of water, osmotic pressure, PH value etc. Although PH, temperature and other environmental aspects are important, factors within the egg probably play a major role in hatching process. Released miracidia can live for a few hours. When meeting snail (*Oncomelania hupensis*), miracidia enter the snail's foot or body with the aid of histolytic gland secretions, within the snail, the cilia are shed, and the miracidia become sporocysts.
Development in snail. Germ cells within sporocysts enlarge and develop into second generation of sporocysts, then the germ cells within second generation of sporocysts develop into cercariae. There is no redicial generation. From miracidial penetration of snail to emergence of cercariae at least 44 days, one miracidium can develop more than thousands of cercariae.
Penetration of host. In water, infested snails with cercariae can release mature cercariae. The cercariae usually emerge during the early part of the night, the suitable conditions include temperature of water and sun light etc. **Cercariae is the infection stage.** They are active swimmers, but may have motionless from the surface of the water. T**he life span of cercariae is about 1-3 days**, and **penetration of host occur within 48 hours**. When contacting with the skin of an host, cercariae attach skin by their suckers, and release enzymes from glands at their anterior ends, then enter skin combining with muscular movements of the parasite body. In the process the tail is cast off. The entry time is about 3 to 7 minutes.

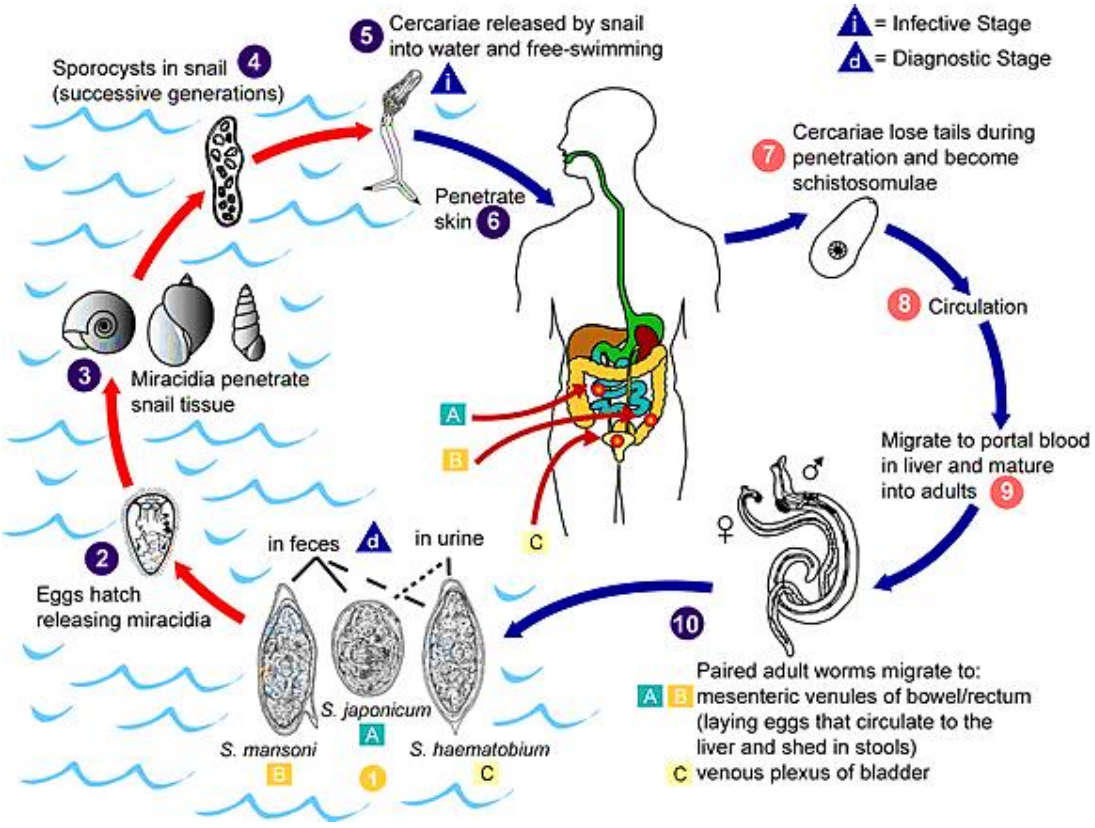


Figure 33. Life cycle of *Schistosoma* (from Parasite image library of CDC, USA)

Migration and inhabiting. When a cercaria has penetrated the skin, it becomes a schistosomules (Once the organism penetrates the skin, lose their tails, and secrete the substances from the various glands, they are considered to be schistosomules). The schistosomule leaves the skin and develops into young worms. The young worm enters into lymphatic or blood vessels, then to the heart and circulatory system, then reaches the mesenteric artery and capillaries, proceed to feed, grow and migrate into the superior mesenteric venules. Here **copulation takes place**, and females enter the gynecophoric canals of males, and lay eggs. *About 24 days' elapses from the time of penetration by cercariae to the oviposition*. A month late the eggs appearance in feces. The life span of adult worms is usually 4.5 years.

Pathogenesis and clinical manifestation

E**ggs deposited** by *S. japonicum* adult female worms are the chief **cause of tissue damage**, consisting of an **inflammatory granulomatous reaction and pseudotubercle formation** followed by fibrosis and the sequel of the disease syndromes. But, besides eggs, other developmental stages of schistosome such as cercariae, schistosomule, adult worm can also have caused pathological changes in host.

Cercariae. Cercarial dermatitis with rash and tingling sensation is due to skin invasion and is likely a result of host sensitization. This pathological changes belong to immediate hypersensitivity**,** mononuclear and eosinophil involve in the reaction.

Schistosomules. The organs passed through by schistosomula such as lung, appear venulitis, lymphangitis, hemorrhages, giant cell reaction and eosinophils infiltration. The characteristic clinical features include fever, cough, bloody cough and eosinophilosis etc.

Adult worms. In generally, adult worm doesn't cause pathological changes significantly, but the **waste products, secretions, metabolic products and toxins products** of worm can induce to form **immuno-complex**, which damages host.

Eggs with miracidium can release **some antigenic and enzymatic secretions,** which can induce **a granulomatous cell-mediated responses** of lymphocytes, macrophages and eosinophils, about 100 times the volume of the egg. The formation of granuloma is closely related to development of eggs. As eggs is not mature, no inflammatory reaction or light reaction appears around the eggs. Because *S. japonicum* eggs were deposited in cluster, the volume of egg-granuloma is larger in which about half the cells are eosinophils and some cells is plasmacyte. Meanwhile, antigen-antibody complex response named *Hoeppli phenomenon* appears around eggs.

In summarizing the recent research results concluded" T-cells are of major importance for the formation of large granuloma around the eggs of *S. japonicum* like *S. mansoni,* but modulation of size of granuloma is primarily antibody medicated." So the mechanism of granulomatous formation is considered to be VI type delay hypersensitivity.

Following the progress of egg-granuloma, miracidium in egg is to be died and egg is to be calcified, then fibroblasts cluster and synthesize collagen. The granulomatous formation transit to fibrous tissue, then the permanent fibrochanges due to schistosoma infection was established. Late in the disease,

periportal pipestem fibrosis and fibrosis in intestinal walls may result in a clinical picture of cirrhosis, including portal hypertension and splenomegaly. So the most important pathological changes in the liver are notice in the portal trials, widening of portal trials, fibrosis, and numerous new capillaries in the fibrosis portal tissues are seen. Apart from the portal system, egg granulomatous lesions have been found in lungs, brain, the skin, breast, kidney, ureter and reproduction organs of both sexes etc. Although *S. japonicum* egg are rarely in endocrine glands, the infection itself, but not the egg deposition, can cause schistosomiasis dwarfism. In these patients, body growth and development are retarded and significant pathological changes are apparent in the skeleton, endocrine glands as well as reproductive organs.

Clinical presentation

Most individuals with *S. japonicum* infection are asymptomatic. The frequency and severity of clinical finding were positively correlated with the intensity of infection, especially with heavily infection. The main clinical finding include weakness, abdominal pain, diarrhoea, hepatomegaly and splenomegaly etc.

In generally, schistosomiasis can be divided into three phases, **acute phase, chronic phase and late phage.**

Acute stage. Penetration of the skin by cercariae may appear **cercariae dermatitis** with local pruritus, erythema and popules. When egg deposition, symptoms with **fever, chills, ache and gastrointestinal complaints, hepatomegaly, splenomegaly and eosinophilia** etc. Frequently mimicking typhoid fever is commonly seen within a month of infection. *In this time, eggs can be found in faces of the patients.* **Acute** cases are usually observed in persons entering the endemic area for the first time.

Chronic stage. **I**n endemic areas 90% infected persons are chronic cases of schistosomiasis. Usually more than half of the chronic cases area symptomatic although stool examination may reveal egg of *S. japonicum*. The general symptoms: weakness, fatigue, abdominal pain, irregular bowel movements and blood in stool, hepato-splenomegaly, anemia and emaciation etc. *Late stage* in general, some chronic cases with heavy infection will become advanced cases of schistosomiasis 5 years after infection. Advanced cases have three types, splenomegaly, ascites and dwarfism. The common clinical finding is *hepato-splenomegaly, ascites, portal hypertension, abdominal collateral vein dilatation and oesophagogastric varices.* Blood loss due to bleeding of oesophagogastic varices is the major cause of death in advanced cases.

Ectopic lesion S. japonicum more commonly invades the central nervous system and other organs than do the other schistosomes. Ectopic parasite can cause cerebral schistosomiasis, and pulmonary schistosomiasis etc.

Immunity

Schistosomal antigens. S. japonicum is a multicellular organism, with complex life history, so it's antigens is complication. Some antigens come from cercaria, some from schistosomula, some from adult worm and some from egg. The secretions, waste products, metabolic products and integument shed of worms possess antigenicity. Among these antigens e.g. SEA can

induce immuno-pathological response and some e.g. GST and paramyosin induce protective immunity, some can be use for diagnosis. Circulating antigens are useful for diagnosis, including GAA (gut associated antigens), MAA (membrane-associated antigens) and SEA (soluble egg antigens).

Concomitant immunity **"Concomitant immunity"** implied that the adult worms were generating an immune effector mechanism that could really destroy schistosomula but to which they themselves were resistant. It is considered to be a common immune phenomenon in helminthes infection. In schistosome infection, the mechanism of concomitant immunity is related to "immune evasion" of schistosome.

The ways of immune evasion are as follows:

1) ***Masquerade by the uptakeof host antigens.*** One mechanism of special way is the ability of schistosomes to disguise themselves by taking host antigens onto their surface. It was found that when adult worms were transferred from the blood vessels of a mouse to a monkey, they lived perfectly well, whereas worms transferred from the blood vessels of a mouse to a monkey that previously had been immunized with mouse red blood cell were killed. Analysis of this form of masquerade has shown that various host molecules are taken up by schistosomes; these include blood group glycolipids, MHC glycoproteins and nonspecific host immunoglobulins.

2) ***Host molecule mimicry Schistosomes*** were capable of synthesizing antigens that cross- reaction with host molecules.

3) ***Shedding of schistosome surface antigens*** *c*an protect from binding specific antibody of host.

4) ***Resistance due to intrinsic membrane changes.*** Resistance to immune attack at the lung stage may be due to intrinsic membrane changes and not the lack of surface antigens secondary to shedding or host antigen acquisition.

5) ***Resistance to complement.*** Freshly transformed skin schistosomula are susceptible to complement, but they rapidly lose this sensitivity. The reasons may be due to surface change that no longer allow the activation of the alternate complement pathway.

6) ***Other mechanisms for escaping immune attack.*** Other mechanisms may *involve the destruction the antibodies themselves and specific immune suppression in host.*

Mechanisms of protective immunity. The information about the mechanisms of protective immunity related to schistosoma infection come from animal experience, vitro observation and epidemiolog ical evidences. *Specific antibodies* e.g. IgG and IgE, complements and cells e.g. eosinophils and mononuclear phagocytes involved in host protection. Experimental studies found that CTL (cytotoxic T lymphocyte) has not the active of killing worm The main immune effector mechanism is ADCC (antibody- dependent, cell-mediated-cytotoxicity) with macrophages, plateles, neutrophils and eosinophils. The acquired resistance in host main attack schistosomula directly. The acquired resistance take place in skin and lungs mainly. The acquired immunity is not complete immunity, some worms can evade immune attack and develop to be mature. Recent years, epidemiolog ical studies showed that humans can slowly develop an acquired resistance to reinfection, which immunity is age-depending. The immunity is lower in younger and stronger in older.

Diagnosis

History of the patient residing in the endemic or contacting the infected water in the endemic area, can help to diagnose the infection. Parasitological examination is considered as definitive diagnosis, and the serodiagnosis methods are useful in the diagnosis of schistosomiasis.

Parasitic diagnosis. In acute cases, eggs can be demonstrated by direct faecal smear examination. In chronic cases, as the number of eggs excreted in the faeces are scanty and intermittent, hatching of miracidium is useful in the diagnosis. Kato-katz's method (Kato's cellophane faecal think smear) is frequently used now to quantify the number of eggs passed in the faeces. Identification of eggs in rectal biopsy is also another procedure for the detection of light and asymptomatic infection as well as late stage cases.

1) ***Direct smears*** After 35-48 days of infection with cercaria, eggs can be found in the faece by direct smears, the method is simple, but low sensitivity.
2) ***Hatching of miracidium*** as index of viable egg
3) ***Kato-katz's method*** as quantitative examination, but the sensitivity is low in lightly infection.
4) ***Microscopical examination of rectal biopsy*** the method is a highly sensitive clinical diagnostic technique, but this invasive procedure is neither simple nor convenient for population- based surveys

Immunodiagnosis It include Skin test (Interdermal test, IDT), antibody detection and antigen detection etc.

1) ***Interdermal test*** **Antigen:** adult worm antigen is used for IDT commonly, the **s**ensitivity is more than 95％, false positive rate is about 2％. The test can be done 2 weeks after infection, so it possesses the **v**alues for early diagnosis and screening test
2) ***Antibody detection.*** **In** China, different antigens and test systems of antibody detection have been used, such as COPT, IHA, ELISA and IFA. The sensitivity of these tests is usually higher than by any current stool examination technique but the specificity is lower. Because the specific antibodies in sera of infected individual can last more than one year, no current serological test can distinguish between past and active infection. So determination of specific antibodies may be used diagnostically only in special situation such as, for example, in people migrating from non-endemic areas. It can also be used for estimating the prevalence in not previously treated populations but continued antibody production after cure makes this approach impractical for monitoring chemotherapy.

Epidemiology

S. japonica is found only in mainland of China, Japan, the Philippians and Indonesia. Japan has controlled and eliminated schistosomiasis since 1978.

Epidemicfeatures

1) *Main reservoirs of infection S. japonica* is a zoonoses. Infected livestock are important reservoirs of infection besides infected persons. Meanwhile 31 species wild mammals e.g. field mice etc are found to be reservoir hosts. Inmost endemic areas, infected cattle or water buffalloes are majorreservoirs.
2) *Way to infection* Contacting infected water containing cercaria is only the way to acquire the infection. Ways to contacting infested water include three types, contacting for production e.g. boating and fishing; for life e.g. washing

3) clothes; for playing e.g. bathing.
4) *Susceptible population* Humans is a susceptible host of S. japonicum. In most endemic areas, both prevalence and intensity increase gradually by age to peak about at 10-20 years of age. But by the beginning the third decade of life, a slight or moderate decrease in prevalence may be noted; the egg counts are markedly lower in those above 30 years of age.
The evidence suggested the occurrence of immunity in older population after repeated infection.
Snail **(***Oncomelania hupensis* Gredler, 1881) is the ***only intermediate host of S. japonicum,*** which normally inhabit flooded areas e.g. the banks of irrigation ditches and canals, marshes of lakes, and beaches of river etc. **Snail** has female and male; female lay eggs in Spring. Baby snail grows under water, and develop to be adult snail in Autumn. The life span of snail is from one to two years. **Snails** are infested by miracidia and release cercaria during the periods of immersion in water. So the ***transmission season is from April to October in China.***
Epidemic factors related to transmission of schistosomiasis include natural and social factors. **Natural factors**: environment, temperature, level of water, nature of soil, and vegetation etc. **Social factors**: economic level, sanitary condition, medical care, ways to produce in local and local costumes etc. ***So environment modification, development of economy, health education and national program of schistosomiasis control will impact on the transmission significantly.***
Control and prevention
The specific objectives control of the technical strategy is to: a) to **reduce morbidity** in areas of the high endemicity to **control morbidity** in areas of medium endemicity (areas with prevalence >3％ and 〈15％〉 and c) **control morbidity, and transmission**, in areas of low endemicity (areas with prevalence<3％)
Control measures. There are different methods used at different endemic areas.
1) ***In areas of high endemicity*** The methods include mass chemotherapy, limited snail control by molluscidng with niclosamide, environment modification where appropriate chemotherapy for livestock and health education are also preformed.
2) ***In areas of medium endemicity and of low endemicity*** The main measures include selective chemotherapy for human, chemotherapy for livestock, snail control and health education.
3) ***Individual prevention*** For the individual or population traveling to endemic areas due to schistosomiasis, avoiding contact with infested water bodies is the practical preventive measure.
If you can not avoid exposure to infested water, Artemether and Artesunate which are artemisinin 's derivatives are recommended to take.

Cercarial dermatitis
Cercarial dermatitis is called **"rice-field dermatitis"** in Chine, and named "**swimmer's it** in USA and Canada, which is caused by secondary exposure of human skin to cercariae of several **birds or domestic animal schistosome species**. *After invading, these larvae of schistosomes only can remain*

in skin, and cann't develop mature worm. The disease is prevalent in many parts of both developed and developing worlds, it is considered as a common disease in some areas.

