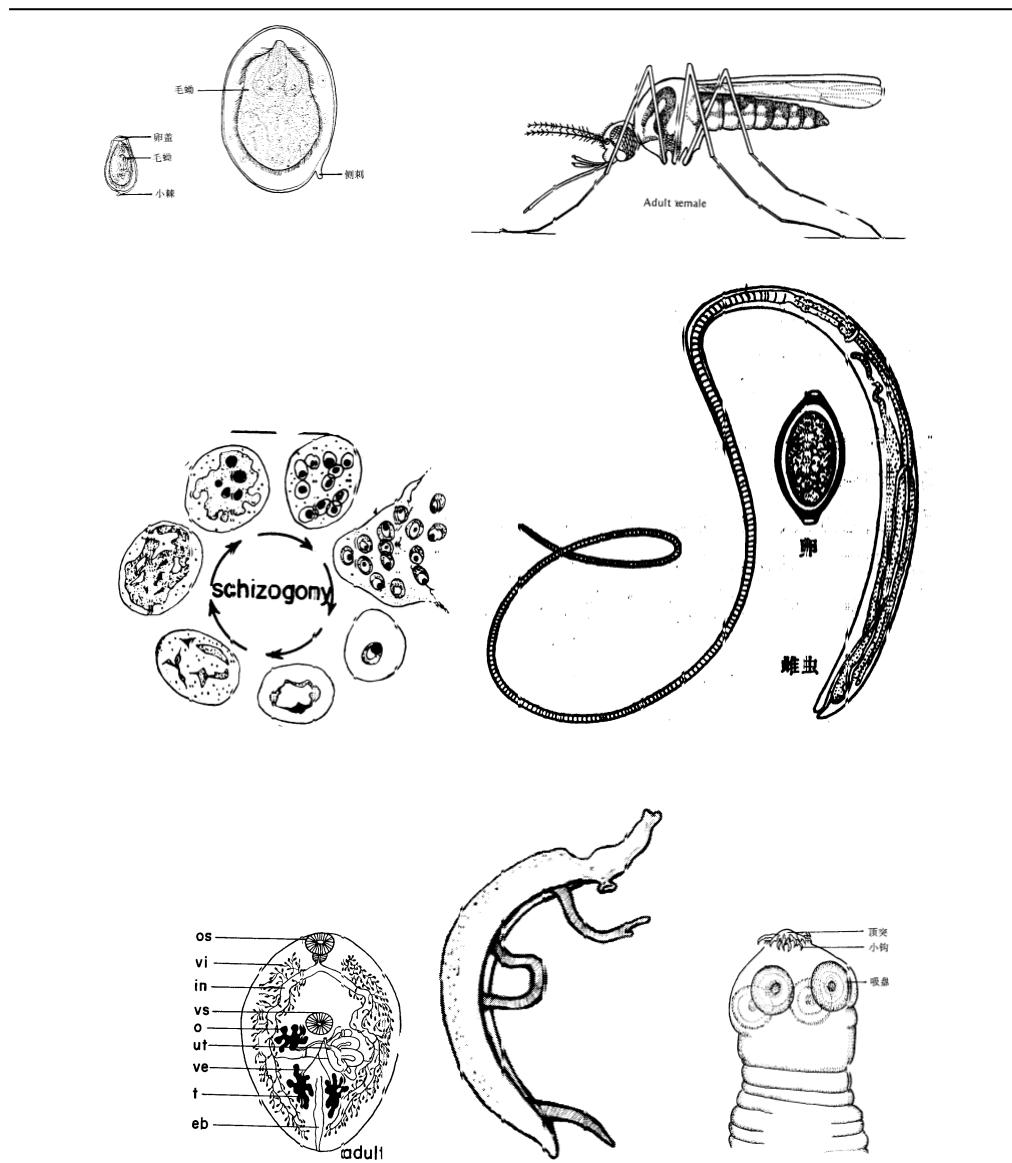


Text Book of Human Parasitology

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Section I INTRODUCTION TO PARASITOLOGY

Parasitology (寄生虫学), the study of parasites (寄生虫) and their relationships to their hosts (宿主), is one of the most fascinating areas of the biology. While it is entirely proper to classify many bacteria (细菌) and fungi (真菌) and all viruses as parasites, parasitology has traditionally been limited to parasitic protozoa (原虫), helminthes (蠕虫), and arthropods (节肢动物), as well as those species of arthropods that serve as vectors (媒介) for parasites. It follows, then, that parasitology encompasses elements of protozoology (原虫学), helminthology (蠕虫学), and medical arthropodology (医学节肢动物学).

Human parasitology, an important part of parasitology, study the medical parasites including their morphology(形态学), life cycle (生活史), the relationship with host and environment. The objectives are to study the way or the measurement of parasitic diseases control.

IMPORTANCE OF PARASITOLOGY

Why do students need to learn the course now? In past time, parasitic infections or parasitic diseases were the most common diseases in the world. Therefore, parasitology played important role on the medicine and public health, none neglect the important of parasitology. With the nearly simultaneous development of antibiotic drugs, synthetic pesticides (杀虫剂), and various antiparasitic agents, it was for a time widely believed that the infectious diseases would for all practical purposes disappear from the clinical scene. Someone has asked the question, why do medical students still need to learn parasitology?

Before answer the question, let me review the epidemic situation of parasitic diseases in the world. According to the WHO (世界卫生组织) 2001 year report, parasitic diseases is still an important human diseases. In the world, 210 million people reside in the endemic areas of malaria (疟疾), 10 million cases with malaria occur every year; 20 million infected individuals was estimated in the world. So TDR/WHO has proclaimed that 10 major unconquered human tropical diseases (热带病), African trypanosomiasis (非洲锥虫), Dengue (登革热), Leishmaniasis (利什曼病), Malaria (疟疾), Schistosomiasis (血吸虫病), Tuberculosis (结核病), Chagas disease (夏格病又称美洲锥虫病), Leprosy (麻风), Lymphatic filariasis (淋巴丝虫病), Onchocerciasis (盘尾丝虫病). Among them 7 diseases are parasitic in the

traditional sense. In addition, DDT and other insecticides (杀虫剂) not only have failed to eliminate the vectors of malaria, schistosomiasis, and other parasitic diseases but have themselves brought on problems too well-known to require mention here. The development of resistance to the synthetic antimalarials has been an ominous occurrence in recent years. The increased mobility of large segments of the population, and popularity of the tropics and subtropics as vacation areas, exposes them to a largely undiminished threat of parasitic infection, and the speed of transportation ensures that many return to their native shores before their infections become patent. For these reasons it remains necessary that all physicians have some familiarity with the parasitic diseases, no matter how “exotic”.

Modifications of the environment maybe have brought about major increases in parasitic diseases, flooding of vast areas has resulted in new habitats for the snail hosts of schistosomiasis. Global warming is suggested as a possible reason for the eventual spread of diseases now seen primarily in the tropics to more temperate climes. An important development of recent years has been the appearance of the human immunodeficiency virus(HIV) and its sequel, the acquired immunodeficiency syndrome(AIDS), which results in greatly increased prevalence and severity of a number of parasitic, viral, and bacterial diseases. As immunosuppression becomes more widespread, not simply because of AIDS, but also as necessitated by organ transplantation, the result of cancer chemotherapy, or the indiscriminate release of toxic chemicals and carcinogens into environment, heretofore unknown or extremely rare infections are being reported from human.

These are the reason why the course on Human or Medical Parasitology has been keeping

Table I – I – 1Current disease portfolio(from WHO report 2001)

	TDR disease category	Disease burden DALYs* (thousands)			Deaths (thousands)		
		Total	Male	Female	Total	Male	Female
African trypanosomiasis	1	1,585	1,013	572	50	32	18
Dengue	1	433	286	147	12	8	4
Leishmaniasis	1	1,810	1,067	744	41	23	18
Malaria	2	40,213	19,237	20,976	1,080	522	558

Schistosomiasis	2	1,713	1,037	676	11	8	3
Tuberculosis	2	35,792	21,829	13,962	1,660	1,048	613
Chagas disease	3	680	360	320	21	12	9
Leprosy	3	141	76	65	2	2	1
Lymphatic filariasis	3	5,549	4,245	1,304	0	0	0
Onchocerciasis	3	951	549	402	0	0	0

* DALY: disability-adjusted life years(失能调整生命年)

In our country, various parasites have long been recognized as one of the important endemic diseases for many years. In the early 1950s, the estimated number of people suffered from schistosomiasis totaled cases 10 million, and that from malaria and filariasis, 30 million each. Since the founding of the People's Republic , the Chinese government has paid great attention to investigation and control of parasites, with particular emphasis on the five major ones, i.e. schistosomiasis, malaria, filariasis, hookworm diseases and kala azar. Through 50 years' endeavor, outstanding achievements have obtained.

Nevertheless, schistosomiasis is still prevalence in lake-marsh and mountain regions along Changjiang River; at the present, falciparum malaria (恶性疟疾) has not been under effective control in several southern provinces due to the emergence of multi-drug resistant strain and ecological characteristics of the vector mosquito, Anopheles dirus (大劣按蚊) , as well as population migration. Besides, a nationwide survey conducted in 1988-1992 disclosed a striking number of parasite-infected population, and a high proportion of polyparasitism (多虫寄生) as well, e.g., overall prevalence of parasites was 59.67%(62.632 ± 0.339%), and more than 700 million cases of infected individuals was estimated in China Although soil-transmitted (土源性) parasites infection have been reduced significantly with improvement of living conditions, food-transmitted (食源性) parasite infection, such as infection of clonorchis, have been become a new public health problem for dietary habits.

So it is considered that parasitic infection /or parasitic diseases are still one of the important problems in public health in our country. As a candidate for doctor, to learn some knowledge of parasitology is necessary.

GENERAL CONSIDERATION

Medical (human) Parasitology consists of **medical protozoology**, **medical helminthology**, and **medical arthropodology**.

Symbiosis (共生) 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 symbote), 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(living together)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 (共栖) 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 (互利共生) 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(partners).

Parasitism (寄生) Parasitism is onother type of symbiotic relationship between two organisms: a parasite(寄生虫), usually the smaller of the two, and a **host**(宿主), 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 (寄生虫) Its biological definition is an animal or plant which lives in or upon another organism(technically called its host) and draws its nutrient directly from it. By this definition all infectious agents, viruses, bacteria, fungi, protozoa, and helminths are parasites, but traditionally protozoa , helminths and medical arthropod (节肢动物) , 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 (体内寄生虫) and Ectoparasite (体外寄生虫). The parasite which lives within the host is called the endoparasite(e.g, leishmania). Invasion by the parasite is called infection. Usually, the endoparasites cause most human diseases. The endoparasites include 3 types, such as obligate parasite, facultative parasite and accidental parasite. **Obligatove parasites**(专性寄生虫) are physiologically dependent upon their hosts and usually cannot survive if kept isolated from them(e.g., *Toxoplasma gondii* 弓形虫). **Facultative parasites**(兼性寄生虫), on the other hand, are essentially free-living organisms that are capable of becoming parasitic if placed in a situation conducive to such a mode. An example of a facultative parasite is *Strongyloides stercoralis* (粪类圆线虫). **Accidental parasites** (偶然寄生虫), the parasite which attacks an unusual host. Ectoparasite, the parasite which lives on the outer surface or in the superficial tissues of the host(e.g., lice). The infection by these parasites is called infestation(侵扰).

Host Host is defined as an organism which harbours 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 (终宿主) The hosts which harbour the adult parasites(e.g., *Taenia saginata* causing intestinal taeniasis), most highly developed form of the parasite(e.g., *Trypanosoma cruzi* causing African sleeping sickness) or where the parasite replicates sexually(e.g., *Paragonimus westermani*) are called the definitive hosts. The definitive hosts may be human or non-human living things.

2) Intermediate host (中间终主) The hosts which harbour the larval stages of parasite development or the asexual forms of the parasite are called intermediate host. Some times 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 钉螺 are the first intermediate hosts and fresh water fish are the second intermediate hosts for *Clonorchis sinensis* 肝吸虫s).

3) Reservoir host (保虫宿主) 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 血吸虫病).

4) **Paratenic host or transport host** (转续宿主) The larva of some parasites can invade a non-normal host, but can not develop, and only keep the larva stage. If the larva enter 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 (分类学)

According to the binomial nomenclature (命名法) as suggested by Linnaeus("Systema Nature" 1758), each parasite has two names: a Genus(属名) and a Species name (种名). 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* 管圆线虫(Chen, 1935) Dougherty, 1946).

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

Phylum Sarcomastigophora (肉鞭毛虫门). This phylum is divided into two subphyla: the Mastigophora or flagellates(鞭毛虫纲), and the Sarcodina or amebae (肉足纲) .

Phylum Apicomplexa (顶复门) Members of this phylum are tissue parasites..Apicomplexa have a complex life cycle with alternating sexual and asexual generations.

Phylum Microspora (微孢子门) 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 (纤毛虫纲) 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(扁形动物门) 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(蛔线虫门) 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(棘头虫门) The thorny-headed worms are all endoparasite organisms. While thorny-headed worms are widely distributed among wild and domestic animal, only three genera have been reported in human beings including *Macracanthorhynchus hirudinaceus*(猪巨吻棘头虫).

Phylum Arthropoda(节肢动物门) The phylum is subdivided into a number if classes, many of which are of medical importance. The classes main include the Class Arachnida(蛛形纲) and Class Insecta(昆虫纲). 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 (形态学)

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 particular function such as reproduction, digestion or excretion is performed by a group of special cells.

Arthropods are segmented and bilaterally symmetrical(对称的) animals with a body enclosed in a stiff, chitinous (甲壳质) covering or exoskeleton (外骨骼) and bearing paired, jointed

appendages. The digestive system is well developed. Sexes are separate.

LIFE CYCLE (生活史)

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 (e.g., *E.histolytica* 溶组织阿米巴, *Trichuris trichiura* 鞭虫, etc). Some of the parasites require two different hosts to complete their various stage of development (e.g., *Schistosoma japonicum* 日本血吸虫, etc). In a complex life cycle many parasites require two different hosts, one definitive host and one intermediate host to complete their life cycle(e.g., Schistosoma species require man as definitive host and snail as intermediate hosts). Few of the parasite require two different intermediate hosts apart from a single definitive host(e.g., *Paragonimus westermani* 卫氏并殖吸虫 requires snails as the first intermediate host and fresh water fish and crabs as the second intermediate host, apart from man and the fish eating mammals as the definitive host.).