In our country, cercarial dermatitis is caused by infection of genera ***Trichobilharzia*** and ***Orientobilharzia. Trichobilharzia i***nclude ***T.pao, T. jianesis,*** and *ducks are their definitive host.* Eggs with duck's feces enter water, hatch miracidium and then develop cercaria with in snails. The cercaria is similar to cercaria of human schistosome. ***When human skin contacts the water containing the cercaria in rice fields or pools, the cercaria can penetrate the skin andcause cercarial dermatitis.***

The clinical symptoms include initial tingling sensation, erythema, maculopapular rash, vesicles, and edema. The **pathological changes** usually appear the skins of hands, or feet, which parts of body contact infested water frequently. The pathogenesis belongs to **immediate hypersensitivity, and delay hypersensitivity.**

LESSON 11. PARASITOLOGY. WORMS. TAPEWORM

INTRODUCTION

Tapeworms belong to Class Cestoda, and live by parasitizing life. The cestodes parasitizing humans constitute a very disparate group of parasites. Taxonomically they belong to two distinct orders, ***Cyclophyllidea*** and ***Pseudophyllidea***, with essential differences in their morphology and life cycles.

MORPHOLOGY

The cestodes are long, segmented and a tape-like worms. They differ trematodes in may ways.

Adult worm Adult worm is flat, long, white or milk white in color. It consists of **scolex**, **neck**, and **strobilu**. Strobilus is a specific structure of tapeworm, consisting of a linear series of sets of reproductive organs of both sexes; each set is referred to as a **proglottid** or **proglottos.** Most tapeworms bear a "head", or scolex, at the anterior end that may be equipped with a variety of holdfast organs to maintain the position of the host in the gut. The scolex may be provided with suckers, grooves, hook, spines, lands, or combinations of these.

The scolex of *Cyclophyllidea* is like ball, in which there are four circular-suckers with rostellum. The scolex of *Pseudophyllidea* is shuttle-like, its holdfast organs are two grooves named bothrium.

Commonly, between the scolex and the strobila lies a relatively differentiated zone called neck, which may be long or short. At contains germinal cells that apparently are responsible for giving rise to new proglottids.

Tapeworms are hermaphrodite/ monoecious with the exception of a few rare species. Usually each proglottid has one complete set of both male and female systems. The proglottid nearby neck is called young proglottid, which reproductive systems is immature. As a proglottid moves toward the posterior end of the strobila, the reproductive systems mature, which one is called **mature proglottid.** As it becomes crowded with eggs, this is **gravid proglottid.**

Tegument of adult worm. Tapeworms lack any trace of a digestive tract and therefore must absorb all required substances through their external covering, which tissue was preferred the term "tegument". Tegumental structure is generally similar in all cestodes, and is covered by minute projections called "microtriches" that are underlaidby the tegumental distal cytoplasm. The microtriches are similar in some respects to the mi found on gut mucosal cells and other vertebrate and invertebrate transport epithelia, and they completely cover the worm's surface, including the sucker.

Calcareous corpuscles. The tissues of most cestodes contain curious structures termed calcareous corpuscles, they are secreted in the cytoplasm of differentiated calcareous corpuscle cells, which are themselves destroyed in the process. The corpuscles are from 12 to 32 um in diameter, depending on the species, and consist of inorganic components, principally compounds of calcium, magnesium, phosphorus, and carbon dioxide embeded in an organic matrix. The possible function of the **calcareous corpuscles** has been the subject of much speculation. For example, mobilization of the inorganic compounds might buffer the tissues of the worm against the large amounts

of organic acids produced in its energy metabolism. Another suggestion has been that they might provide depots of ions or carbon dioxide for use when such substances are present in insufficient quantity in the environment, such as upon initial establishment in the host gut.

Reproductive systems. Tapeworms are hermaphroditic**.** Usually each proglottid has one complete set of both male and female systems, but some genera have two sets of each system.

The male organs mature first and produce sperm that are stored until maturation of the ovary. The male reproductive system consists of one to many testes, vas efferens, seminal vesicle, deferens cirrus pouch, and cirrus etc. The female reproductive system consists of an **ovary** and associated structures, including **vitelline follicles**, **vitelline duct, uterus, seminal receptacle and vagina** etc.

LIFE CYCLE

Cestodes complete their life cycle in two or their different hosts (exception: Hymenolopis nana complete the life cycle in a single host only). Adult worms live in the intestine of vertebrates, and the life cycle need one or two intermediate hosts. It is called as "**metacestode**" when larva tapeworm lives in intermediate hosts. Among the life histories that are known, much variety exists in the juvenile forms and details of development, but there seems to be a single basic theme: embryogenesis within the egg to result in a larva, the **oncosphere** ; hatching of the oncosphere after or before being eaten by the next host, where it penetrates to a parenteral site; metamorphosis of the larva in the parenteral site into a juvenile usually with a scolex; development of the adult from the metacestode in the intestine of the same or another host.

The development stages of Pseudophyllidea include **egg, coracidium, procercoid**， in first intermediate host such as freshwater insects), **plerocercoid / sparganum**， in second intermediate host such as fish), **adult worm** (in the intestine of definitive host).

The development stages of Cyclophyllidea: **egg, oncosphere**, **bladder worm/coenuru/hydatid cyst** containing **proto-scolex** and **alveolar hydatid cyst/multilocular hydatid cyst** in the tissues, adult worm in definitive host.

PATHOGENESIS AND PATHOLOGY

Adult worm. The majority of tapeworms cause intestinal infection, the pathological changes are related to the physical stimulation of the sucker in the holdfast and chemical damages of worm's secretions. The clinical symptoms due to adult worm are not serious, abdominal discomfort, diarrhea, nausea and weakness are common symptoms.

1) Competing with the host for nutrients, such as vitamin B12 (e.g., *Diphyllobothrium latum*).

2) Causing mechanical obstruction or migration to the unusual sites (e.g., *Taenia species*).

3) Evoking local inflammatory reactions (e.g., Taenia solium).

Larvae. Tapeworm larvae can invade almost any internal organ in a human being, and may cause serious pathological damages. Bladder worms or plerocercoidsmigrate in subcutaneous tissue and develop cysts. If invading

eye or brain, the infection will have serious consequences. Hydatid cyst parasitize in liver, or lungs. If the cyst is broken, the content of the cyst will cause hypersensitivity or shock. This is one of the major cause of death due to tapeworm infection.

EPIDEMIOLOGY

Tapeworm infections in human are relatively restricted in their distribution in comparison to the infections caused by flukes. Tapeworm infections are acquired either by ingestion of the eggs or of the larval stages, present in the meat etc. Reinfection with the larvae is rare but common with adult worms.

DIAGNOSIS

Intestinal infection with adult worms are usually diagnosed by demonstration of the eggs and sometimes the segments in the faeces. Stool microscopy is not useful for extra-intestinal infection caused by the larvae. They are best diagnosed by radio-imaging procedures, biopsy and serology.

PREVENTION AND CONTROL

Avoidance of eating raw or inadequately cooked food, meat, or of ingestion of contaminated water will prevent transmission of the infection to man. Thorough cooking of various food as well as health education are essential to control the infection.

TAENIA SOLIUM

Kingdom: Animalia
Phylum: Platyhelminthes
Class: Cestoidea
Order: Cyclophyllidea
Family: Taeniidae
Genus: *Taenia*
Species: *solium*

It is necessary to remember

T. solium called the pork tapeworm can cause the infection of *T. solium taeniasis*, and its cysticercus cause human cysticercosis. The life cycle of the parasite was first described by Kuchemeister and Leukart. He demonstrated that the larval stage (*Cysticercus cellulosae*) of the parasite present in the muscles of the pig is infective to man. It is the only cestode for which man acts as both the definitive host (harbouring the adult worm) and the intermediate host (harbouring the larva of the parasite).

Morphology

Adult worm measures 2 to 4 meters in length and has a scolex, neck and segments (Fig. 34).

1) ***Scolex.*** Typically, it is provided with hooklets, hence characteristically known as armed tape worm. Scolex is round, measures 0.6-1 mm in diameter and has four suckers and is armed with a rostellum. The latter consists of two small and large hooks.

2) ***Neck.*** The neck is short and 5-10 mm long and about one-half as thick as head.

3) ***Proglottid.*** The strobila consists of 700-1000 segments or proglottids (immature, mature and gravid). The immature segments are broader than long while the gravid proglottids are longer than the broad. The gravid segments look grayish-black and transparent when fully developed. The uterus has 7-13 lateral branches and is completely filled with 40000 eggs

4) ***Egg.*** The are brown colored, round shaped and measure 31-43 µm in diameter. It is provided with a two layered shell. The outer shell is thin, transparent and does not always remain with the eggs. The inner embryophore is a thick, brown, roughly structured wall which surrounds the embryo.

5) ***Cysticercus cellulosae.*** Cysticercus cellulosae is the larval stage of *T. solium*. Cysticerci are small, oval and milky white bladder-like structure. They are filled with some fluid rich in albumin and salts. It shows a small white spot representing the future head invaginated into the bladder. This stage is found both in human and pigs, and is the infective stage of the parasite.

Life cycle

***Definitive host*:** Man

Intermediate host: Pig, at time man. Humans is the definitive host as well as intermediate host of *T. solium.* Adult worm lives in the intestine, and fix the wall by its scolex. The gravid proglottids become detached from the strobila, usually in groups of three to five, and are excreted passively in the human feces.

The eggs are scattered from the proglottids which are damaged or macerated. The *T. solium* eggs can survive in the environment for several months. Pigs are infected after ingestion of the proglottid or eggs present in an environment contaminated by humans.

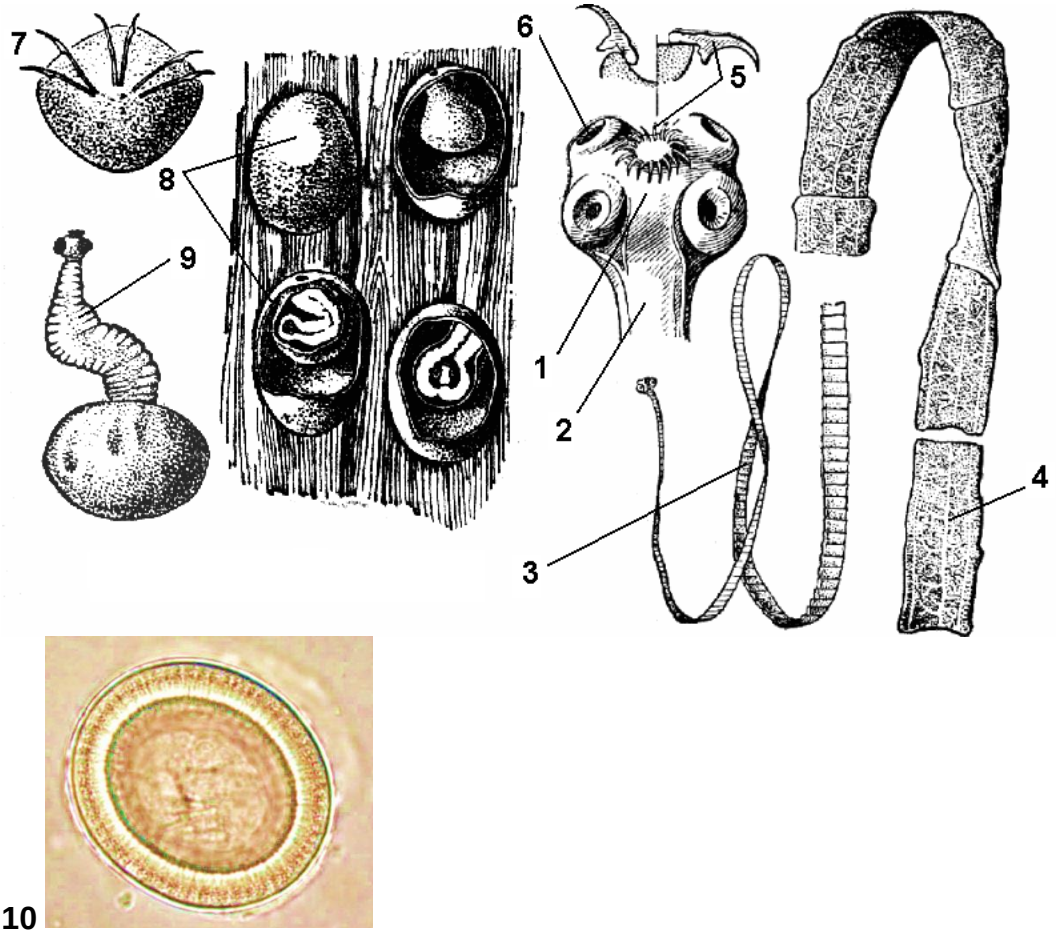


Figure 34. Scolex, mature and gravid proglottids of *T. solium.*
1 – scolex, 2 – neck, 3 – jointed body, 4 – proglottid, 5 – hooks, 6 – oral saccker,7 – onkosfera, 8 – finna, 9 – ainn turned with the head in the human body, 10 – egg of taeniidaes (form is characteristic of all taeniidaes).

The oncosphere leaves the embryophore in the pig intestine and migrates to the tissue; the bladder larva cysticercus develops mainly in the muscle tissue and the myocardium but often also in the brain and liver. The cysticerci become fully grown and invasive for humans 2 months after ingestion of the eggs.
A human acquires taeniasis ingesting *T. solium* **cysticerci** in raw pork. In the human small intestine, the scolex attaches itself to the mucosa and within 2 months develops into an adult tapeworm producing eggs. It is reported that *T. solium* can live more than 25 years in humans.
If humans swallow eggs or gravid proglottids, the oncosphere can develops bladder worm like in pig. But it cann't continue to develop adult worm.

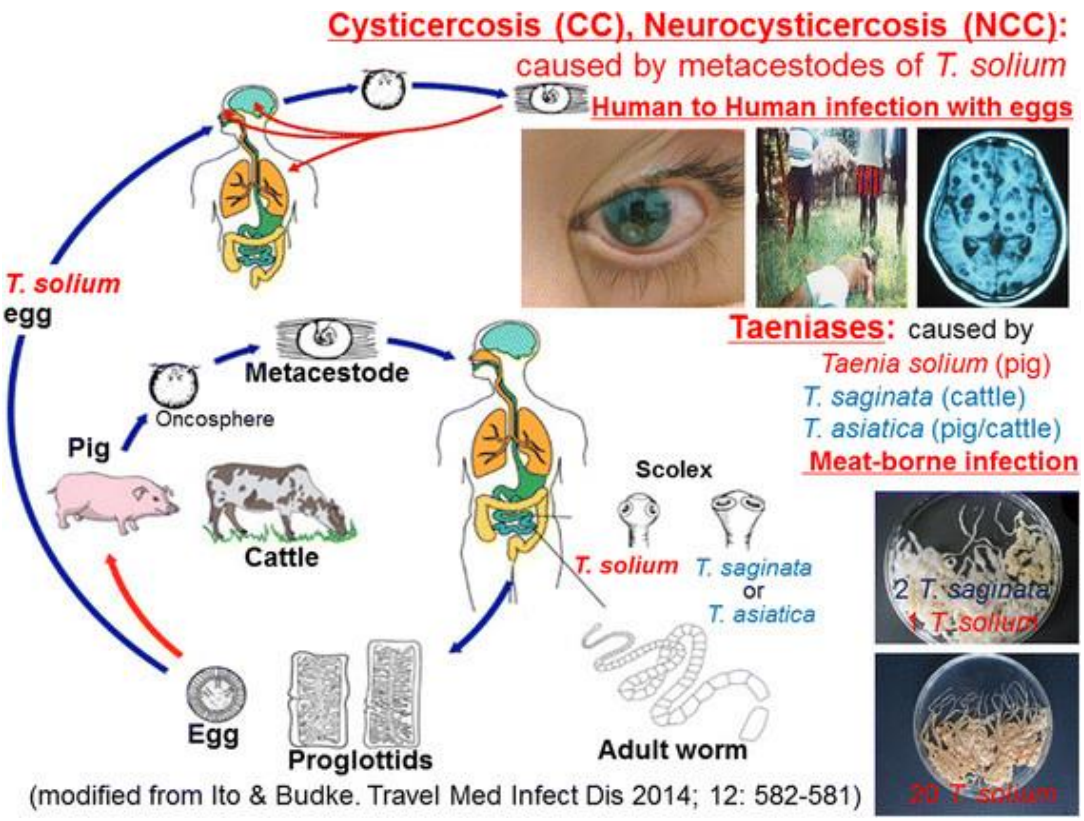


Figure 35. Life cycle of *Taenia solium* (from Parasite image library of CDC, USA)

Pathogensis and clinical manifestation

Both adult and larvae (*Cysticercus cellulosae*) are pathogenic.

The adult worm. The adult worm occasionally may cause mild irritation or inflammation of the intestinal mucosa by their armed scolex. The clinical manifestation of intestinal ***T. solium*** **taeniasis is** relatively mild. Vague abdominal discomfort, hunger pangs, and chronic indigestion have been reported but are undoubtedly seen more often in patients who are aware of their parasitic infection than in those who are not. Moderate eosinophilia frequently occurs.

The larvae or Cysticercus cellulosae. The *Cysticercus cellulosae* frequently cause a serious diseases known as Cysticercosis in man. The number of bladder worms parasitizing in humans range from 1 to thousands. Virtually every organ and tissue of the body may harbor bladder worms. Most commonly they are found in the subcutaneous, connective tissues; followed site is the eyes, brain, muscles, heart, liver, lungs, and coelom. A fibrous capsule of host origin surrounds the larvae. The seriousness of the disease depends upon:

a) The sites of location of *cysticerci*, and
b) Numbers of *cysticerci*.

Cysticerci can develop in any other organ and issue of man, but are commonly present in the following sites:

a) Muscle, subcutaneous tissue and viscera are affected in disseminated cysticercosis. The viable cysticerci evoke a moderate tissue reaction while the dead cysticerci evoke a strong inflammatory reaction in the tissues.

b) Eye is affected in ophthalm ic cysticercosis. The cysticerci are often present in the subcutaneous tissue, vitreous humou, anterior chamber of the eye, and
c) Brain and spinal cord of the central nervous system are involved in neurocysticercosis. Cystic lesions are usually 2 cm in diameter and found chiefly in the meninges, cerebrum, ventricles and subarachnoid space, at the base and ventricles of the brain. Subcutaneous or muscular cysticercosis is usually asymptomatic. The presence of a large number of cysticerci in the muscles and subcutaneous tissues may cause muscle pain, cramp and fatigue.
Neurocysticercosis is the most serious clinical manifestation of the condition. The human brain can be invaded by one, by several, or even by more than two thousand cysticerci. Some cases have not any symptoms, but some may die suddenly. Usually its process is slow, the incubation period ranges from one month to one year, but can last 30 years in a few cases. The symptomatogy of cerebral cysticercosis is characterized by three basic syndromes: convulsions, intracrnial hypertension, and psychiatric disorder, occurring separately or in combination. The prognosis of cerebral cysticercosis is highly variable and unpredictable. Ocular cysticercosis may cause irreparable damage to the retina, iris, uvea, or choroids. This disease constitutes about one-fifth of human neurocysticercosis cases. Host reactions to cysticerci vary from slight to severe inflammation with complication such as chorioretinitis, and iridocyclitis.

Diagnosis

Intestinal taeniasis. It depends on the demonstration of gravid segments and eggs in the faeces and perianal scrapings by microscopy.
a) Questioning the history of eating raw pork;
b) Tool examination for finding eggs;
c) Recovery of gravid proglottids and count of the main lateral arms of the uterus;
d) Evacuation of the scolex following medication.

Cysticercosis. The laboratory diagnosis includes the following:
a) Radio diagnosis: In subcutaneous cysticercosis, plain X-ray of the soft tissues may show oval or elongated cysts if they are calcified. X-ray of the skull may demonstrate cerebral calcification and reveal intracranial cysticercosis in the neurocysticercosis. CT and MRI (magnetic resonance imaging) are very useful in the diagnosis of neurocysticercosis. They detect both calcified and non-calcified cysts and also show intracranial cysts.
b) Biopsy: the easiest type to diagnose by biopsy is subcutaneous cysticercosis.
c) Serological diagnosis: At present, serological examination is considered to be useful for diagnosis. These methods include **IHA, ELISA, and Dot-ELISA for detecting specific antibodies.**

Epidemiology

The *T. solium* infection is prevalent in Europe, Central and South America, Central and South Africa, and Southeast Asia. It was reported that among 1978 the patients, 83.8% case is adult persons aged from 20 to 39 years old. In some areas they are more prevalent in malesand in others more in females, depending on the eating habits.

Prevention and control of cysticercosis

Praziquantel is effective for the treatment of intestinal taeniasis caused by *T. solium*. Surgery is indicated for cysticercosis of the brain and eye. Praziquantel is usually given to reduce the inflammatory reactions caused by the dead cysticerci.

The measures to prevent and control *T. solium* taeniasis include:

a) Avoidance of eating raw or insufficiently cooked pork;
b) Inspection of pork for the cysticerci;
c) Changing pig hushandry methods and avoiding human fecal contamination;
d) Treatment of infected persons.

The cysticercosis canbe prevented by

a) Early detection and chemotherapy by taeniacides, such as praziq uental;
b) Avoidance of food contaminated with eggs of *T. solium* and Changing the habits of eating raw or uncooked pork;
c) Improvement of personal hygiene.

TAENIA SAGINATA

Kingdom: Animalia
Phylum: Platyhelminthes
Class: Cestodes
Order: Cyclophyllidea
Family: Taeniidae
Genus: Taenia
Subgenus: *Taeniarhynchus*
Species: *saginata*

It is necessary to remember

T. saginata, called the beef tapeworm, is similar to *T. solium* in life cycle and morphology. It only cause *T. saginata*. Taeniasis in human, but can not cause human cysticercosis.

Morphology

T. saginata is a long flattened ribbon-like tapeworm that is white in color (Fig. 36). It is about 6 to 7 milimeters in width. The adult T. saginata usually grows to be about 4 to 8 meters in length, with about 1000 segments called proglottids. Unlike the *T. solium*, the scolex (or mouth) is "unarmed" because it has 4 suckers but no hooks.

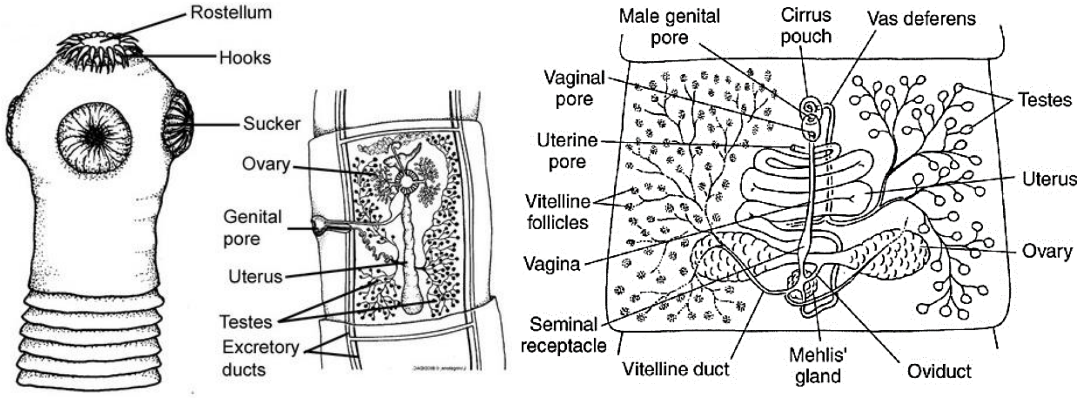


Figure 36. Scolex, mature, gravid proglottids and sexual system schem of *T. saginata.*

Life cycle

A human being is the only definitive host of *T. saginata*, which live in the intestine. With *T. saginata* infection, about 6 gravid proglottids, each containing 80,000 to 100,000 eggs, pass daily through the anus. The eggs can survive for several months or years. The eggs develop further when ingested by cattle, a intermediate host. The oncosphere leave its embryophore in the cow's intestine and migrates to the muscles, where within 60-70 days the next larval stage-the cysticerus-develops. The cysticercus is an oval bladder, filled with fluid and containing the invaginated scolex of the tapeworm. It can survive in the muscle of the cattle for 1 to 3 years and can infect humans when ingested with raw meat. The quadrangular scolex of the *T. saginata* then attaches itself to the jejunal mucosa, and within 3 to 3.5 months a fully grown tapeworm is developed.

Pathogenesis and clinical manifestation

No significant pathological phenomena usually occur. Eosinophils may be moderately or, occasionally, markedly increased; this may be followed by a slight neutropenia.

Many cases of the infection are asymptomatic. Verminious in toxication, caused by absorption of the worm's excretory products, is common, with the characteristic symptoms of dizziness, abdominal pain, headache, localized sensitivity to touch, and nausea. Neither diarrhea nor intestinal obstruction is uncommon. There may be in creased or loss of appetite, weakness or weight loss.

Diagnosis

Question the history of passing proglottids. Usually, the gravid proglottid passed in the feces is first noticed and taken to a physician for diagnosis.

Stool examination for the egg.

Recovery the scolex and gravid proglottid after treatment.

Epidemiology

T. saginata is a world distribution. It is especially prevalent in countries or localities where raw beef is a common article of diet. Human infection is acquired from eating raw beef containing the viable bladder worm.

Prevention and control

The main measures are as fellows:

a) Chemotherapy with areca nut plus pumkin seed or **praziquantel;**
b) Restriction of cattle from grazing on contaminatedland;
c) Inspection of beef for cysterci;
d) Changing the habit of eating raw beef by health education.

ECHINOCOCCUS GRANULOSUS

Kingdom: Animalia
Phylum: Platyhelminthes
Class: Cestoda
Order: Cyclophyllidea
Family: Taeniidae
Genus: *Echinococcus*
Species: *granulosus*

It is necessary to remember

The adult worm of ***Echinococcus granulosus*** live in the intestine of carnivores, and the larval stages parasitize in various mammalian intermediate hosts. The larval or metacestode forms are referred to as hydatid cysts and the diseases caused by them as **hydatidosis** or **hydatid disease.** Adult worm was described by Hartmann in the intestine of the dog. The larval form subsequently was described by Goeze.

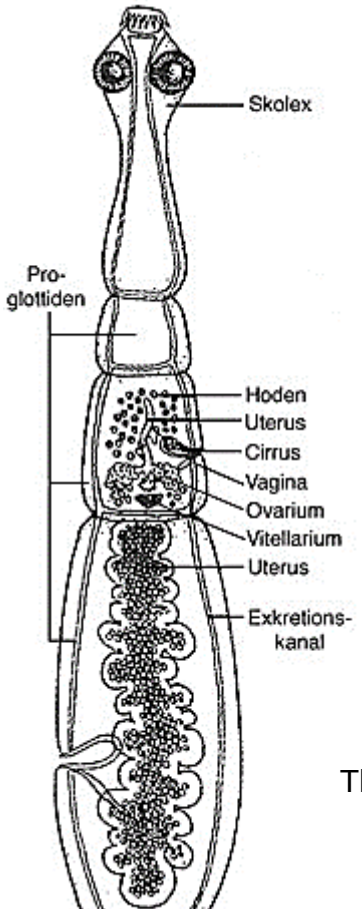
Morphology

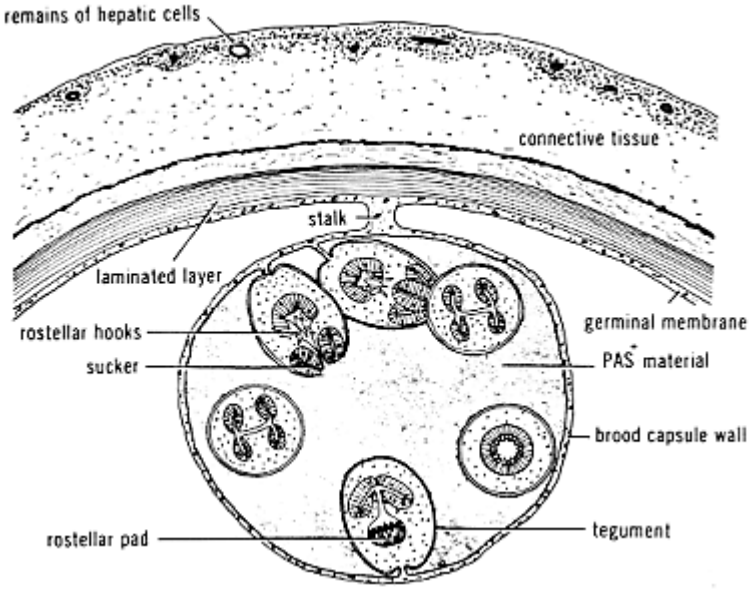
Adult worm. It is a mall tape worm and measures 2-7 mm in length; having a pyriform scolex provided with 4 suckers and armed with 28-48 hooklets; an attenuate neck; usually only 1 immature proglottid, only 1 mature proglottid, and only 1 gravid proglottid (Fig. 37). The morphology of mature proglottid and egg are similar to that of Taenia.
Egg**:** It is indistinguishable from those of *Taenia* species.
Larvae: Hydatid cyst with two layers, an outer and an inner layer, is fluid-filled and typically unilocular. The outer layer of the cystic friable, laminated, milky-opaque, non-nucleated layer; the inner layer is called germinal layer, which canbud many protoscoles, brood capsules and daughter cysts. The daughter cyst can also develop from protoscolex or brood capsule. Gradually the protoscoles, brood capsules, and daughters break down from the inner layer to hydatid fluid, which fluid is called as "hydatid sand".

Life cycle

The adult *E. granulosus* lives in the intestine of dogs and other canine hosts. Its intermediate hosts include sheep, cattle, and humans etc. Sheep is the optimum intermediate host; man is an accidental host (Fig. 38).
Ovoid eggs containing single, fully differentiated oncosphere are shed with the feces of infected definitive host. When the eggs are ingested by a suitable intermediate host, digestive processes and other factors in the host's gut cause hatching and release of activatedoncospheres. After penetration of the intestinal mucosa, oncospheres enter venous and lymphatic and are distributed passively to other anatomic sites. Most larvae develop in the liver, but some may reach the lungs, and a few develop in the kidney, spleen, central nervous system, or other organs. After 3 months, oncospheres develop hydatid cysts. If definitive hosts such as dogs eat the meat containing hydatid cysts, each protoscolex develop an adult worm in the intestine.

Figure 37. Morphology of *E. granulosus*





The cist of *E. granulosus*

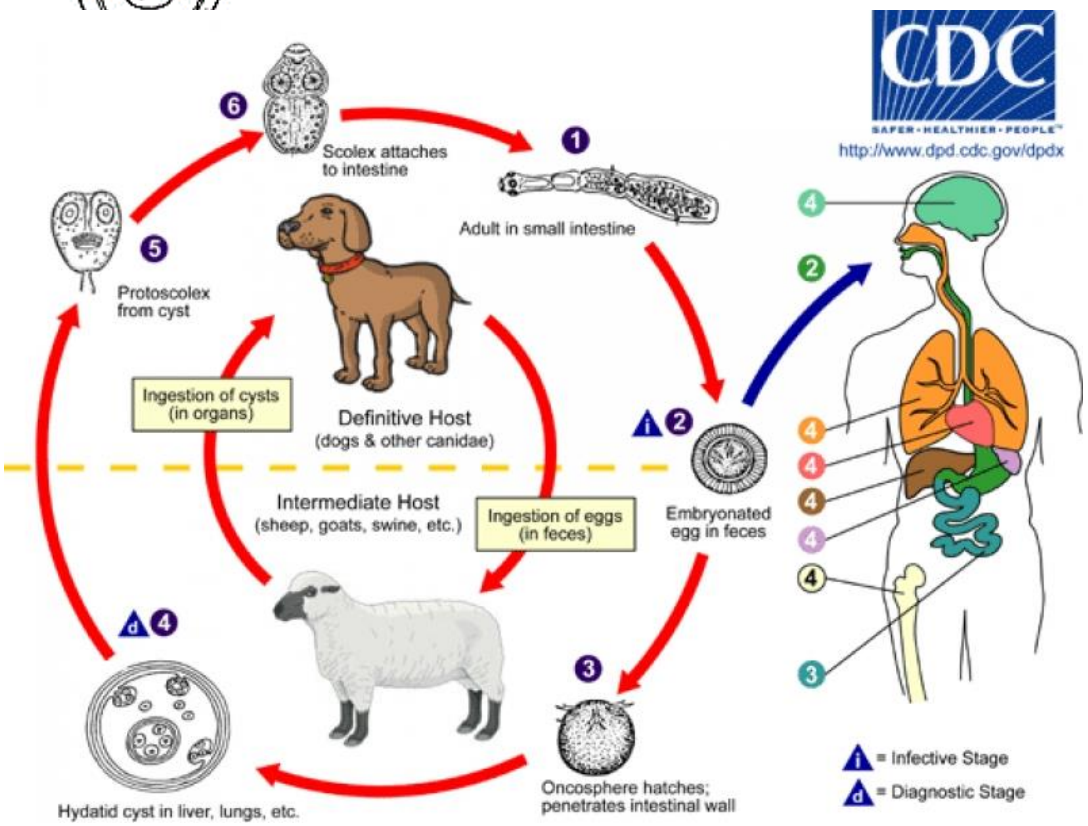


Figure 38. Life cycle of *E. granulosus* (from Parasite image library of CDC, USA)

Pathogenesis and clinical manifestation

Hydatid cyst, the larval form of the parasite, primarily is responsible for the pathogenesis of the diseases in man. In human the most frequently reported site of hydatid cysts is the liver, followed by the lungs, and less frequently, the spleen, kidneys, heart, bones, central nervous system and elsewhere. The slowly enlarging *E. granulosus* cyst is well tolerated by human hosts until it becomes large enough to cause pain or dysfunction.

The damage produced by the hydatid cysts in human body is both mechanical and toxic.

1) Physical burden or pressure causedby tremendous size of the cyst;
2) Toxicosis due to the resection of the worm;
3) Serious allergic reaction due to rupture of the cyst.

The condition remains asymptomatic through out the life in a majority of the cases. It is detected only at autopsy or when the cyst ruptures giving rise to anaphylactic reactions. The clinical manifestation of hydatid disease depends on the size, location and number of hydatid cysts. Occasionally, due to high intracystic pressure, the cyst may rupture. A ruptured hydatid cyst presents two risks:

1) First, it sets free an unusually large volume of hydatid fluid, which when partially absorbed in the circulation, bronchi, peritoneum or pleura, produce a sudden anaphylactic shock which may be fatal.
2) Secondly, this results in the formation of new secondary hydatid cysts in various parts of the body due to dissemination of scolices by the circulation.

Diagnosis

Because the clinical feature of the disease is not characteristic, laboratory diagnosis is important for the diagnosis of the hydatid disease. Of course, questioning the history of contacting with dog and sheep at endemic areas may be suggestive of the disease.

a) Parasitological examination for finding the scolices, broodcapsules or daughter cysts etc in the cystic aspirated from a surgically removed cyst. Diagnostic aspiration of intact cysts is not recommended because of the danger of anaphylactic reactions due to rupture or spillage of the cyst or its products.
b) X-ray, CT, and B ultrasound examination are frequently helpful.
c) Serological examination for detecting antibodies or Cag play on important role in establishing the diagnosis of hydatid disease. These methods include ELISA, Dot- ELISA, IHA, IFA and LAT etc.

Epidemiology

The distribution of this species is coincident with that of the reservoir intermediate hosts, especially sheep. The dog is the common definitive host and the chief reservoir of infection; the common intermediate hosts are sheep, cattle, pigs and occasionally man.