TRANSMISSION OF PARASITES

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

Source of infection (传染源)

1) Humans 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(人类传染病).

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 人兽共患病 (e.g., hydatid disease 包虫病).

Mode of transmission (传播方式) *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 贾第虫 and *Entamoeba histolytica* 溶组织阿米巴; ova of *Ascaris lumbricoides* 蠕虫, *Trichuris trichura* 鞭虫 and *Enterobius vermicularis* 蜷虫).

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* 猪囊尾蚴, the larval stage of *Taenia solium* 猪带绦虫).

Ingestion of raw or uncooked fish and crab: Infection is transmitted by ingestion of raw or undercooked 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 westermani* 卫氏并殖吸虫), fish harbouring the metacercariae 囊蚴 of *Clonorchis sinensis* 华支睾吸虫/肝吸虫, etc).

Ingestion of raw or under cooked water plants: Infection can be transmitted by eating raw or undercooked water plants harbouring the infective form of the parasite(e.g., water chest nuts, etc., containing metacercariae of *Fasciolopsis buski* 布氏姜片虫 and *Fasciola hepatica* 肝片形吸虫).

2) Penetration of the skin and mucous membrane The infection is transmitted by

- A) Penetration of the intact skin by filariform larvae (丝状蚴) of hookworm, *Spirongyloides stercoralis* on coming in contact with faecally polluted soil, and
- B) Piercing the skin by cercariae of *Schistosoma japonicum* 日本血吸虫, *S. mansoni* 曼氏血吸虫 and *S. haematobium* 埃及血吸虫 on coming in contact with infected water.

3) Inoculation by an arthropod vector The infection also can be transmitted by

- A) Inoculation into the blood by Anopheles(vector for *Plasmodium* 疟原虫).
- B) Inoculation into the skin by mosquitoes(vectors for *Wuchereria bancrofti* 斑氏丝虫, *Brugia malayi* 马来丝虫 etc).

4) Sexual contact

Trichomonas is transmitted by sexual contact. Frequently, Entamoeba also is transmitted by sexual contact among homosexuals (同性恋).

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(a) sub-clinical latent infection, (b) clinical disease or (c) carrier (携带者).

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 (爆发性的), 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 ZONOSES(人兽共患病)

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 (发病机制与病理学)

Pathogenesis (发病机制) 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: trauma by adult worm, larva, and egg(e.g., hookworm cause oozing of the blood at the site of attachment); 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); Inflammatory reaction(many of the parasite induce inflammatory reactions in the host leading to the formation of

various pathological lesions); Toxin(parasites like bacteria also produce toxins but they appear to have a minimal role in the pathogenesis of the disease processes; 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). Various pathogenic mechanisms in parasitic diseases are summarized in the below table I – I –2.

The parasitic infections usually are designate by generic names of the parasites ending with -iasis or -osis. (e.g., *Schistosoma* infection is called as schistosomiasis)

Table I – I –2 Pathogenic Mechanisms in Parasitic Diseases

Mechanism	Parasitic diseases
Trauma	Strongyloidiasis, enterobiasis, taeniasis, clonorchiasis, schistosomiasis and hookworm infection
Invasion and destruction of host cell	Malaria, leishmaniasis, trypanosomiasis, toxoplasmosis and amoebiasis
Inflammation	Trichinellosis, lymphatic filariasis, paragonimiasis, Amoebiasis
Toxin	Amoebiasis, Chaga's disease and sleeping sickness
Allergic manifestation	Schistosomiasis, hydatid disease

HOST IMMUNITY

The host resistance or immunity in parasitic infections refers to the resistance offered by the host towards the injury caused by the parasites and their products. It may be classified into: a) Innate, and b) Acquired immunity.

Innate immunity (先天性免疫) It is the inherited but non-immune type of the host defence against a parasite., e.g., Haemoglobin-S thalasaemia (地中海贫血) and glucose- 6-phosphate dehydrogenase (葡萄糖 6-磷酸脱氢酶, G6PD) deficient erythrocytes (红细胞) are resistant against *Plasmodium falciparum* 恶性疟原虫; persons with Duffy-negative genes (Duffy 阴性基因) are resistant to malaria, etc.

Acquired immunity (获得性免疫) It may be a) Non-specific or acquired immunity, or b) Specific acquired immunity.

1) **Non-specific or acquired immunity** It confers protective immunity against many

protozoal and helminthic infections. It is developed during exposure of persons to antigenically unrelated micro-organisms, microbial extracts or some synthetic products. Non-specific immunity has been shown to supplement the specific acquired immunity. These appear to be mediated by macrophages or their active products and also by interferons.

2) Specific acquired immunity It is mediated by both humoral and cell mediated immunities.

a) Humoral immunity It is mediated through the production of specific antibodies. These antibodies are serum proteins and gamma globulins in nature. These antibodies may be protective or non-protective. The antibodies may offer protection in following ways:

- ① The antibodies prevent the parasites from attaching and penetrating the host cells by binding the specific sites on the surface of parasites.
- ② The antibodies neutralize parasite toxins and inactivate parasite enzymes by binding with the determinants of parasitic antigens.
- ③ The secretory IgA antibodies found in various body secretions prevent attachment of some protozoal parasites in the gut wall epithelium.
- ④ In a few parasitic infections(e.g., trypanosomiasis), the parasites are killed by lysis of antibody-coated cells mediated by the complement, and
- ⑤ The antibody dependent cell mediated cytotoxicity(ADCC) helps in the killing of a few helminths coated by specific antibodies. It is an important mechanism by which the parasites are killed. This is mediated mainly by the lymphocytes and to some extent by neutrophils, eosinophils and macrophages. The antibodies are mainly of IgG, IgE and bind specifically to the parasites.

b) Cell mediated immunity(CMI) It is mediated through T cell which are cytotoxic. The CMI offers protection against many parasitic infections in following ways.

- ① Cytotoxic T lymphocytes alter the osmotic permeability of parasitic cells causing swelling and disruption of cells thereby lysing the cells(e.g., *Plasmodium falciparum* infection in man).
- ② Activated macrophages kill parasites in various ways such as producing enzymes or activated substances(e.g., *Toxoplasma*, *Leishmania*, the schistosomule of *Schistosoma species* or by producing hydrogen peroxide(*Leishmania species* etc).
- ③ Natural killer cells These cells appear to be helpful in the initial host resistance against many parasitic infections.

IMMUNE RESPONSES

The immune response of man against parasitic infections are variable. It may be: a) Protective, or b) Harmful to host.

Protective immune response

1) Sterilizing immunity The sterilizing or complete immunity is associated with the clinical cure, complete elimination of the parasite from the host and life long resistance against subsequent infection. It occurs rarely in humans. It occurs only in the cutaneous leishmaniasis.

2) Incomplete immunity It is associated with the clinical recovery from the disease and the development of immunity to specific challenge with the parasite. The parasites always persist in the host, even though relatively at a low level. This incomplete immunity also known as “premunition(带虫免疫)” typically is found in many protozoal infections(e.g., malaria), as “concomitant immunity(伴随免疫)” typically is found in helminthic infection(schistosomiasis).

3) Absence of an effective immunity It is seen after complete clinical cure from infections(e.g., amoebiasis, visceral leishmaniasis, American trypanosomiasis).