Human infection results from ingestion of the eggs, such eggs reach the mouth of man by hands, food, drink or containers contaminatedwith feces of infected dogs.

Prevention and control

Surgery still remains the mainstay of the treatment of hydatid disease. Surgical removal of the cysts is indicated for:

a) The cysts located in the operable sites such as the liver, lung, etc., and
b) The cysts which may enlarge and likely to interfere with functioning of the vital organs.

Albendazole, mebendazole and praziquantel have proved to be efficacious against hydatid cysts both in amn and animal. The preventive and control measures for hydatid disease include:

a) Regular treatment of infected dogs to reduce worm load.
b) Elimination of infected dogs.
c) Prevention of dogs from eating infected offals of domestic animals in he endemic areas.
d) Health education and strict personal hygiene.
e) Avoidance of unnecessary contact with infected dogs.

HYMENOLEPIS NANA

Kingdom: Animalia
Phylum: Platyhelminthes
Class: Cestoda
Order: Cyclophyllidea
Family: Hymenolepididae
Genus: *Hymenolepis*
Species: *nana*

It is necessary to remember

Morphology

Adult worm. The dwarf tapeworm, is the smallest cestode with an adult length of 15-44mm. Scolex is small, 0.3 mm in diameter, globular (rounded), cup-like, situated at the anterior end, has four suckers and retractile rostellum with a single row of 20–30 hooks (Fig. 39). Gravid (mature, full of eggs) proglottids are 0.2–0.3 mm long and 0.8–0.9 mm wide. Proglottid is filled with eggs, uterus is not visible. Each proglottid has both male and female reproductive organs making Hymenolepis nana hermaphroditic. A proglottid copulates with itself or with other segments of the same individual or nearby Hymenolepis nana tapeworms. Proglottids usually disintegrate in the gastrointestinal tract and are rarely present in the feces.

Egg**:** *H. nana* egg is colourless, almost transparent, oval, 30–50 μm (micrometers) in diameter, has polar filaments. When shed in stool they are immediately infective and survive up to 10 days in the external environment, they are embryonated and have a 6-hooked oncospheres inside the shells. Shell consists of two distinct membranes. On inner membrane there are two small “knobs” or poles from which 4–8 filaments arise and spread out between the two membranes.

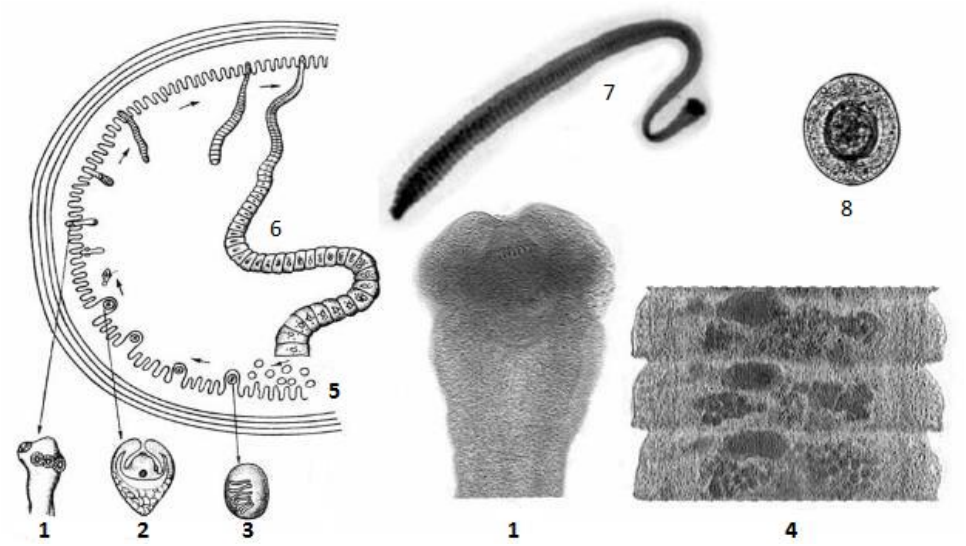


Figure 39. Morphology of H. nana.
1 – the scolex and its attachment to the intestinal wall, 2 – cistecercoid, 3 – emerged from eggs hexacanth embedded in the villi of the gut, 4 – proglotiden, 5 – the villi of the intestine, 6-7 – the body of H. nana and his egg – 8.

Life cycle

The life cycle of *H. nana* does not require an intermediate host, complete development occurring within the villi of a single host, resulting in a 'direct' life cycle (Fig. 40). It can also utilize an insect as an intermediate host.

The eggs that are released from mature proglottids in the upper ileum are usually passed out in the feces. If swallowed by another human, they develop into hexacanth oncospheres and burrow into the villi of the small intestine. This is where they develop into tailless cysticercoids and then migrate towards the ileum and attach to commence the formation of proglottids.

The eggs which are ingested by insects, such as fleas, beetles or cockroaches hatch to form tailed cysticercoids which remain unmodified as long as they are inside the insect. If they are accidentally swallowed by a human, they pass down to the ileum and establish themselves.

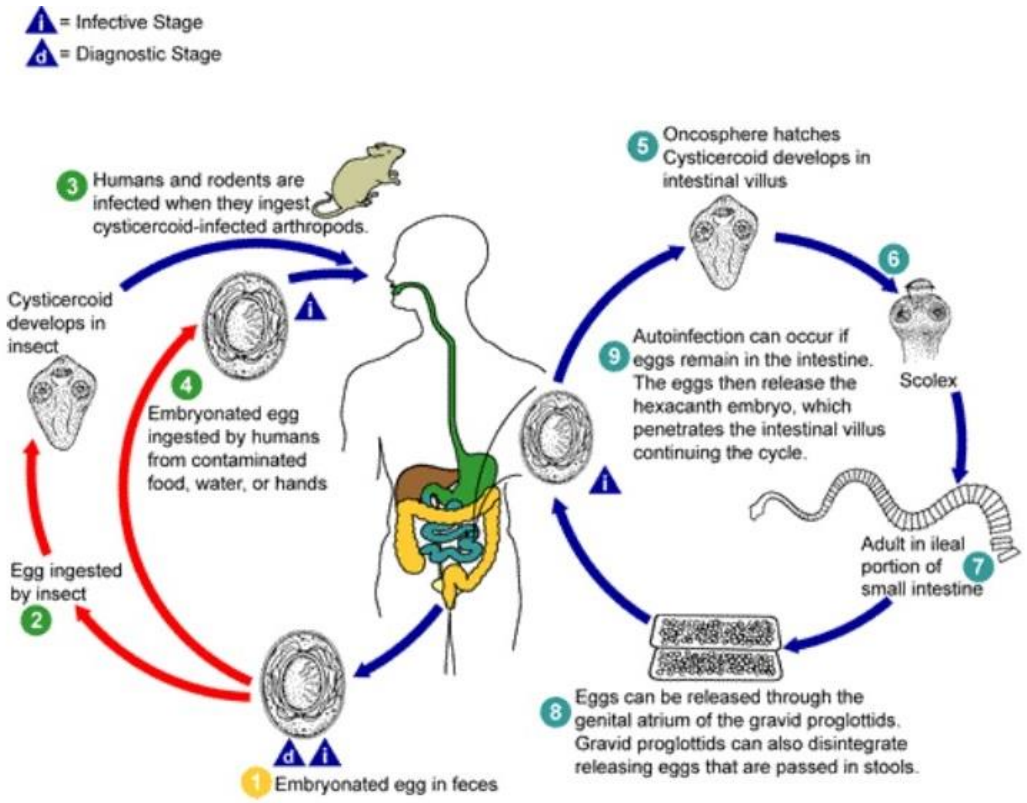


Figure 40. Life circle of *H. nana* (from Parasite image library of CDC, USA)

Pathogenesis and clinical manifestation

Pathology due to adult worms results from the physical presence and activity of the large worms (*Taenia* species), occasional erosive action (causing local inflammation) by scolex hooks (*T solium, H nana*), or reduced host intake of vitamin B12 (*Diphyllobothrium latum*). Allergic reactions may also be responsible for symptoms such as headache, dizziness, inanition, and anal and nasal pruritus.

Diagnosis

Adult Worms*: Taenia* infections are diagnosed by finding gravid segments in stool specimens; the eggs of these species are indistinguishable. Other species are diagnosed on the basis of eggs in stool specimens.

Larval Worms*:* Cysts in tissues may be identified in biopsy specimens, by radiography (calcified cysts), and by computed tomography (brain cysts). Serology (indirect hemagglutination, ELISA) is useful but of variable sensitivity and specificity. A history of travel in endemic areas is often of great importance.

Epidemiology

Infective larvae are acquired by eating contaminated raw or undercooked meat, grains, or fish. *Taenia solium* cysticercosis or *H. nana* can be transmitted in a direct cycle via ingestion of eggs from human feces. *Echinococcus* eggs from dog or fox fur cause human hydatid disease (humans are the intermediate host; canids are the definitive hosts). Reinfection with adult tapeworms is common; second infections with larvae are rare. *T solium* cysticercosis may be acquired by autoinfection; internal autoinfection with *H nana* from a cysticercoid infection is possible.

Prevention and control

Meat should be cooked thoroughly or frozen at -10°C for 10 days; beef and pork should be inspected for *Taenia* ("measly meat"); human feces should not contaminate drinking water; sheepdogs should be treated and should not be fed sheep viscera. Humans may be treated with praziquantel or niclosamide.

LESSON 12. PARASITOLOGY. WORMS. NEMATODE

The nematode belong to the Class Nematoda, which is larger population of invertebrates. It is estimated there are about 10 thousand species of the nematode. Most nematodes live in fresh-water, or sea-water, or soil freely (free living, e.g, *Caenorhabditis elegans*), a few are parasitic. Parasitic nematodes that infect humans have about 10 species, including *Ascaris lumbricoides*, hookworm, filarial and *Trichinella spiralis*.

MORPHOLOGY

Structure of the adult Nematodes are generally elongate, cylindrical, and tapered at both ends. The basic body design is a tube within a tube, the outer tube being the body wall and underlying muscles, and the inner tube the digestive tract. Between the tubes is the fluid-filled pseudocoelom，in which the reproductive system and other structures are found. Sexual dimorphism is evident: at the curved, posterior end of the male are a copulatory organ and other specialized organs, male is also usually smaller than females.

Parasitic nematodes vary widely in size according to species. Nematodes are colorless and vary from translucent to opaque when examined alive. It is not uncommon for some to absorb colored matter from surrounding host tissues or fluids.

Structure of egg Eggs of parasitic nematodes ordinarily consist of three layers enclosing an embryo that may range from a few blastomeres to a completely formed larva. Immediately following sperm penetration, the oocyte secretes a fertilization membrane, which gradually thickens to form the chitinous shell/chitinous layer. The inner membrane, the lipid layer/ascaroside, is formed by the zygote.

A) Embryo member: consist of lipid protein;

B) Chitonous layer: consist of chitonous and protein, and process the function of resisting the mechanic pression;

C) Lipid layer/ascaroside: consist of lipid protein and ascaroside, and process the function of regulating.

LIFE CYCLE

The basic processof development include egg, larva and adult.

Eggs of parasitic nematodes may hatch either within the host or in the external environment.

Under the suitable stimuli conditions, the hatching of some nematodes' eggs, e.g. hookworm eggs, can occur in the external environment, and a first-stage larva usually emerges. The process is controlled partly by the maturity of the larva and partly by ambient factors such as temperature, moisture, and oxygen tension. The eggs of nematodes hatch only after ingestion by a host may be related to carbon dioxide tension, salts, pH, or temperature. These conditions stimulate the enclosed larva to secrete enzymes that partially digest the enveloping membranes, such as Ascaris. A few nematodes lay larva directly in host, which larva should parasitize in intermediate host for developing to infestive-stage larva, e.g. filarial. Nematodes undergo four molts; the sequence of events is controlled by exsheathing fliud secreted by the larva. This fluid digests the cuticle at specific sites on the inner surface, causing it to

loosen. The larva's ability to form a new cuticle in the hypodermis before shedding the old one allows the nematode to develop continuously between molts; however, growth occurs most rapidly just after molting. Larval stages in the life cycle of parasitic nematodes are generally referred to as first, second, third, and fourth-stage., named Rhabditiforum larva filariform larva or microfilaria. The first stage larva of parasitic nematodes such as hookworms are called rhabditiform larvae. After molting twice, the rhabditiform larvae of hookworms become third stage or filariform larvae. The prelarvae or advanced embryos of filarial nematodes such as *Wuchereria bancrofti* are known as microfilariae. This larva, generally found in circulating blood.

The adult worm of nematodes parasitizes in digestive tract such as intestinal or blood and tissue of the host. The worms that reside in intestinal are called intestinal nematodes, the worms reside in blood or tissue are called blood and tissue nematodes.

Some parasitic nematodes have simple life cycle, consisting of egg, larva, and adult worm, these nematodes are considered as direct development type of nematodes or soil-transmission nematodes, such as hookworm. Some parasitic nematodes need intermediate host to complete the life cycle, these nematodes are called vector-transmission nematodes or bio-source nematodes, such as filaria.

PHYSIOLOGY AND PATHOGENESIS

Physiology. Parasitic nematodes derive much of their energy from the metabolism of glycogen. Most larva of nematodes derive their energy from the metabolism of lipid.

Pathogenesis Parasitic nematodes cause the damage to humans by mechanical disruption and toxicity. The damage related with species of nematodes, worm burden, development stage, parasitic site, and physiological condition or immunological response of host. Penetrating and migrating of infective stage larva can cause dermatitis or local inflammatory response. The adult worm causes the pathological changes, which related with the parasitic site, these changes may include erosion, bleeding, inflammatory and proliferation of tissue.

CLASSIFICATION OF PARASITIC NEMATODES

Common human parasitic nematodes belong to Class Nematoda, including *Ascris lumbricoides, Enterobius vermicularis*，*Trichuris trichura*， *Ancylostoma duodenale*， *Necator americanus*，*Wuchereria bancrofti*，*Brugia malayi*，*Trichinella spirialis.*

ASCARIS LUMBRICOIDES

Kingdom: Animalia
Phylum: Nematoda
Class: Rhabditea
Order: Ascaridida
Family: Ascarididae
Genus: *Ascaris*
Species: *lumbricoides*

It is necessary to remember

Ascaris lumbricoides is one of most common human parasites, which adult worm parasitize in the intestinal tract of human, and cause Ascariasis.

Morphology

In *Ascris lumbricoides*, known as the large intestinal round-worm of humans, females may attain a length of 40 cm while male worms may reach 20～35 cm. In both sexes, the mouth is surrounded by one dorsal and two ventrolateral lips (Fig. 41). The posterior end of the female is straight while that of the male curves ventrally. The females are a prodigious egg producer, depositing about 200,000 eggs daily; the uterus may contain up to 27 million eggs at a time.

The fertilized egg measures 45～75 × 35～50μm, there are three layers in the shell and one

embryo cell in the egg. Some time the protein membrane may be found outside of egg shell. The shell is relatively thin, hyaline and transparent. The embryonated eggs are infective to human. Unfertilized egg measures 88～94 × 39～44μm, there is no ascaroside in the shell and embryo cell in unfertilized egg.

Life cycle

The life cycle of Ascris consist of two parts, one is eggs development in the soil, another adult worms inhabit humans body.

Adult worms inhabit the lumen of the small intestine and draw nourishment from the semidigested food of the host. Copulation occurs at this site, and eggs are passed with host feces. The outer, albuminous coat of the fertilized egg is golden brown due to bile pigment adsorbed from feces. Among the oval, fertilized eggs are found numerous unfertilized eggs, identifiable by their elongated shape and the absence of albuminous coat. When fertilized eggs are deposited, the zygote is uncleved, and it remains in this state until the egg reach soil. Eggs deposited in soil are resistance to desiccation but are very sensitive to environmental temperature at this stage of development. The zygote within the eggshell develops at a soil temperature of about 21～30℃. Development ceases at temperatures below 15.5℃, and eggs cannot survive at temperatures more than slightly above 38 ℃℃.

After 2-4weeks in moist soil at optimal temperatures and oxygen levels, the embryo molts at least once in the shell and develops to an infective secound-stage larva. Eggs containing infective larvae may remain viable in the soil for two years or longer.

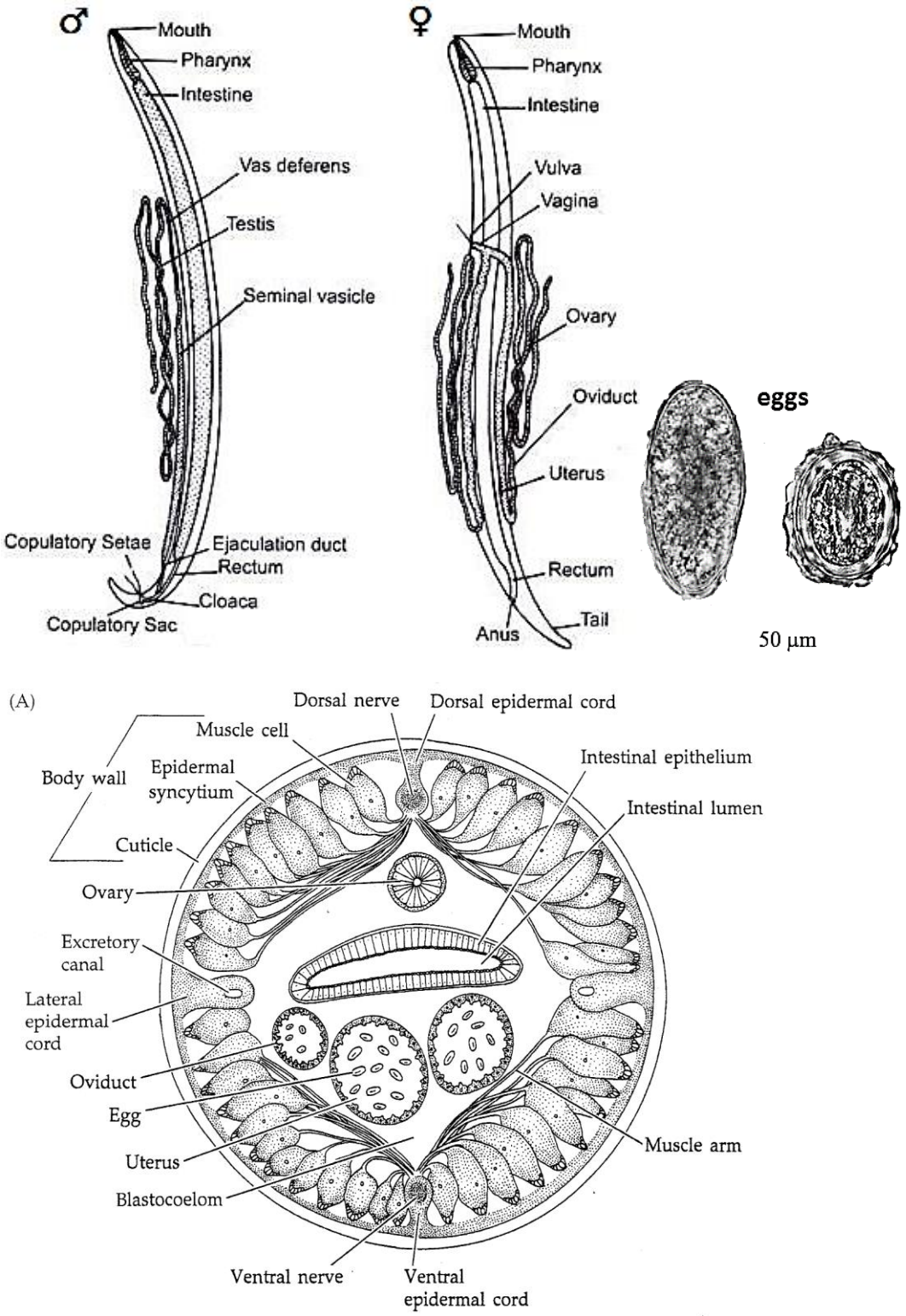


Figure 41. The morphology of *Ascris lumbricoides.* (A) Cross section of female *Ascris lumbricoides.*

After being ingested by a human, eggs containing infective larvae hatch in the duodenum. The larvae actively burrow into the mucosal burrow into the mucosal lining, enter the circulatory system, and are carried via the portal circulation to the liver, through the right side of the heart, and to lungs by the pulmonary

artery. This migration requires approximately one week. The larvae remain in the lungs for several days, molting twice, and eventually rupture from the pulmonary capillaries to enter the alveoli. From there, the four-stage larva move up the respiratory tree and trachea to the epiglottis to be coughed up, swallowed, and passed again to the small intestine. During this complex migratory process, individual worms grow from 200-300μm in the small intestine is essential to the worms' survival, and those worms that undergo this molt develop to sexual maturity. The interval from the ingestion of infective eggs to the appearance of sexually mature worms in the small intestine is about 60～75 days.

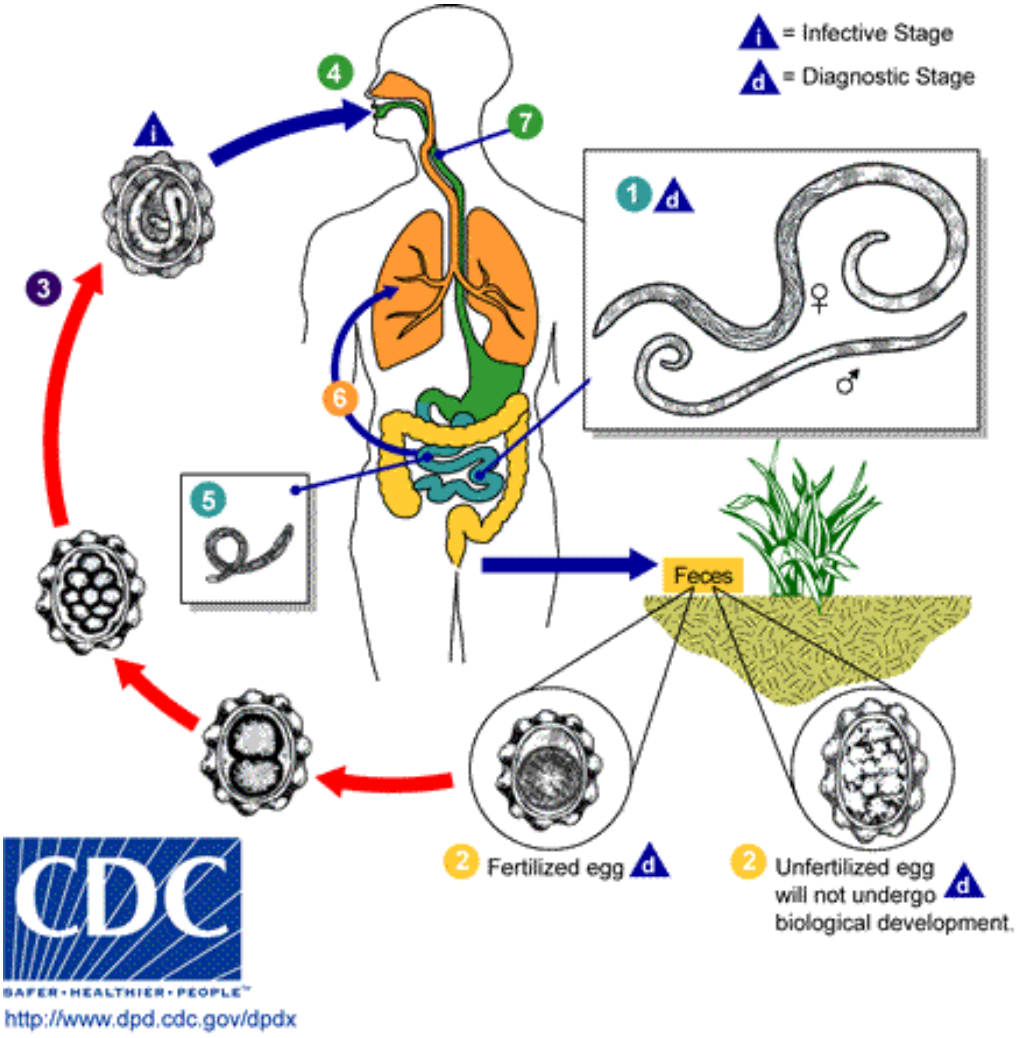


Figure 42. Life cycle of Ascaria lumbricoides (from Parasite image library of CDC, USA)

Pathogenesis and clinical manifestation

Both adult worms and larva can cause pathological changes of humans by mechanical disruption and toxicity.

Migrating larva. Minute hemorrhages occur at the penetration sites of the larvae through the intestinal wall and into the alveoli of the lungs. During the passage through the liver and lungs, the larvae may be immobilized, covered with eosinophile, enveloped in eosinophilic granulomas.

Especially in lungs, the pathological changes may be more significant. Larvae from large numbers of infective eggs, or repeated ingestion of eggs, produce pathologic changes in the lungs characterized by a lobular pneumonitis.
Local reactions are usually accompanied by general hypersensitivity reactions such as bronchial asthma, transient eosinophilic pulmonary infiltrates (Loeffer's syndrome). Angioneurotic edema, and urticarial.
Adult worm. The adult worms can cause no pathology in the small intestine. If, however, they are present in sufficient numbers, they can cause below damage to humans.
1) ***Intaking nutrients and negatively affect the absorption.*** Because adult worms of Ascaris not only take food from the digested food in the intestine of host but also produce the metabolic toxicity, the presence interferes with the digestion and absorption protein, fat, carbohydrate ， vitamin, and cause the poor nutritional status, especially in children with lower nutritional intake.
Clinical symptoms include anepithymia, nausea, vomiting, vague abdominal pains.
2) ***Allergy*** The Ascaris allergen is one of the most potent allergens of parasitic origin. An increase in circulating IgE globulins in response to Ascaris infection is common, but only a small number of IgE globulines have antibodies specific for Ascaris. Exposure to Ascaris allergen may cause hypersensitivity reactions in lungs, skin, conjunctiva, and intestinal mucosa. The most common skin change is urticaria, itchand Angioneurotic edema.
3) ***Complication of Ascariasis*** The adult Ascaris worm is a relatively common cause of severe complications due to its characteristically large size and aggregating and/or migratory activities. The migratration of adult Ascaris may be promoted by some drugs, including some antihelminthics and those used for anesthesia, but also by fever and peppery food.
Large numbers of adult worms sometimes cause mechanical blockage of the intestine, which produces partial or complete obstruction. The usual site of obstruction is the ileocecal region. The symptoms usually start suddenly with vomiting and colicky, recurring abdominal pain; intestinal perforation are less common. Among the most common signs are abdominal distension and tenderness, abnormal abdominal sounds and X-ray evidence of intestinal obstruction.
Ascaris worms can invade the bile duct, pancreatic duct, appendix etc, and cause biliary or hepatic, pancreatic, and appendix ascariasis or ascariasis granulomas, which occurs most frequently in children. Among the most common complication is biliary ascariasis. The symptoms usually include right upper abdominal pain, which is characterized by a sudden onset, and a very strong intensity. Vomiting with bile-stained gastric contents frequently coexists with the pain. A typical sign is pain at the pressure point just below the xiphoid process. Serious case may occur biliary necrosis or perforation.

Diagnosis
Diagnosis is made by identification of eggs in feces. Because egg production per female is fairly constant, egg counts can provide reasonably accurate estimates of the number of adult worms present, provided uniform samples are used.

Epidemiology

Distribution of *A. lumbricoides* is worldwide, but it is most prevalent in the areas with warmer climates, moister and poor sanitation. The infection in the population of rural areas is higher of c ity, children higher adult.

An estimated 531 million people are infected, making it the most common nematode parasitizing humans.

The patients who passing fertilized eggs are the infective source. The fertilized egg can develop to infective-stage eggs in soil without intermediate host. Because per female of Ascria produces large number of eggs and the eggs process the strong ability to resist environmental conditions, humans are easy to expose to the eggs. This is main reason why the infection of Ascria is one of the most common parasitic diseases.

It is common ways to contaminate soil, and vegetables that human feces is used as fertilizer or children excrete feces freely. Hand to mouth is common transmission way.

Of course, the prevalence of Ascria is considered to be very closed relation with the social- economic condition, a mode of production, education level and sanitation etc.

Prevention and control

For treatment of individuals in whom adult worms have been vein the intestine but who do not require hospitalization, a single dose of pyrantel pamoate and Mebendazole are highly effective. The case with complication should be sent to hospital. The preventive and control measuresinclude:

a) Treatment of infected children and other members of the family. For optimal effectivness, such a program should be combined with treatment of the population with broad-spectrum antihelmintics two or three times annually.

b) Improved personal hygiene and cleanliness such as cutting the nails short, washing the hands before eating, washing the bed linens and night dress daily, and

c) Avoidance of putting the fingers in the mouth.

TRICHURIS TRICHIURA (Trichocephalus trichiurus)

Kingdom: Animalia
Phylum: Nematoda
Class: Enoplea
Order: Trichocephalida
Family: Trichuridae
Genus: *Trichuris*
Species: *trichiura*

It is necessary to remember

Trichuris trichura, called whipworm also, is one of most common human parasites. Human infection with *T. trichiura* cause Trichuriasis. The condition is an intestinal infection caused by invasion of the mucosa of the colon by the adult worm. T. trichiura was first described Linnaeus in the year 1771.

Morphology

Adult worm Adult worms characteristically are whip-shaped, the anterior three-fifth being long, thin and hair-like and the posterior one-to-two fifth being short, thick and stout (Fig. 43). Males are slightly smaller than females, the latter measuring 35～50 mm in length. In both sexes, a capillary- like esophagus extends two-thirds of the body length and is encircled along much of its length by a series of unicellular glands. The cells can excrete some enzymes which possess antigenicity.

Egg. The egg is typically barrel-shaped with two polar plugs. These are yellowish brown and double shelled. The eggs measure 50～54×22～23μm. The eggs contain an unsegmented ovum each, when passed in the faeces. These freshly passed eggs are not infective to humans.

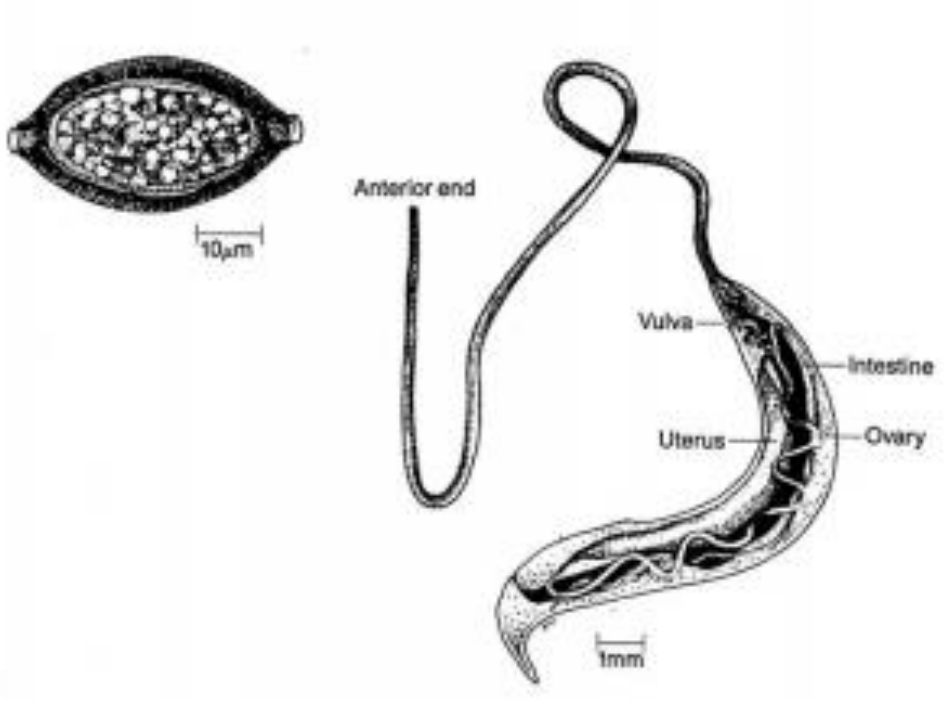


Figure 43. Morphology of *Trichuris trichiura* and his egg (from Parasite image library of CDC, USA)

Life cycle

The life cycle of *T. trichiura* is simple, complete in a single host, the man (Fig. 44). The change of host is needed for the continuation of species.

Adult whipworms occur primarily in the human host's colon but also inhabit the appendix and rectum. The female deposits up to 1000 ~7000 eggs daily; after passing to the exterior in feces, the eggs develop slowly in warm, damp/moist soil. An unhatched, infective, third-stage larva develops in three to five weeks. New human hosts become infected when these embryonated eggs are ingested with contaminated food or water or from fingers. The larvae hatch in the upper portions of small intestine and quickly burrow into the cells of the intestinal villi, where they mature, undergoing two molts in about 3-10 days. Subsequently, they migrate to the caecal region and develop to sexual maturity in 30-90 days from the time the eggs were ingested. Adult worms embed the long, slender, anterior ends of their bodies deeply into the colon submucosa. While these worms normally survive approximately 3-5 years in the human host.

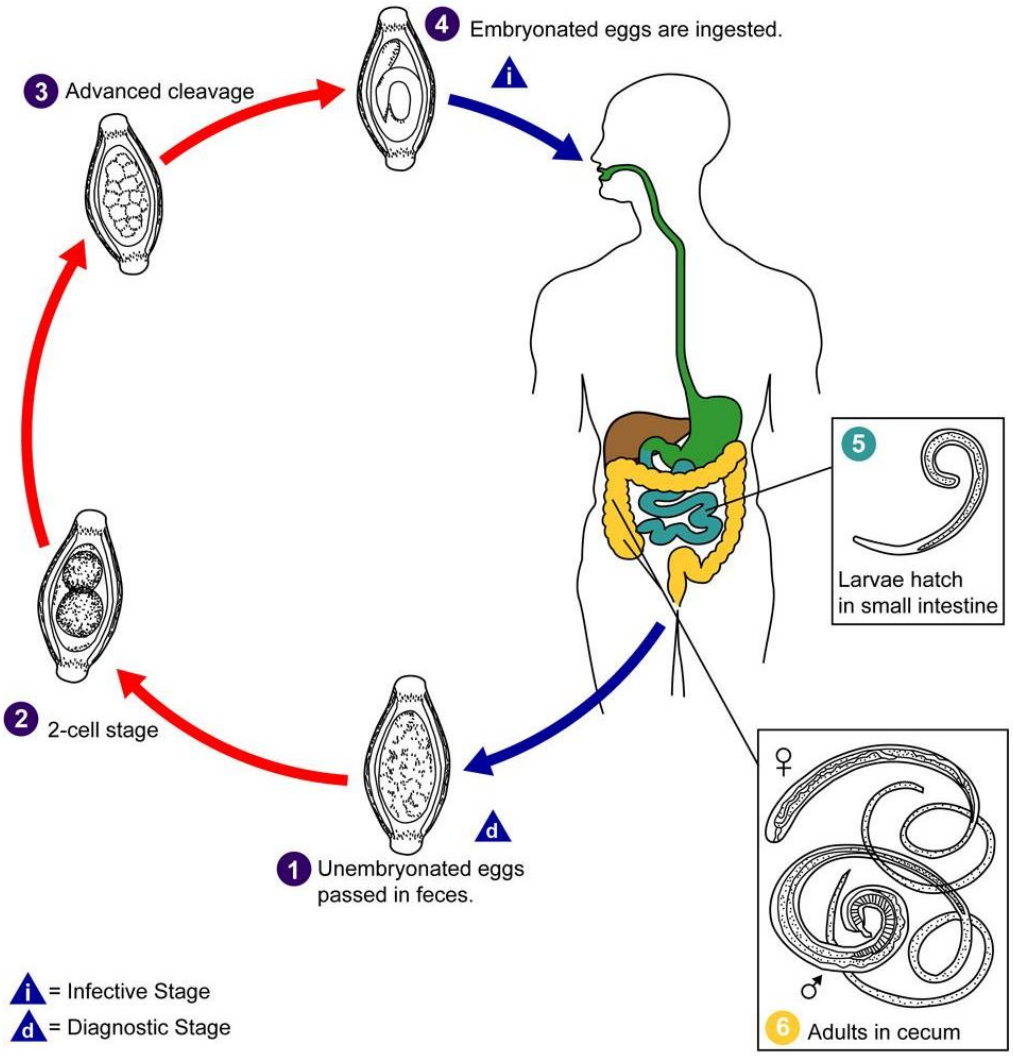


Figure 44. Life cycle of *Trichuris trichiura* (from Parasite image library of CDC, USA)

Pathogenesis and clinical manifestation

The major pathology resembles that of inflammatory bowel disease due to mechanical disruption and toxicity of whipworms. The pathological changes include hyperemia edema or hemorrhage/bleeding. In few cases, there are cellular proliferationand thickness of the intestinal wall causing by inflammatory and granulomas.