Harmful immune responses In this condition, immune regulatory system shows a negative effect by inhibiting protective immune responses and instead produces harmful effects in the host. It is manifested development of hypersensitivity reactions.

This is of four reaction: Type I, II, III, IV hypersensitivity.

① Anaphylactic reaction(过敏反应) It is type I hypersensitivity reaction. It is involved in the pathogenesis of tropical pulmonary eosinophilia, Loefflers pneumonia, swimmers' itch and anaphylactic reaction of ruptured hydatid cyst inside the body. The skin manifestations of the anaphylactic reactions characteristically are seen during the phase of invasion of the skin by the larvae of hookworm (钩虫), *Strongyloide* (类圆线虫), *Schistosoma* and other parasites.

② Cytotoxic (细胞毒) It is type II reaction. It is responsible for a) anaemia in malaria, b) chronic myocarditis (心肌炎) and megacolon (巨结肠) in Chagas' disease , c) quinine (奎宁) induced massive haemolysis (溶血) and haemoglobinuria (血蛋白尿) in malaria .

③ Immune complex mediated (免疫复合物介导) This is type III reaction and is responsible for development of glomerulonephritis (肾小球肾炎) seen in malaria. It is also responsible for immune complex mediated nephritis in leishmaniasis, trichinosis and

schistosomiasis.

④ **Delayed hypersensitivity** (迟发性变态反应) This is type IV reaction and is responsible for development of pathological lesions in a) Schistosoma species infections, b) local lymphatic inflammation in filariasis, c) inflammation of muscle tissue around *Trichinella* and d) survival and proliferation of parasites.

IMMUNOEVASION OF PARASITES (寄生虫免疫逃避)

Many parasites survive and proliferate in immunologically competent host by of mechanisms. These include.

- 1) Intracellular location(e.g., *Toxoplasma* 弓形虫, *Leishmania* 利什曼原虫)
- 2) Antigenic shedding(e.g., *Entamoeba* 内阿米巴, *Toxoplasma* 弓形虫, *Fasciola* 片形吸虫, *Trichinella* 旋毛虫)
- 3) Antigenic variation(*Trypanosoma* 锥虫)
- 4) Antigenic mimicry(*Schistosoma* 血吸虫), and
- 5) Modification of host immune responses. This is caused by inactivation of complement(e.g., *Taenia* 带绦虫), immune suppression(e.g., *Plasmodium* 疟原虫, *Leishmania* 利什曼原虫, *Toxoplasma* 弓形虫, *Wuchereria* 吴策线虫属, *Brugia* 布鲁线虫属), activation of lymphocytes(e.g., *Trypanosoma brucei* 布氏锥虫, *Schistosoma species*), modified leucocyte function(e.g., *Fasciola hepatica* 肝片吸虫) and immune complex(e.g., *Leishmania* 利什曼原虫, *Trypanosoma brucei* 布氏锥虫, *Toxoplasma* 弓形虫, *Plasmodium* 疟原虫).

CLINICAL MANIFESTATION (临床表现)

Clinical manifestation of parasitic diseases are variable. It may be acute or chronic. However, many of the diseases are chronic in nature. The onset of the disease is slow. In a few parasitic diseases, the onset may be sudden. For example, in ascariasis, pneumonitis develop immediately few days after ingestion of *Ascaris* 蛲虫 eggs. Ingestion of pork infected with the larvae of *Trichinella spiralis* 旋毛虫, causes gastro-intestinal disturbances within a few hours, simulating food-poisoning.

Allergic manifestation are important in many a helminthic infections. *Schistosoma* eggs produce an allergic reaction in the host tissue. Similarly, the adult *Brugia* and *Wuchereria* worm in

the lymphatics cause frequent attacks of filarial fever, lymphangitis, etc. A variety of localized and generalized allergic and neurological manifestations may be caused by inoculation of toxins into the skin by arthropods, during the bite.

DIAGNOSIS

The diagnosis of parasitic infections depends upon: 1) Clinical diagnosis, and 2) Laboratory diagnosis.

Clinical diagnosis In areas where the disease is endemic, clinical diagnosis may be made by the characteristic clinical manifestations of the disease. However, in some situations even in endemic areas, the clinical diagnosis is hindered by:

- 1) Low prevalence of major clinical signs
- 2) Late development of clinical signs
- 3) Lack of specificities of clinical signs
- 4) Occurrence of asymptomatic carriers

In non-endemic areas, clinical diagnosis may even be more difficult.

Laboratory diagnosis It plays an important role in establishing the specific diagnosis and supplementing the clinical diagnosis of the condition. It depends upon

- 1) Morphological recognition of parasites in relevant specimens(parasitic diagnosis)
- 2) Immunological tests(immuno diagnosis)
- 3) DNA probes or PCR etc
- 4) Other laboratory

a) Parasitic diagnosis The definitive diagnosis is made by demonstration of parasites in appropriate clinical specimens. The parasitic diagnosis can be made by a) microscopy, b)cultivation, c) animal inoculation (动物接种) and b) xenodiagnosis(异体接种诊断法). The nature of clinical specimen to be collected depends upon the habitat of the parasite.

① Luminal parasites (腔道寄生虫) *of the gastrointestinal, genitourinary and pulmonary tract* Infections with these parasites are confirmed by the presence of their characteristic forms in the faeces, urine, sputum and other body secretions.

Faeces (排泄物/粪便) : It is an important clinical specimen for the diagnosis of a) intestinal parasitic infections and b) helminthic infections of the biliary tract in which eggs are discharged

in the intestine. In protozoal infections, the cysts(包囊) and trophozoites(滋养体) of *Entamoeba histolytica*, *giardia intestinalis*, can be demonstrated in the faeces. The trophozoites are usually excreted in acute infections whereas cysts are found in chronic infections. In helminthic infections the eggs, larvae and adult worms are found in the faeces as follows:

.Non-faecal specimens (标本) : These include a) anal swabs(e.g., for the eggs of *Enterobius vermicularis*), b) genital specimens(e.g., for Trophozoites of *trichomonas vaginalis* 阴道滴虫), c) sputum (e.g., the eggs of *Paragonimus westermani*), d) urine(e.g., the eggs of *Schistosoma haematobium*), e) cerebrospinal fluid(e.g., Trypomastigotes 鞭毛体 of *Trypanosome brucei*), f) aspirations and biopsies(e.g., duodenal fluid, sigmoidoscopy aspirates, abscess aspirate, and biopsies etc).

② Blood and tissue parasites The infections caused by these blood and tissue parasites are confirmed by the presence of their morphological stages in the blood, tissue and other specimens.

Blood: The blood can be examined by direct smear, concentration , culture and animal inoculation etc.

Tissue biopsy and aspiration: for example, the larvae of *Trichinella spiralis* and *Taenia solium* can be demonstrated in muscle biopsy.

b) Immunological diagnosis These methods are particularly useful in the latent or asymptomatic infections as well as in some chronic infections. The immunological tests broadly are of two types, a) skin test, and b) serological test.

① Skin test Skin test are performed by the intradermal injection of a low volume of sterilized parasitic antigen and noting whether erythema and induration occurs after 30 mins(immediate hypersensitivity) or after 48 hrs(delayed hypersensitivity).

② Serological test Serological tests important both for mass screening and case detection particularly during the chronic phase of infection. These essentially are based on: a) Antibody detection and b) Antigen detection.