Most infections are light with no clinical symptoms. Chronic infections, however, produce symptoms such as bloody stools/chronic diarrhea, pain in the abdomen, weight loss, rectal

prolapse, anemia. It was reported that 73% of persons infected with whipworm were identified to be the cases of chronic colonitis by fibrescopy.

Diagnosis

The clinical manifestation is not specific, so identification of eggs in fecal material constitutes diagnosis. It is based on the demonstration of the characteristic barrel-shaped eggs in the faeces by light microscopy.

Epidemiology

Whipworm infection occurs worldwide, most frequently in tropical countries.

Warm climate, moist, dense shade, sufficient oxygen in soil are the environmental conditions for egg development. So the prevalence in south part is higher than in north part of China.

The species *T. trichiura* is almost exclusively a human parasite, with rare records of occurrence in other primates. Hand to mouth is major way to acquire infection.

Prevention and control

Mebendazole or albendazole, the drugs of choice, are most effective when administered orally for three consecutive days.

The control measures are similar to that measures for Ascria control, e.g. health education and sanitation.

ENTEROBIUS VERMICULARIS

Kingdom: Animalia
Phylum: Nematoda
Class: Rhabditea
Order: Oxyurata
Family: Oxyuridae
Genus: *Enterobius*
Species: *vermicularis*

It is necessary to remember

This nematode *Enterobius vermicularis,* commonly known as pinworm or seatworm, is parasitic only to humans. It is familiar to parents of young children worldwide. The infection of *E. vermicularis* may cause Enterobiasis. Leuckart was the first to describe the complete life cycle of the parasite.

Morphology

Adult worm. The adult worms are small, white, spindle-shaped and thread-like (Fig. 45). True buccal capsule is absent. Female pinworms, measuring 8-13 mm by 0.3-0.5mm, are characterized by the presence of winglike expansions of body wall at the anterior end, distension of the body due to the large number of eggs in the uteri, and a pointed tail. Males are 2-5mm long and posses a curved tail.

Egg. The eggs are ovoid but asymmetrically flattened on one side, measuring 50～60× 20 ～ 30 μm; a colorless, thick shell covers the larva. The embryonated eggs are infective to humans.

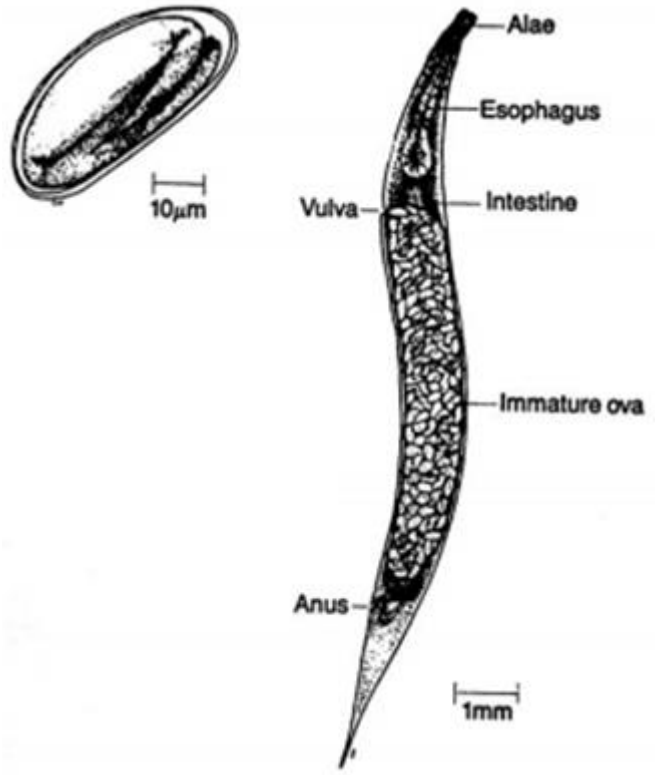


Figure 45. Morphology of *E. vermicularis*

Life cycle

Life cycle of *E. vermicularis* is simple and is completed in a single host (Fig. 46). Man is the natural host. No intermediate.

Sexually mature worms usually inhabit the human intestinal tract, but they can spend to adjacent regions of the small and large intestines (blindgut, cecum, appendix, colon, rectum or below portion of ileocaecal). Adhering to the mucosa, the worms feed on bacterial and epithelial cells. Males die following copulation, while egg-bearing females, with up to 15,000 eggs in their uteri, migrate to the perianal and perineal regions. There, stimulated by the lower temperature and aerobic environment, they deposit their eggs and then also die. More eggs are released when the female 's body ruptures.

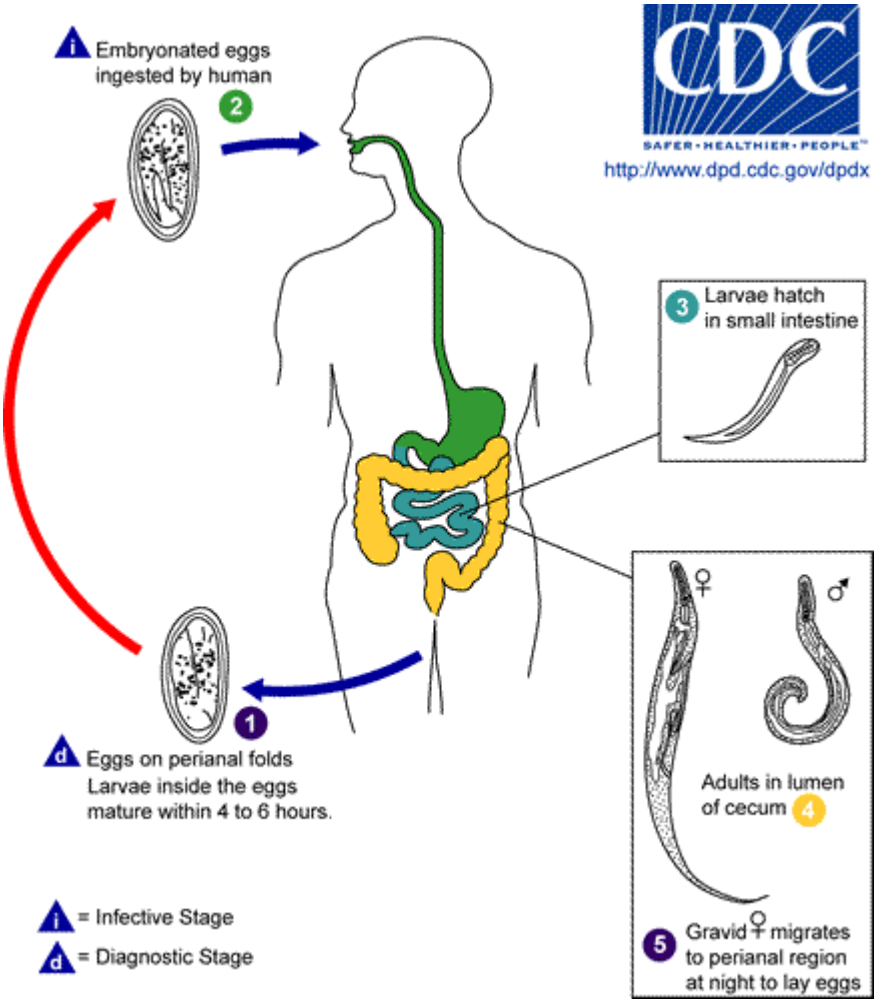


Figure 46. Life cycle of *E. vermicularis* (from Parasite image library of CDC, USA)

Upon deposition, each contains an immature larva. The infective, third-stage larva completes development within the egg several hours after leaving the body of the female worm.

Infection and reinfection occur when eggs containing the infective larvae are ingested by the host. This may happen when eggs are picked up on the hands from bed-clothes or beneath fingernails contaminated when the host scatches

the perianal zone to relieve itching caused by noctural migration of the female worms. However, the lightweight eggs are sometimes airborne and, therefore, can also be inhaled. Retroinfections occur when third – stage larvae hatch from perianally located eggs and enter the host's intestinal tract through the anus.
Ingested eggs usually hatch shortly after reaching the duodenum. The escaping larvae molt and develop as they migrate posteriorly, reaching sexually maturity by the time they arrive at the colon. The life cycle of *E. vermicularis* spans 2-6 months. The females survive 2 months in host.

Pathogenesis and clinical manifestation
Pinworm are not highly pathogenic as the parasite causes little mechanical injury to the colonic mu and the toxemic or allergic action is disputable. Most of the evident pathological changes due to itching and irritation caused by the migration of gravid females around the perianal, perineal, and vaginal areas. Enterobiasis is usually asymptomatic. Heavy infections in children may also produce such symptoms as sleeplessness, weight loss, hyperactivity, grinding of teeth, abdominal pain, and vomiting.
Gravid females may also migrate up the female reproductive tract, become trapped in the tissues, and cause vaginitis, endometritis and granulomata in the uterus and fallopian tubes. They may also migrate to the appendix, the peritoneal cavity, or even the urinary bladder.

Diagnosis
The history of *pruritus ani* and demonstration of small white thread-like worms in the undergarments is suggestive of *E. vermicularis* infection in Children.
Female worms emerge at night and are frequently visible observed on feces as well; however, eggs are found in feces in only about 5% of cases.
The most reliable procedure for finding eggs is to apply a strip of cellophane tape to the perianal skin, remove the tape, and place it on a clean microscope slide for examination. Negative results from this protocol for seven consecutive days constitute confirmation that the patient is free of infection.

Epidemiology
E. vermicularis is one of the most common human parasites. Children, especially of early school-age, are most vulnerable to pinworm infection. The geographic distribution of the worm is global. In Alaskan of USA a 51% prevalence in children was displayed.
Humans is only host of pinworm. Infections occur in one of four ways: retroinfection, when hatched larvae migrate back into the large intestine; self-infection, when the patient is reinfected by hand-to-mouth transmission; cross-infection, when infective eggs are ingested, either with contaminated food or from fingers that have been in contact with contaminated surface or body parts from infected humans; and inhalation of airborn eggs. In household with heavily infected individuals, infective eggs have been found in samples of dust taken from chairs, tabletops, dresser tops, floors, baseboards, etc. In a survey to determine the distribution of airborn pollen in public places, pinworm eggs were found in theaters, not only on arm rests and baseboards but also on chandeliers high above the seats; Experiments show that at room temperature, eggs survive about 3 weeks.

Prevention and control

Following positive diagnosis in any individual, treatment should be administered to all members of the household. Pyrantel pamoate or mebendazole, usually administered in a single dose and repeated once after 2 weeks, is the treatment of choice.

Complete eradication of pinworm infection from a population is highly unlikely, so personal hygiene combined with chemotherapy is the most effective deterrent.

LESSON 13. PARASITOLOGY. WORMS. NEMATODE

ANCYLOSTOMA DUODENALE AND NECATOR AMERICANUS

Kingdom: Animalia
Phylum: Nematoda
Class: Rhabditea
Order: Strongylida
Family: Ancylostomatidae
Genus Species: *Ancylostoma duodenale*
Genus Species: *Necator americanus*

It is necessary to remember

The hookworms parasitizing humans include *Ancylostoma duodenale* and *Necator americanus.* Because these worms are similar in morphology and life cycle, they will be described together, with notations on dissimilarities.

Morphology

Adult worm. The worms are cylindrical, grayish white and slightly curved (Fig. 47). The anterior end of the worm is bent slightly, in the same direction of the body curve and gives in its name “hook worm”. Adults of *A. duodenale* are somewhat larger than those of *N. americanus*. Female adults measure about 1 cm long. The posterior end of the male has an umbrella-shaped bursa (copulatory bursa), with riblike rays. The mouth or buccal capsule of *A. duodenale* has two pairs of curved teeth on the ventral wall of its buccal capsule, *N. americanus* has a conspicuous pair of semilunar cutting plates on the dorsal wall.

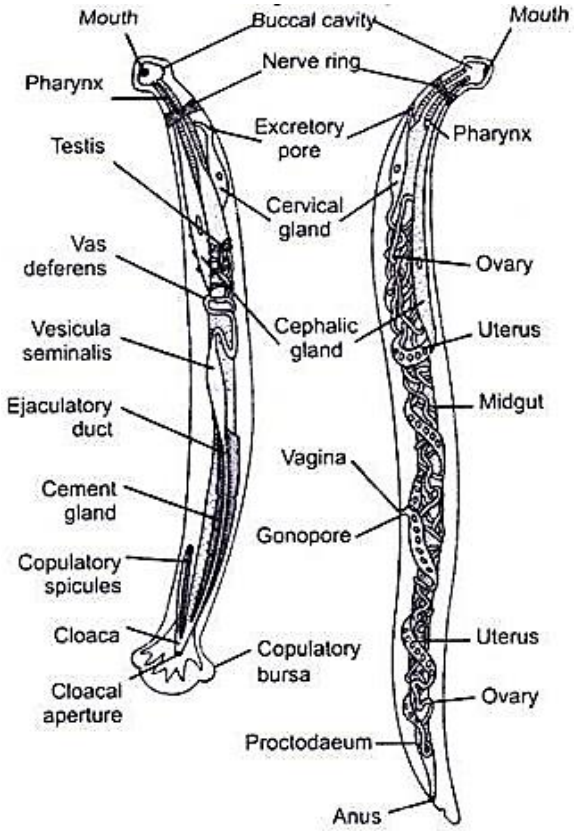
Egg. The eggs are oval thin–shelled and colourless. These are surrounded by a thin transparent membrane. The eggs usually contain two or four blastomere in faeces. When passed in the faeces, these eggs are not infective to man and a clear space is always present between the segmented ovum and the egg shell.

Infective form. Third stage larva is the infective form. It is slender and measures 0.5～0.7 ×0.025 mm. The mouth is closed, oesophagus is present in the anterior third of the body. The tail is pointed.

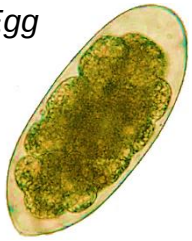
Life cycle

Life cycle is completed in a single host. Man is the only host. No intermediate host is needed. Eggs are passed in the stool, and under favorable conditions (moisture, warmth, shade), larvae hatch in 1 to 2 days. The released rhabditiform larvae grow in the feces and/or the soil, and after 5 to 10 days they become become filariform larvae that are infective.

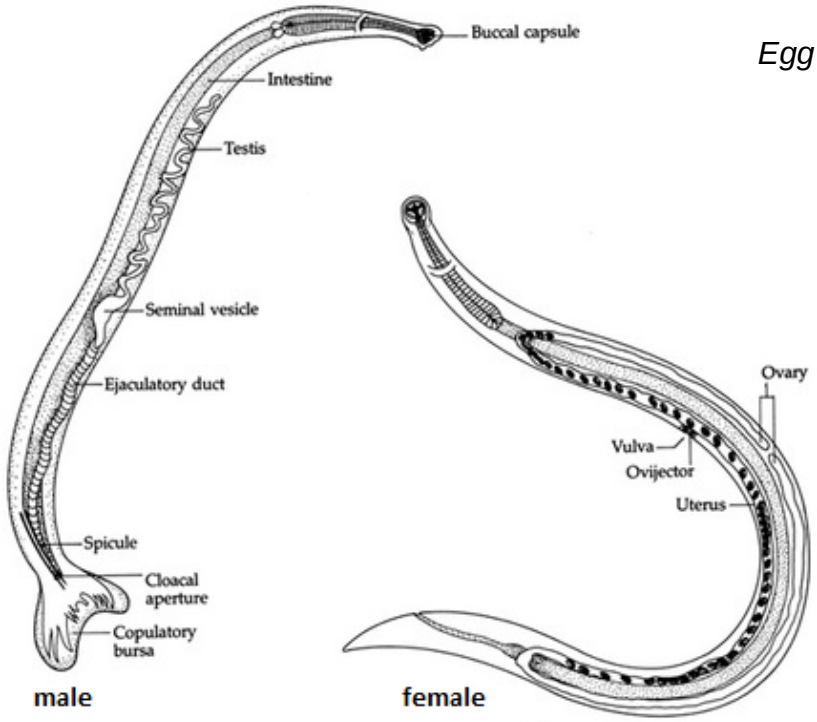
These infective larvae can survive 3 to 4 weeks in favorable environmental conditions. On contact with the human host, the larvae penetrate the skin and are carried through the veins to the heart and then to the lungs. They penetrate into the pulmonary alveoli, ascend the bronchial tree to the pharynx, and are swallowed. The larvae reach the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, where they attach to the intestinal wall with resultant blood loss by the host. Most adult worms are eliminated in 1 to 2 years, but longevity records can reach



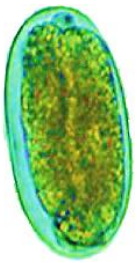
Egg



Ancylostoma duodenale



Egg



Necator americanus

Figure 47. The morphology of *Ancylostoma duodenale* and *Necator americanus* several years. Some *A. duodenale* larvae, following penetration of the host skin, can become dormant (in the intestine or muscle). In addition, infection by *A. duodenale* may probably also occur by the oral and transmammary route. *N. americanus,* however, requires a transpulmonary migration phase.