TREATMENT

Treatment of parasitic diseases primarily is based on chemotherapy and in some cases surgery.

Chemotherapy The chemotherapy is employed for the treatment and prophylaxis of the parasitic infections. Many parasitic diseases can be treated by chemotherapy. Albendazole (阿苯达

唑), mebendazole (甲苯咪唑) and pyrantel pamoate (噻嘧啶) are some of the commonly used anthelmintics. Praziquantel (吡喹酮) is used against many cestode(tape worm 绦虫) and trematode(fluke 吸虫) infections.

An ideal agent for use in chemotherapy against a parasitic infection should be:

- 1) Of high therapeutic index
- 2) Administered orally, preferably in a single dose or divided doses on the same day
- 3) Stable over a long period
- 4) Inexpensive, and
- 5) Also the parasite should not develop drug resistance.

Surgical management Management by surgery is indicated in the parasitic diseases for which anti-parasitic drugs are not yet available or if available are not completely effective. It is recommended especially for the hydatid disease, paragonimiasis, etc.

PREVENTION AND CONTROL

Prevention of parasitic disease refers to its interception. Control refers to check the possibilities of dissemination of infection and epidemics, reduce and maintain a low level of parasitic infections prevalent in human population.

Control methods can be aimed at controlling and eradicating the disease at its reservoir and source. It can be achieved by

- 1) Chemotherapy and isolation. It is useful to prevent the spread of infection and eliminate the reservoir and source of infection.
- 2) Immunoprophylaxis(免疫预防). It is carried out by artificial immunization such as vaccines but has not been possible for most of the parasitic diseases. In falciparum malaria and a few other infections pilot studies of the vaccine have been carried in the field.
- 3) Control of infection in animal reservoir for zoonotic prophylaxis (预防).

PARASITOLOGY IN FUTURE

Human parasitic diseases caused by protozoans, helminths and arthropods include a wide variety of infectious organisms, a broad spectrum of disease processes, and some of the most

fascinating scientific and important public health challenges that we will still face in the 21st century. Many of these diseases represent very well adapted host/parasite/vector relationships that imply a degree of co-evolution of these "partners in disease" that stretches the imagination. This is often exemplified by chronic infections that, in a high percentage of hosts, cause little morbidity, and life-cycles that are exquisitely timed and organized for optimal, but regulated, transmission of the pathogen. A few of these diseases are more fulminant, and some clearly lead to more disease in recently infected populations of hosts than in long-standing situations of medium-to-high prevalence. Work with these parasites and their hosts and vectors spans the gamut from very basic scientific research, to extremely practical implementation of eradication programs. It is, however, critical to remember that a sound scientific understanding of the organism, the vector and the host, and how they interact biologically, spatially, and temporally, is always the foundation upon which effective control, elimination, or eradication programs are based. Until the proper understanding of the situation is in hand, and the appropriate tools are assembled, sheer determination is simply usually not enough to effectively dominate these well-established and spreading diseases. These seemingly all-encompassing characteristics make *Parasitology* facing scientists, clinicians, and public health officials as we move into the 21st century.

Section II PROTOZOA (原虫)

I . INTRODUCTION

Protozoa are usually defined as singly celled animals, they belonging to the animal kingdom, subkingdom Protozoa. Structurally, protozoa resemble single metazoan (多细胞动物) cells; a

protozoan cell has a full complement of **cellular organelles** (细胞器), i.e. **nucleus** (细胞核), **mitochondria** (线粒体), **endoplasmic reticulum** (内质网), **Golgi apparatus** (高尔基体) 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 world 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** (原生质膜), cytoplasm (细胞质) and nucleus.

Plasma membrane: The membrane appears three layered in electron micrographs because the central lipid portion looks light or clear (electron lucent) and is enclosed by the darker (electron dense) 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 a low-density medium with physical properties of a colloid. In some species, the cytoplasm is divisible into two portions: **ectoplasm** (外质) and **endoplasm** (内质).

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** (细胞器): There are several membrane organelles characteristic of eukaryotes (真核细胞), 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** (运动细胞器): **flagella** (鞭毛), **cilia** (纤毛), and **pseudopodia** (伪足). 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 (肉足类) (and other organisms) 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** (核仁) 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** (顶复门) (e.g.: malaria parasites) contain DNA; in contrast, the karyosomes of **trypanosomes** (锥虫) and parasitic amoebae do not contain any DNA. Chromatin granules giving the appearance of condensation on thin 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 (micro-nucleus) and a large nucleus (macro-nucleus). They are homogeneous in composition. In certain protozoa such as trypanosomes, a non-nuclear DNA-containing body called **kinetoplast** (动基体) is also present in addition to the nucleus.

Trophozoite(滋养体): 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(包囊): 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 cysts formed sexually are called oocysts.

TYPE OF LIFE CYCLE

The life cycle of protozoa may be simple or complex. According to the characteristics of transmission, they can be divided into three types

***Person to Person transfer* (人际传播型)**

In this type, no intermediate host is needed. The transmission is only from person to person. For example, the life cycle intestinal amoebae, flagellates and ciliates are simple and completed in a single host only. The parasites replicate only by asexual method of reproduction mostly in the trophozoite stage. Sometimes, they multiply in cystic stage (e.g. *Entamoeba*, *Giardia*)

Under unfavourable circumstance, the actively growing trophozoites secrete a resistant cyst wall and are transformed into cysts. This process is called **encystation** (成囊). When the condition becomes favourable the cyst wall are lysed to liberate trophozoites. This process is called **excystation** (脱囊). Both the processes of encystation and excystation take place in a single host. The parasites are transformed from one host to another host after encystation only.

***Circulation transfer* (循环传播型)**

Some species of the parasitic protozoa, like *Toxoplasma gondii* (刚地弓形虫), it needs more than one vertebrate to finish its life cycle, in which cat is the definitive host, man and others are the intermediate host, with sexual and asexual reproduction. The intermediate host and the definitive host can infect each other.

***Vector transfer* (虫媒传播型)** Some protozoa need an insect vector in their life cycle. The transfer manner of these type protozoa is person to insect and insect to person. In *Plasmodium* species, sexual method of reproduction occurs in one host (*Anopheles* mosquito) and asexual replication in another host (man). This process is known as **alternation of generation** (世代交替) accompanied by alternation of host.

PHYSIOLOGY

The physiologic processes of protozoa include locomotion, ingestion, metabolism, reproduction and others.

Locomotion(运动): Protozoa move by four basic types of organelles: flagella, cilia, pseudopodia, and others.

Movement by means of pseudopodia is a complex form of protoplasmic streaming; Flagella are hair-like projections of cytoplasm; arise from a kinetoplast within the cytoplasm and serve as locomotor organelles in flagellates; In the ciliates, numerous short threads called ‘**cilia**’(纤毛) which are distributed over the surface of the body, perform the function of locomotion.

In most sporozoan parasites there no specific locomotion organelles, locomotion is accomplished by undulating ridges. The **merozoites** (裂殖子), **ookinetes** (动合子), and **sporozoites** (子孢子) appear to glide through fluids with no subcellular motion whatever, but electron microscope studies reveal tiny undulatory waves that form in the cell membrane and pass posteriad.

Nutrition(营养): There are several type nutrition are found in protozoa.