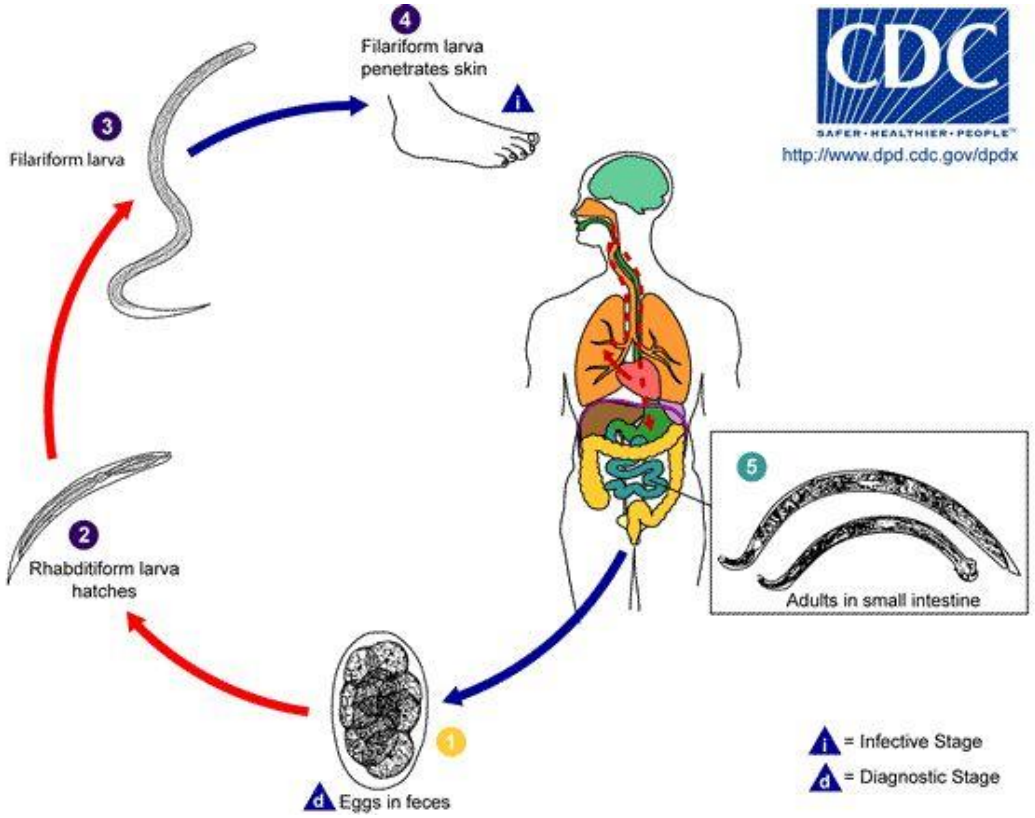


Figure 48. Life cycle of hookworm (from Parasite image library of CDC, USA)

Pathogenesis and clinical manifestation

The infection with *A. duodenale* is more serious than that caused by *N. americanus*. Pathogenic changes in hookworm infection is the adult worms and less frequently, by the infective larvae.

By adult worm The major pathological changes are caused by the attachment of the adults worms in the small intestine by their buccal capsules. These worms cause considerable loss of blood and tissue fluids, during their feeding on the intestinal mucosa. One *A. duodenale* adult worm is responsible for loss of 0.15 to 0.26 ml blood per day. One *N. americanus* adult worm is responsible for loss of 0.02 to 0.10 ml blood per day. The blood loss is caused by:

a) Ingestion of the blood by the worm.
b) Seepageof the blood around the site of attachment of the worm.
c) Oozing of the blood from the burrowed site previously attached by the worm, and
d) Anticoagulants secreted by the buccal capsule of the worm, which prevent clotting of the blood at the wound site.

Excessive blood loss caused by heavy and prolonged worm infection leads to *hypochromic microcytic* anaemia. The anaemia can frequently become serious and even fatal in the persons with low iron intake and low level of inor absorption. Loss of protein leads to hypoproteinemia and oedema. The early phase manifests as low-grade fever, anaemia, nausea, vomiting, diarrhoea and abdominal discomfort. Iron-deficiency anaemia and hypoalbuminaemia are major clinical manifestation. Development of anaemia depends on the

worm load of the intestine and nutritional status of the host. Infection in children is associated with desire to eat the soil and other unusual substances.
By larva The infective filariform larvae at the site of the penetration of the skin produce a local reaction called ground itch, frequently complicated by secondary bacterial infections. The migration of a large number of larvae, through the lung, produce minute haemorrhage and infiltration of leucocytes resulting the entrapment of the larvae in lung tissues. Both eosinophilia and leucocytosis occur at this stage.
Ground-itch is important manifestation in skin phase. In lung phase, fever, cough, dyspnoea, pharyngitisand occasionally, haemoptysisare the important symptoms.

Diagnosis
Eosinophilic leucocytosis and hypochromic microcytic anaemia may be suggestive of the condition in the endemic areas.
Laboratory diagnosis. It includes parasitic diagnosis and immunodiagnosis. Parasitic diagnosis is made by demonstration of the hookworm eggs in the faeces by microscopy and concentration.
1) Microscopy Direct microscopic examination of faeces is adequate to detect moderate or serve infections.
2) Concentration Concentration of stool by formalin-ether or simple salt floatation stool is essential to detect light hookworm infection.
3) Third-stage larvae in the faecal culture

Epidemiology
Distribution. Hookworm diseases is widely epidemic parasitic disease in the world. Hookworm distribute these areas between northern latitude 45℃ to southern latitude 30℃. *A. duodenale* is chiefly found in tropic areas and subtropic areas, *N. americanus* is commonly found in warm zone. In the endemic areas, mix infection with both hookworms can be found, but the infection with single species hookworm is most common. According to the report from 1988-1992 national survey, in our country, the cases with hookworm infection were detected except for Bejing, Jilin Heilongjiang, Qinghai etc, the average infection rate is 17.16%. In Hainan, the infection rate was upto 60.89%, which was the highest in the prevalence.
Reservoir, source and transmission of infection Human faeces is the only source of infection. Human is the only reservoir of infection. Non-human mammalian reservoir is absent. The infection route is the infective filariform larvae penetrate the skin. Persons walking barefoot are infected while they work in the area contaminated with the faeces containing eggs which hatch out to the filariform larvae.
The transmission of hookworm is correlated natural environment crop planting, ways to production and life conditions etc. The hookworm infection is common in the warm, tropical areas where people defecate indiscriminately in the open ground or use faeces as fertilizer directly. The hookworm infection is more prevalent in the rural areas especially in farmer in tea garden, vegetable garden. It was reported that the infection rate in miners is higher in some mine district.

Prevention and control

Treatment of hookworm infection consists of a) treatment of worm infection by anthelmintics such as mebendazole; b) treatment of anaemia.

The most important control measuresconsist of reducing the contamination of the soil by

1) Sanitary disposal of human faeces;
2) Treatment of infected persons;
3) Health education with improved use of sanitary latr ines and use of foot wears.

WUCHERERIA BANCROFTI (FILARIA)

Kingdom: Animalia
Phylum: Nematoda
Class: Secernentea
Order: Spirurida
Suborder: Spirurina
Family: Onchocercidae
Genus: *Wuchereria*
Species: *W. bancrofti*

It is necessary to remember

Filaria is one species of nematoda. Adult worm resides in lymph nodes and adjacent lymphatics or in the subcutaneous tissue. Female worm produce microfilaria, which belong to viviparous. The microfilariae migrate into lymph and blood stream. When insect's bits' infection person with microfilaria, the microfilaria invades the insect such as mosquitoes, and develop to the infective larval stage. The infective larvae enter human through skin while biting, and then become adult worm slowly. Though eight filaria l parasites commonly infect humans, two species account for most of the pathology associated with these infections in China. They are filariae *Wuchereria bancrofti* and *Brugia malayi*. *W. Bancrofti* is one of the most common human filariae, which worm was found in the lymph nodes and lymphatic channels of humans in 1876. *B. malayi* is only endemic in Asia, the worm was found in human in 1940. They cause lymphatic filariasis.

Morphology

Adult worm. Both of filariae are similar, such as milk white, threadlike with smooth surfaces, less 1.0 mm long (Fig. 49). Their mouth with papillae is located at the top of the head. On the ventrally curved tail of male worm, there are pairs of papillae. Female is larger than male, uterus with embryos and larvae occupies almost the whole body.

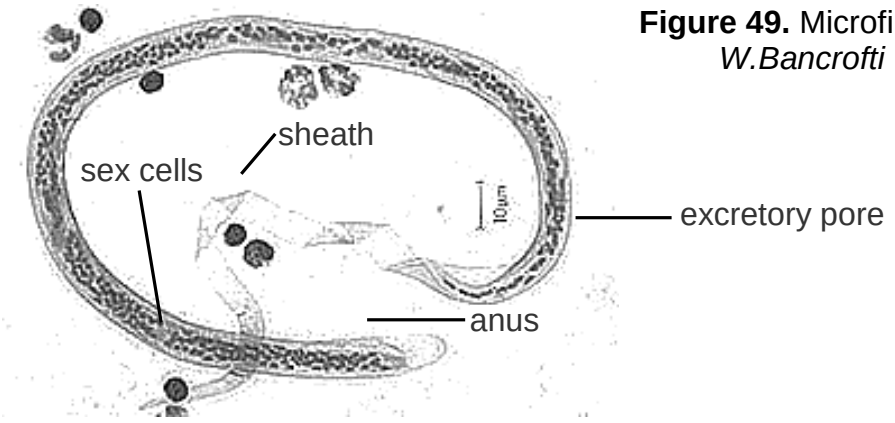


Figure 49. Microfiliariae of *W.Bancrofti*

Microfilariae. Microfilariae with sheaths are bluntly rounded anterior end and and pointed tail end. Internal structures can be visualized by the use of fixed stained preparations. In a stained preparation, it shows a central column of nuclei consisting of few anatomical" land marks". These land marks are use to differentiate the Microfilariae of *W. bancrofti* from *B. malayi* These are: a) nerve ring, b) body cell, c) tail nuclei.

Life cycle

The development includes two stages; larva in mosq uito, adult in man.

Development in the human host. It is commonly accepted that the infective-stage larvae, after penetrating the skin, pass through peripheral lymphatics, in which they migrate and grow, then settle down in certain lymphatic vessels retrograde to lymph nodes, grow to maturity and mate, followed by parturition of the gravid females. *W. bancrofti* usually resides in deeper lymphatic system besides in lower lymphatic system; *B. malayi* usually resides in lower lymphatic system of limb. The life spans of both filaria is about 4～10 years, and of both microfilaria is about 2～3 months. Human is the only known definitive host of *W. bancrofti*, there is no natural or reservoir host for *W. bancrofti*. But *B. malayi* can be transmitted to cats and rhesus monkeys except man.

Microfilarial periodicity is about in patients harboring living adults there is a nocturnal surge of the microfilariae into peripheral circulation. Microfilarial of *W. bancrofti* begin to appear in the blood from 10 PM to 2 AM; of *B. malayi* is from 8 PM to 4 AM. It was considered that microfilarial periodicity are correlated with factors of hosts and the biology of microfilaria.

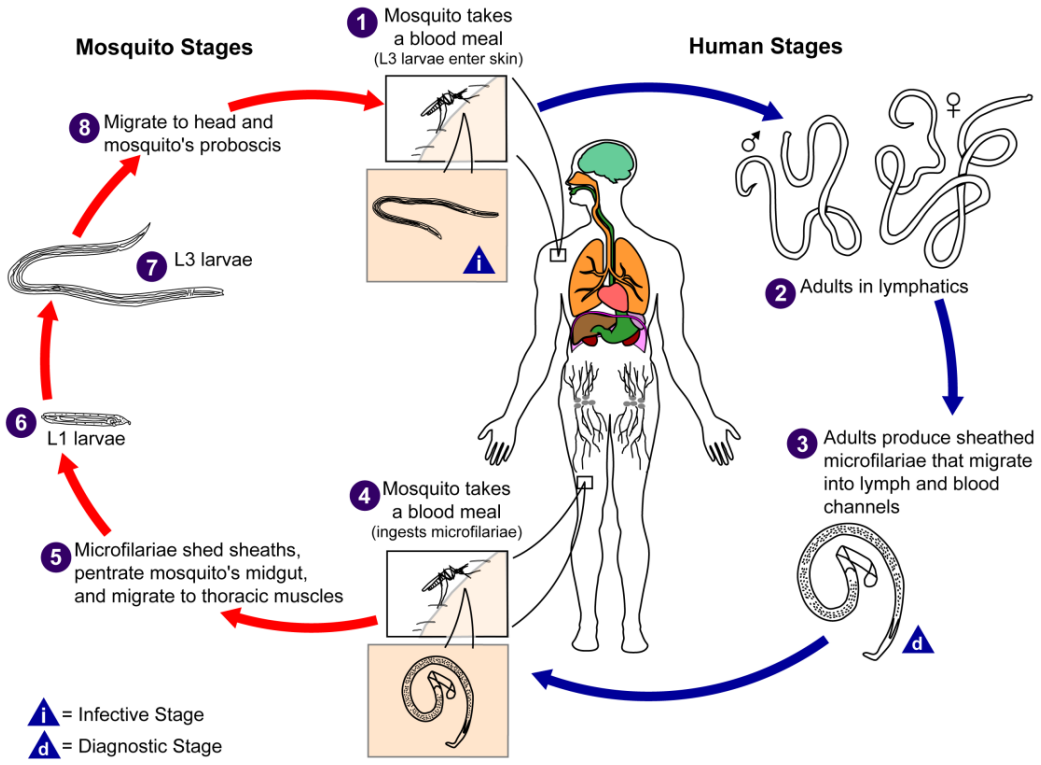


Figure 50. Life cycle of *Wuchereria bancrofti* (from Parasite image library of CDC, USA)

Development in mosquito When the microfilaria is ingested by an appropriate species of mosquito during its blood meal, they enter the anterior end of the stomach. In the gut, they lose their sheath, penetrate the gut-wall within an hour or two to enter the haemocoele. From there they turn anteriorly to penetrate the thoracic musculature, where they rest and begin to grow.
The larva moult to the first stage larva, second stage larva (sausage –shaped larva), and finally third stage larva. The third stage larva migrate to the salivary glands of mosquito. When the mosquito bites a man during blood meal, the larva is released from the lip of proboscis of mosquito and the life cycle is continued. Development in mosquito is usually completed within 10-14 days or 6-6.5 days.

Pathogenesis and clinical manifestation

Pathogenesis. The microfilariae are doing not harm the human host. Light infections may remain symptomless but are likely to be associated with an eosinophilia, tropical pulmonary eosinophilia, TPE). In more intense and repeated infections the presence of mature worms in the lymphatic vessels and nodes leads to allergic inflammation around the lymphatics and to temporary lymphatic obstruction. Eventually, after repeated attacks, in some of which secondary bacterial infections may play a part, permanent obstruction of a main lymphatic trunk may be produced. Lymphatics rupture and lymph spills into tissues. Progressive enlargement of the limb or region below the obstruction then follows with thickening and fibrosis of the tissues.
Acute lymphatic pathology. The secretions and metabolites of microfilariae and adult worms can cause acute allergic reaction, which belong to type I or type III of hypersensitivity. In early stage, there are edema and thickening of lymphatic vessels. And then, the wall and tissue around vessel were infiltrated by eosinophils, plasma cells, lymphocytes, and macrophages which tended to form into nodules.
Frequent early manifestations of filariasis are fever, lymphangeitis, lymphadenitis and dermatitis. The characteristic symptom of lymphangeitis is erythema along the course of inflamed lymphatic.
Chronic lymphatic pathology. Chronic lymphatic symptoms mainly result in lymphatic obstruction. Adult worms and microfilariae cause. inflammation and allergic reaction, which leads to obstruction of lymphatic vessels. The press of lower lymphatic vessel obstructed become higher, then lymphatic rupture and lymph spills into tissues. The infected people have different clinical manifestations based on the location of obstruction.
1. ***Elephantiasis*** Elephantiasis is a common symptom of chronic filariasis.
Lymphatic rupture and lymph spills into tissues, and then progressive enlargement, coarsening, corrugation and fissuring of the skin and subcutaneous tissue, with warty superficial excrescences, develop gradually until a leg resembles that of an elephant. The name “elephantiasis” may also occur in an upper limb.
Hydrocele testis. Obstruction of spermatic cord and testis lymphatics may be caused to hydrocele testis. The manifestation is commonly found from the patient with *W. bancrofti* infection.
Chyluria. Obstruction of Obstruction of the abdominal or thoracic lymphatics may be lead to chyluria, chylous ascite or a chylous pleural effusion. The manifestation is commonly found from the patient with *W. bancrofti* infection.

The interval between infection and the onset of elephantiasis is usually not less than 10 years, after which the condition tends to be slowly but remorselessly progressive. Gross elephantiasis develops only in association with repeated infections in highly endemic areas.