1) Permeation(渗透), the passing of molecules directly through the out cells membrane: Some protozoa may obtain nourishment from the environment by diffusion or by active transport across the plasma membrane. Diffusion is possible when the cell membrane is permeable to a particular molecule and when the concentration of that molecule is lower inside the cell than outside.

The accumulation of molecules against a concentration gradient requires the expenditure of energy and is called active transport. Active transport appears to operate through a carrier in the cell membrane, but the action of the carrier must be coupled with an energy-yield metabolic reaction. Obviously the ultimate value of the molecule or the energy derived from it must be great than the energy expended in acquiring it. Some important food molecules such as glucose are brought into the cell by active transport.

2) Pinocytosis(胞饮) & **phagocytosis**(吞噬): Protozoa eat fluid food through cell membrane called pinocytosis, and protozoa eat solid food is called phagocytosis. Parasitic protozoa feed by ingesting entire organisms or particles thereof. Their mouth openings may be temporary, as in amebas, or permanent **cytostomes** (胞口), as in most ciliates. Particulate food passes into a food vacuole, which is a digestive organelle that forms around any food thus ingested.

Pinocytosis and phagocytosis are important activity in many protozoa. Both pinocytosis and phagocytosis are example of endocytosis, differing only in that phagocytosis deal with droplets of fluid, whereas phagocytosis is the process of internalizing particulate matter.

Reproduction(生殖): Reproduction in protozoa may be either asexual or sexual, although many species alternate types in their life cycle.

1) **Asexual reproduction**(无性生殖)

① **Binary fission** (二分裂): most often, asexual reproduction is by binary fission, in which the individual divides into two. All the internal structures are duplicated before division of the parasite (e.g., *Entamoeba histolytica*)

② **Multiple fission** (多分裂): multiple fission occurs in the Sarcodina and Sporozoea. In this type of division the nucleus and other essential organelles divide repeatedly before cytokinesis; thus a large number of daughter cells are produced almost simultaneously. During schizogony the cell is called a **schizont** (裂殖体), meront, or segmenter. The daughter nuclei in the schizont arrange themselves peripherally, and the membranes of the daughter cells form beneath the cell surface of the mother cell, bulging outward. The daughter cells are merozoites, and they finally break away from a small residual mass of protoplasm remaining from the mother cell to initiate another phase of merogony or begin gametogony. Schizogony to produce merozoites may be referred to as merogony. Another type of multiple fission often recognized is **sporogony** (孢子生殖), which is multiple fission after the union of gametes (see sexual reproduction).

Several forms of **budding** (芽殖) can be distinguished. Plasmotomy, sometimes regarded as budding, is a phenomenon in which a multinucleate individual divides into two or more smaller, but still multinucleate, daughter cells. Plasmotomy itself is not accompanied by mitosis. **External budding** (外芽殖) is found among some complex ciliates, such as the Suctoria. Here nuclear division is followed by unequal cytokinesis, resulting in a smaller daughter cell, which then grows to its adult size.

Internal budding, or **endopolyogeny** (内芽殖), differs from schizogony only in the location of the formation of daughter cells. In this process the daughter cells begin forming within their cell membranes, distributed throughout the cytoplasm of the mother cell rather than at the periphery. The process occurs in some stages of the schizonts of the Eimeriina. Endopolyogeny is endopolyogeny in which only two daughter cells are formed.

2) **Sexual reproduction**

It occurs by conjugation gametogeny, conjugation. Sexual reproduction involves reduction division in meiosis, causing a change from diploidy to haploidy are the gametes, and the process of

production of gametes is **gametogony** (配子生殖). Cells responsible for the production of gametes are gamonts.

Reproduction may be amphimictic, involving the union of gametes from two parents, or automictic, in which one parent gives rise to both gametes. Uniting gametes may be entire cells or only nuclei. When they are whole cell, the union is called **syngamy** (两性生殖). When only nuclei unite, the process is termed **conjugation** (接合生殖). Conjugation is found only among the ciliates (纤毛虫), whereas syngamy occurs in all other groups in which sexual reproduction is found. Meiosis is known in both types of sexual reproduction.

Metabolism (代谢): the main energy in protozoa, as in other cells, is in the form of high-energy phosphate bonds, primarily in adenosine triphosphate (ATP). Energy is released in the step by step, enzymatic oxidation of food molecules; part of this energy is conserved by coupling these oxidations with the phosphorylation of adenosine diphosphate (ADP) to ATP. Both protozoan and metazoan are unexpectedly variable in their energy metabolism, particularly in the factors to consider are that many parasites must survive in locations in which the oxygen supply is quite limited and that, even in many cases in which oxygen is not limited, neither is glucose; therefore there is no advantage in completely oxidizing glucose. If glucose is in plentiful supply, the organism can live on little more than the energy derived from glycolysis, simply by consuming more glucose, and the partially oxidized products can be excreted as waste.

Excretion (排泄): most protozoa appear to ammonotelic; that is, they excrete most of their nitrogen as ammonia, most of which readily diffuse directly through the cell membrane into the surrounding medium. Other, sometimes unidentified waste products are also produced, at least by intracellular parasites. These substances are secrete and accumulated within the host cell and, on the death of the infected cell, have toxic effects on the host. Carbon dioxide, lactate, pyruvate, and short chain fatty acids are also common waste products.

PATHOLOGIC CHARACTERISTICS 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 (增殖): 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 (机会致病) : 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 Protozoologists (1985). This classification is based on the morphology of the protozoa as demonstrated by light and electron scanning microscopy. The Sarcomastigophora(肉足鞭毛门), the Apicomplexa (顶复门) and Ciliophora (纤毛门), are three important phyla which contain species of medical importance causing disease in man (Table 1). According their locomotion organelles, protozoa can be divided into four groups: Amoebae, flagellates, ciliates and sporozan. The common parasitic protozoa are list in table II - I -1.

Parasitic location	Scientific Species Name	Family	Order	Class
Monony-clear protozoa phagocyte system (单核吞噬系统)	<i>Leishmania donovani</i> 杜氏利什曼原虫 <i>Leishmania tropica</i> 热带利什曼原虫 <i>Leishmania braziliensis</i> 巴西利什曼原虫	Trypanosomatidae 锥虫科	kinetopastida 动基体目	Zoomastigophora 动鞭纲
Blood 血	<i>Trypanosoma</i> sp 锥虫			
Urogenital canal 泌尿生殖道	<i>Trichomonas vaginalis</i> 阴道毛滴虫	Trichomonadidae 毛滴虫科	Trichomonadi 毛滴虫目	
Oral cavity 口腔	<i>Trichomonas tenax</i> 口腔毛滴虫			

Intestine 肠	<i>Trichomonas hominis</i> 人毛滴虫			
	<i>Dientamoeba fragilis</i> 脆弱双核阿米巴			
	<i>Giardia lamblia</i> 蓝氏贾第鞭毛虫	Hexamitidae 六鞭毛科	Diplomonadida 双滴虫目	Lobosea 叶足纲
	<i>Entamoeba histolytica</i> 溶组织内阿米巴	Entamoebidae 内阿米巴科	Amoebida 阿米巴目	
	<i>Entamoeba dispar</i> 迪斯帕内阿米巴			
	<i>Entamoeba hartmanni</i> 哈门氏内阿米巴			
	<i>Entamoeba coli</i> 结肠内阿米巴			
	<i>Iodamoeba butschlii</i> 布氏噬碘阿米巴			
	<i>Endolimax nana</i> 微小内延阿米巴			
Oral cavity 口腔	<i>Entamoeba gingivalis</i> 齿龈内阿米巴			
Brain	<i>Acanthamoebidae sp</i> 棘阿米巴	Acanthamoebidae 棘阿米巴科		
	<i>Naegleria fowleri</i> 福氏耐格里阿米巴	Dimastiamoebidae 双鞭阿米巴科		
erythrocyte	<i>Plasmodium vivax</i> 间日疟原虫	Plasmodidae 疟原虫科	Eucoccidiida 真球虫目	Sporozoea 孢子纲
	<i>Plasmodium malariae</i> 三日疟原虫			
	<i>Plasmodium falciparum</i> 恶性疟原虫			
	<i>Plasmodium ovale</i> 卵形疟原虫			

Alveolus 肺泡	<i>Pneumocystis carinii</i> 卡氏肺孢子虫	Undefined		
Karyocyte 有核细胞	<i>Toxoplasma gondii</i> 刚地弓形虫	Toxoplasmatidae	Eucoccidiida 真球虫目	
Tissue 组织	<i>Sarcocystis sp.</i> 肉孢子虫	Sarcocystidae 肉孢子虫科		
Epithelium of small intestine mucosa 小肠黏膜上皮	<i>Isospora sp</i> 等孢子虫	Eimeriidae 爱美虫 科		Kinetofrag- Minophorea 动基裂纲
	<i>Cryptosporidium sp</i> 隐孢子虫	Cryptosporidae 隐孢子虫科		
Colon	<i>Balantidium coli</i> 结肠小袋纤毛虫	Balantidiidae 小袋科	Trichostomati da 毛口目	

II ENTAMOEBA HISTOLYTICA(溶组织内阿米巴)

Species in the **Endamoebidae** (内阿米巴科) are parasites or **commensals** (共生物) of the digestive of **arthropods** (节肢动物) and **vertebrates** (脊椎动物). The genera and species are differentiated on the basis of nuclear structure. Species of **Entamoeba** (内阿米巴属) are found in both vertebrate and **invertebrate** (无脊椎动物) host. Several species of the protozoan parasite genus *Entamoeba* infect humans, *Entamoeba coli* (结肠内阿米巴), *Entamoeba hartmanni* (哈门氏内阿米巴), *Entamoeba dispar* (迪斯帕内阿米巴) and *Entamoeba histolytica*. *E. histolytica* is the only species known to cause disease. The other (non-pathogenic) species are important because they may be confused with *E. histolytica* in diagnostic investigations.

Entamoeba histolytica causes **amoebiasis** (阿米巴病). 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 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 disease. The larger form 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 species 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, prompted Brumpt (1925, 1949) to suggest that there were in fact two morphologically identical species which he proposed should be named *E. dispar*, as an asymptomatic species and *E. dysenteriae*, the causative agent of symptomatic amoebiasis. This was not widely accepted and led to much discussion on diagnosis and treatment strategies.

The establishment of distinct non-pathogenic and pathogenic strains of *Entamoeba* on the bases of their isoenzyme patterns or zymodemes, by Sargeaunt et al (1987), and more recently by differences observed in antigenic specificity, and at the level of genomic DNA and ribosomal RNA and also in the epidemiological picture, has confirmed Brumpt's original assertion of two distinct species. This has lead to the recognition and acceptance of the establishment of two distinct species. *Entamoeba dispar* - (Brumpt 1925) Asymptomatic commensal in which carriers have no amoebic antigens in their serum, do not mount a serum anti-amoebic antibody response. *Entamoeba histolytica* (Schaudinn 1903) Although most infections with this pathogenic organism are also asymptomatic, carriers develop an anti-amoebic antibody response have amoebic antigens in serum samples may have evidence of colonic invasion. Only about 10% of people infected with this species present with invasive amoebiasis.

Its life cycle, general morphology, and overall appearance, with the exception of size, are identical to those of *E. histolytica*, but their antigenicity and gene are completely different.

MORPHOLOGY

The parasite occurs in 3 stages: trophozoite, precyst and cyst.

Trophozoite(滋养体): it is the invasive form of the parasite and is present in the lumen and in the wall of the large intestine. The diameter of most trophozoites falls into the range of 20 to 30 μ m, occasional specimens are small as 10 μ m or as large as 60 μ m. 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 (铁苏木素). 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 (Fig1)

Food vacuoles (食物泡) are common in the cytoplasm of active trophozoites and many contain host erythrocytes (红血球) 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.

Cyst (包囊) : In a normal asymptomatic infection, the amoebae are carried out in formed stools. As the fecal matter passes posteriad and becomes dehydrated (脱水), 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 (包囊形成), the trophozoite disgorges any undigested food it may contain and condenses into a sphere, called the **precyst** (包囊前期). A precyst is so rich in glycogen (糖原) that a large glycogen vacuole may occupy most of the cytoplasm in the young cyst. The **chromatoid bars** (拟染色体) 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*.

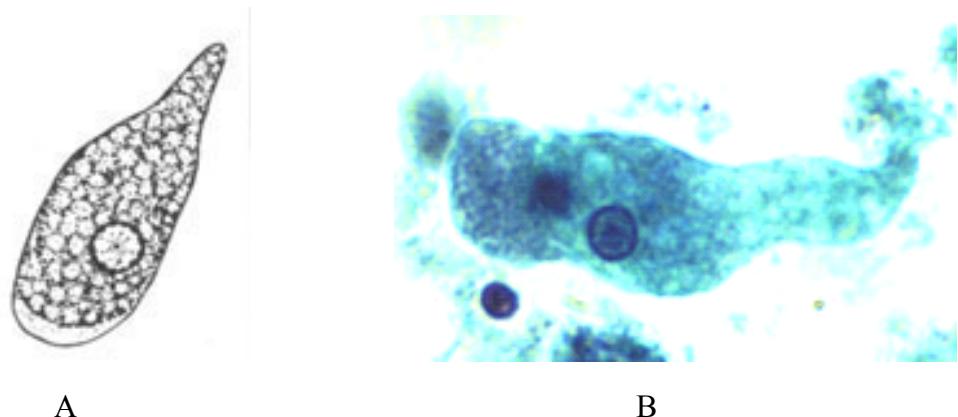


Fig II-2-1 Trophozoites of *Entamoeba histolytica* (A: Line drawing; B Stain specimen)

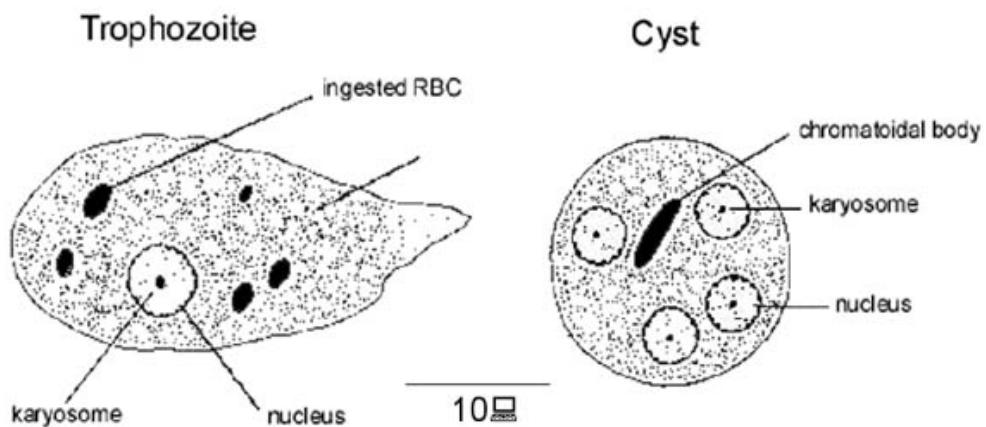


Fig II-2 Trophozoite and cyst of *Entamoeba histolytica*

The precyst rapidly secretes a thin, tough hyaline **cyst wall** (包囊壁) 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 5 μm . 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** (拟染色体) disappear. In semiformed stools one can find precysts and cysts with one to four nuclei, but **quadrinucleate cysts** (四核包囊) are most common in formed stools. The mature cysts can survive outside the host and can infect a new one.