Asymptomatic filariasis. Such patients were only defined/found by finding microfilariae in the nodes or lung. Most common of the symptomatic clinical syndromes are recurrent episodes of 'filarial fever", the tropical eosinophilia syndrome, higher level of IgE etc.

Diagnosis

The clinical manifestations are suggestive for filariasis diagnosis. As most of the manifestation are non-specific, the laboratory diagnosis plays important role.

Laboratory diagnosis include parasitic diagnosis for microfilariae or adult worm in circulating blood, and immunodiagnosis for detecting the specific antigens or antibodies in serum of patients.

Parasitic diagnosis. It is made by demonstration of the microfilariae in the peripheral blood and rarely, in the chylous urine and hydrocele fluid and by demonstration of adult worm. Microfilariae can be demonstrated in the blood by the following methods:

1. ***Thick blood smear.*** The thick blood smear was made using 60□l blood (2 to 3 drops of peripheral blood), after drying, then stained with Giemsa. Optimal blood drawing time is from 10 PM to 2 AM for *W. bancrofti*, from 8PM to 4 AM for *B. malayi*.
2. ***Other methods for microfilariae detecting*** fresh blood drop method, concentration, DEC provocative test, examination of microfilariae in urine and other body fluid.
3. ***Examination of adult worm.*** The cross sections of adult worms are demonstrated in the biopsy specimens of the enlarged lymph nodes immediately proximal to the affected lymphatic vessels.

Immonodiagnosis Immunodiagnosis methods play an important role in the diagnosis of filariasis, especially in the case with low density of microfilariae states. It is commonly used for epidemiolog ical survey.

1) Interdermal test for screening in population,
2) Serological tests Detecting for antibodies such as IFA, IEST ELISA and IHA and detecting for antigens such as dot-ELISA and Sandwich-ELISA.

Epidemiology

Distribution *W. Bancrofti* is the worldwide distribution throughout the tropics and subtropics.

B. malayi is only endemic in Asia. It was estimated that there was 700 million people who reside in endemic areas of lymphatic filariasis.

Epidemic factors

1) *Source of infection.* The infected individuals and patients with microfilariae in peripheral blood constitute the source of infection. But when the density of microfilariae is reduced to below 5 each blood, those persons will loss the role of reservoir.
2) *Mosquito vectors.* There are more than 10 species of mosquitoes have

been proven to be satisfactory intermediate hosts in China. The most important know vectors in our country are *Culex pipiens pallens*, *Culex fatigans* and *Anopheles sinensi* for *W. Bancrofti* and *Anopheles anthropophagus*, *Anopheles sinensis* and *Aedes togoi* for *B. malayi*.

3) *Susceptibility of population* Humans is susceptible to filaria infection. But in endemic areas the peak of prevalence occurs in younger group aged from 21 to 30.

4) *The transmission of infection* is affected by the climatic factors, warm and moist are favorable for the breeding of mosquito vectors and the propagations of parasites in mosquito phase. The transmission season are from May to October.

Provention and control

Screening in population and mass chemotherapy are important measures for filariasis control.

After controlled, regular surveillance become the routine work.

Mass treatment In endemic areas population aged above 1 are screened regularly, and the positive are treated by using hetrazan. In our country, drug salt with DEC was the common measure for masstreatment. The dosage of DEC is 4.2g in 5-7 days for *W. bancrofti* and 1.5-2.0g in 3-4 days for *B. malayi*.

Mosquito control. Mosquito control is aimed to break the cycle of transmission of filariasis by controlling mosquito vectors. These include: a) by spraying insecticides such as DDT, etc; b) by biolog ical control; c) by environment modification; d) by reduction of man-vector contact.

Regular surveillance. When the transmission is blocked or stopped, the regular surveillance should be kept on. The advanced cases should be treated and rehabilited.

TRICHINELLA SPIRALIS

Kingdom: Animalia
Phylum: Nematoda
Class: Enoplea
Order: Trichurida
Family: Trichinellidae
Genus: *Trichinella*
Species: *spiralis*

It is necessary to remember

Trichnella spiralis is a nematode parasite of humans that is cosmopolitan in its geographical distribution. It is nearly unique among helminthic parasites in that all stages of development occur within a single host; over 100 species of mammals have been reported to be susceptible to infection. The infective encysted larvae may remain viable in the host's musculature for many years; they may also survive long periods in decaying and putrefying muscle. *Trichnella spiralis causes trichinellosis, a zoonotic infection in human.* Humans are infected when parasite-infected meat (port in most instances) is ingested.
Tidemann in Germany and Peacock and Owen in London first discovered the encysted larval stage of *Trichinella spiralis* in the muscles of an infected man.

Morphology

Adult worm. The adult worms are very small and slender with slightly tapered anterior ends, white and just visible to the naked eye (Fig. 51). The male measures 1.4～1.6 mm in length and 0.04～0.05 mm in diameter. The female size is 3～4×0.06 mm. Its pharynx is one third or half of worm body long, and posterior part of pharynx consists of a column of cells called of stichocytes. The reproductive system of both sex worm is single tract, and the single uterus is fille d with developing eggs in its posterior portion, where as the anterior portion contains fully developed, hatched juveniles or larva.
Larvae cyst. The cyst are found in skeletal muscle commonly, its size is about 0.25～0.5×0.21～0.42 mm. Usually, there is more than one larvae in a cyst.

Life cycle

All stages of development occur within a single host such humans, pigs, dogs, rats and cats etc. Adult worms reside in small intestine, and larvae reside in skeletal muscle. However, two different hosts are required to complete the life cycle.
Primary host: Pig is the primary host.
Natural host: rodents, carnivores and various other species of omnivorous animals are the other natural hosts.
Man is an accidental host and is the dead end for the parasite.
When man consumes raw or rare flesh infected with cysts of *Trichnella*, the cysts are digested out of the muscle in the stomach; the larvae are resistant to gastric juice. After passage to the small intestine, the larvae penetrate the villi of the small intestine, molt, and develop into mature adult within 48 hours. After fertilization, the gravid female burrow deep into the mucosa, discharging larvae beginning 5 to 46 days after infection and continuing for 2 to 4 weeks or occasionally longer. Widely disseminated via lymphatics and the bloodstream, larvae enter most organs, but persist only in individual skeletal muscle fibers. Increasing almost ten-fold in size over succeeding weeks, larvae

gradually become surrounded by a cyst wall of muscle. Although the capsules calcify within six months to two years, the larvae within remain viable for months to years, rarely for decades.

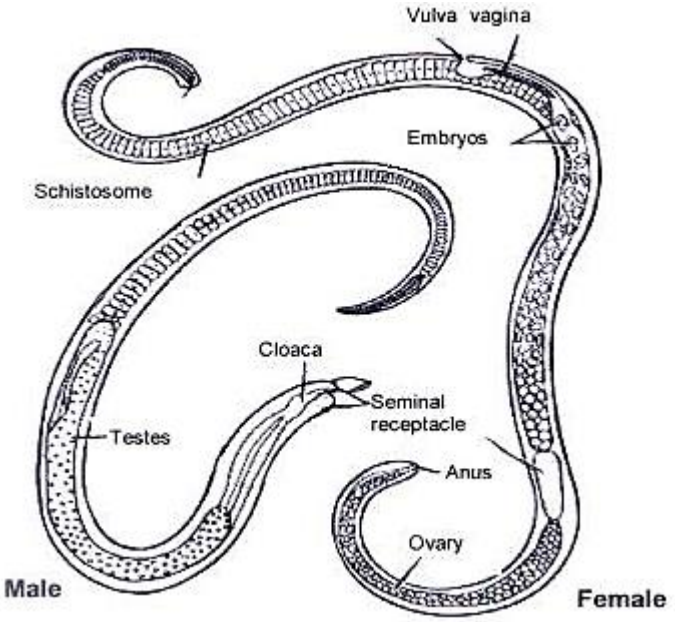


Figure 51. Morpgology of *Trichnella spiralis*

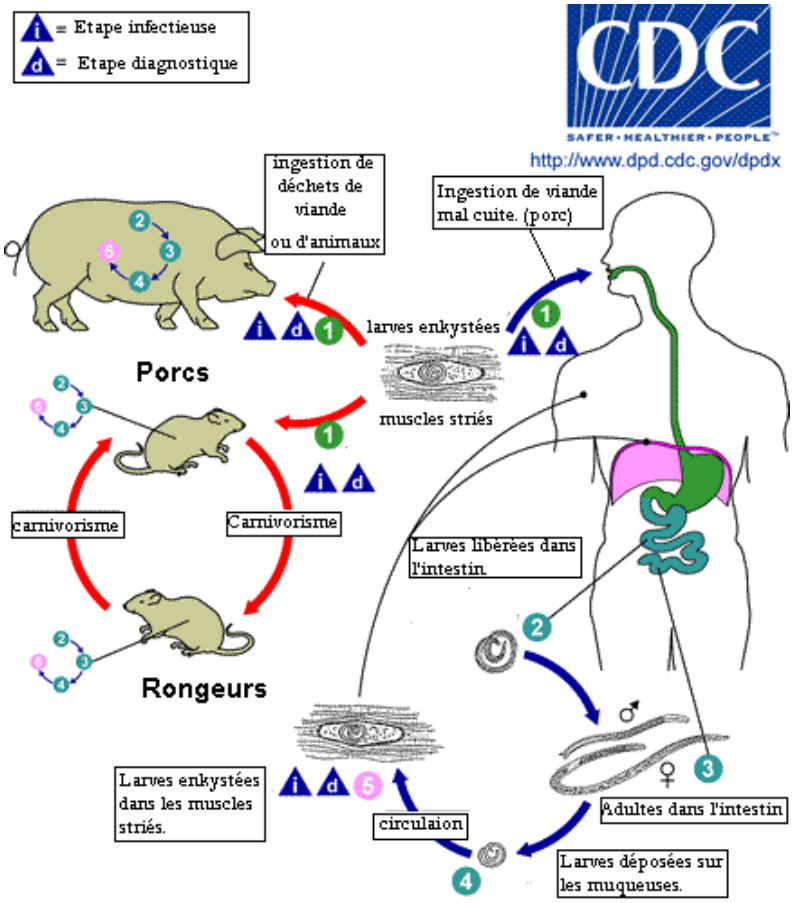


Figure 52. Life cycle of *Trichnella spiralis* (from Parasite image library of CDC, USA)

Pathogenesis and clinical menifestation

Adult worm and both migratory and encysted larvae are pathogenic: a) Adult female worms present in the intestine cause gastrointestinal disturbances;

b) Migrating larvae cause various allergic manifestation such as fever, oedema of the face, eosinophilia, and c) Encysted larvae in the skeletal muscles cause muscular pain.

The process of pathological change can be divided three phases.

Invade phase. The phase occurs within the first week after ingestion of infected meat, during the intestinal phase; this phase is associated with the development of larvae develop into adult. For invading of larvae and adult worms, the wall of intestine is damaged. Microscopic ulceration, mucosal hyperemia, localized edema, punctate hemorrhages, and intestinal inflammation may main pathological changes. Gastrointestinal signs and symptoms may be the first evidence of infection, including fever, disgusting, vomiting, abdominal discomfort, diarrhea etc.

Migratory phase. This phase, beginning about 7 to 9 days after exposure, is associated with penetration of the newborm larvae into muscle cells, initiating a strong inflammatory response. Later, the fibers enlarge, and edema, nuclear proliferation, and intestinal inflammation ensue, and fibrosis. Early symptoms of this stage are swelling of the eyelids and facial edema. Following this, muscle swelling, tenderness, pain on movement, and fever usually develop. Within the first two weeks of severe systemic disease, allergic phenomena such as edema, pneumonitis, and pleural transudate may occur. Serious complications, including myocarditis, and meningoencephalitis, occur most often in the third to ninth week of the disease.

Encystation of the larvae andtissue repair. The formation of cyst result in the stimulation of larvae and tissue reparation. With encystation, the inflammation disappears gradually, the clinical manifestation become light, but the muscular pain can still last for months.

Diagnosis

Diagnosis of Trichinosis depends on a combination of a) clinical manifestations with a history of ingesting meat that may contain larvae; b) immunodiagnosis; c) muscle biopsy.

Parasitic diagnosis. The definitive diagnosis is made by demonstration of free or encapsulated *Trichinella larvae* in the skeletal muscles obtained either in biopsy or at autopsy. Muscle biopsy may be positive as early as the second week of infection but is often not required. A small amount of muscle is excised under local anesthesia from a tender, painful, swollen muscle; a portion is sent for routine pathologic examination; and small amount is crushed between glass slides and examined directly under a scanning or low power objective for motile larvae.

Immunodiagnosis. a) Interdermal test for screening in population; b) Detecting for antibodies such as COP and ELISA etc.

Epidemiology

Human and animal infections of *T. spiralis* is worldwide distribution.

Three types of transmission cycle are seen in nature:

Pig-to-pig cycle. This occurs in human population due to their habit of feeding garbages to pigs. Pigs fed with Trichinella scrap, pig meat or carcase of animals suffer from infection.
Rat-to-rat cycle. This occurs between rats/mouse and is not dependent upon the presence or absence of infection in pigs.
Pig-to-rat cycle. This plays an important role in keep the transmission of infection.
It was reported that the prevalence of the infection in pigs was 50.2% in some endemic areas of Henan province. Eating or ingesting raw pork with larva cyst is major route to infection.
The cyst has stronger resistance to low temperature, freezing at -15 ℃ for 20 days' can destroy the parasites in the pork. Cyst can be killed at 70℃, so eating non-properly processed meat products is the way to require infection.

Prevention and control
Deep freezing at -15℃ for 20 days or -30℃ for 6 days and thorough cooking at 70℃or above kills the larvae in the pork. Smoking, curing or drying of meet are not dependable medthods for killing the larvae.
Regular inspection of meat, avoidance of eating raw or undercooked pork and meat of other wild animals; and avoidance of feeding raw garbage to pigs will prevent transmission of infection to man.
Treatment of the immature worms in the small intestine is usually successful and will abort or markedly inhibit systemic disease, so treatment of the intestinal phase in all cases up to six weeks after infection is advisable. Albendazole is the effective drug for trichinellosis.
Mebendazole is also recommended, it is believing to kill both adult worms and larvae.

LESSON 13. TEST

Test is conducted in the form of a written reply to the ticket.
Each ticket includes two theoretical questions and a diagnostic task.

VOCABULARI

COP – **coatomer-protein.** The **coatomer** is a protein complex that coats membrane-bound transport vesicles. Three types of coatomers are known:
- COPI (retrograde transport from trans-Golgi apparatus to Cis-Golgi and endoplasmic reticulum)
- COPII (anterograde transport from ER to the Cis-Golgi)
- clathrin and its associated adaptins (endocytosis from the plasma membrane, and trans-Golgi to lysosomes).

IFA – **Immunofluorescence** is a technique used for light microscopy with a fluorescence microscope and is used primarily on microbiological samples. This technique uses the specificity of antibodies to their antigen to target fluorescent dyes to specific biomolecule targets within a cell, and therefore allows visualisation of the distribution of the target molecule through the sample. Immunofluorescence is a widely used example of immunostaining and is a specific example of immunohistochemistry that makes use of fluorophores to visualise the location of the antibodies.

EST – **expressed sequence tag** is a short sub-sequence of a cDNA sequence. They may be used to identify gene transcripts, and are instrumental in gene discovery and gene sequence determination.

IEST – **Immunoenzymatic staining o**f haematological samples with monoclonal antibodies.

IHA – **indirect hemagglutination assay** a kind of passive agglutination in which erythrocytes, usually modified by mild treatment with tanic acid or other chemicals, are used to adsorb soluble antigen onto their surface, and which then agglutinate in the presence ofantiserum specific for the adsorbed antigen.

ELISA – **enzyme-linked immunosorbent assay** is a test that uses antibodies and color change to identify a substance.

dot-ELISA – Dot enzyme-linked immunosorbent assay, a qualitative ELISA test, can be performed very quickly with the end detection done visually. Because of its relative speed and simplicity, the dot ELISA is an attractive alternative to standard ELISA. In Dot-ELISA, small volumes of antibodies are immobilized on a protein binding membrane (Nitrocellulose) and the other antibody is linked to an enzyme Horse radish perxoidase (HRP). The test antigen at first reacts with the immobilized antibody and later with the enzyme-linked antibody.

Sandwich-ELISA – is used to detect sample antigen. The sandwich ELISA quantify antigens between two layers of antibodies (i.e. capture and detection antibody). The antigen to be measured must contain at least two antigenic epitopes capable of binding to antibody, since at least two antibodies act in the sandwich. Either monoclonal or polyclonal antibodies can be used as the capture and detection antibodies in Sandwich ELISA systems.

TABLE OF CONTENTS

Educational book

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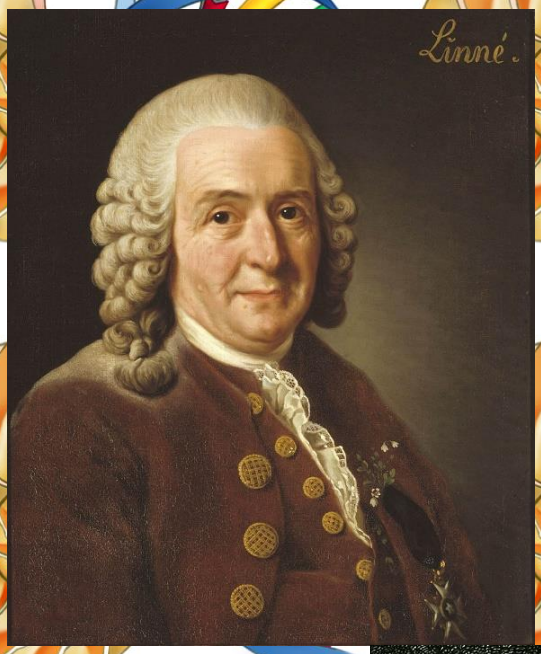
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