LIFE CYCLE

The life cycle of *E. histolytica* is simple and is completed in a single host, the man. **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** (脱囊) in the small intestine. The cyst wall is lysed by intestinal trypsin liberating a single trophozoite with 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 are 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 abscess preceded by non-suppurative infection of the liver.

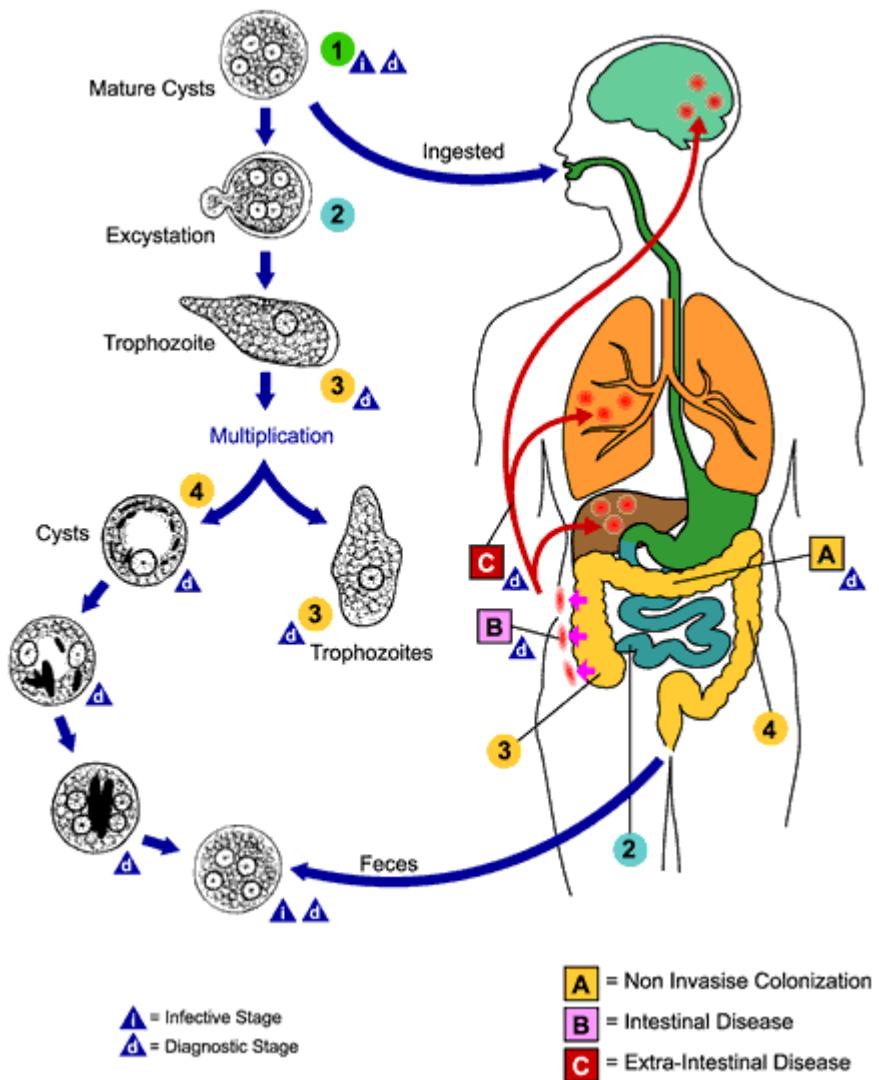


Fig II – II – 3 Life cycle of *E. histolytica*, Adapted from parasite image library of CDC, USA

The **mature cysts** (成熟包囊) 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. They are rapidly killed by putrefaction, desiccation, and temperatures below -5 °C and above 40 °C . 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.

PATHOGENESIS AND CLINICAL MANIFESTATIONS

Pathogenic mechanism: Amoebiasis (阿米巴病) 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 various effector mechanisms of the host.

Initially, the slow transit 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 (区系) 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 (凝集素) of the amoebae known as galactose(Gal) or N-acetyl-O-galactosamine(gal NAC) inhibitable surface lectin (the Gal/gal NAC lectin 半乳糖/乙酰氨基半乳糖凝集素). After adherence, trophozoites kill target cells in the intestinal mucosa, only by direct contact and also by secreted cytotoxins (细胞毒素). The cytolysis occurs within 20 minutes of the amoebic adherence.

E. histolytica also secretes numerous proteolytic enzymes (水解蛋白酶) that appear to be involved in various pathogenic processes. Cathepsin B proteinase (组蛋白酶) is responsible for dissolution of extra cellular matrix containing cells and tissue components. Amoebic glycosidases (糖苷酶) such as β -glucosaminidase (氨基葡萄糖苷酶) and a surface membrane-associated neuraminidase (神经氨酸苷酶) cause degradation of mucosal 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 (胃酸屏障) kills amoebic trophozoites, and rapid intestinal transit reduces the time for amoebae to colonise on the intestinal mucosa. Also colonic mucus inhibits amoebic adherence to epithelial cells.

The specific immunity involves both humoral and cell mediated immunity. Humoral

immunity (体液免疫) 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 (蛋白水解酶), which enable them to penetrate 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 (粘膜肌层) 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 muscularis mucosae and then begin eroding laterally, causing broad, shallow areas of necrosis (坏死). The tissues may heal nearly as fast as they are 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 (粘膜下层), 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 (腹膜炎). Surgical repair of perforation is difficult because a heavily ulcerated colon becomes very delicate.

Sometimes a granulomatous mass called ameboma(阿米巴肿), forms in the wall of the intestine and may obstruct the bowel. It is a result 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: (1) Dysenteric; (2) Nondysenteric colitis

B. Extraintestinal amebiasis:

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

(2) Pulmonary

(3). Other extraintestinal foci (very rare)

E. histolytica infection in man is variable. In endemic area, nearly 90% of individuals harbouring *E. histolytica* in their intestinal tract are asymptomatic cyst passers, while remainders have invasive intestinal amoebiasis or extra-intestinal invasive amoebiasis such as amoebic liver abscess.

Incubation period is usually long and often indefinite. It may be short in rare instances.

1) Intestinal amoebiasis(肠阿米巴病): Intestinal amebiasis is the most common form of infection and may be asymptomatic (无症状的). Certain patients with intestinal amebiasis have vague and nonspecific abdominal symptoms. Acute amoebiasis patients have more definite symptoms, such as diarrhea or dysentery(痢疾, 腹泻), abdominal pain and cramping, flatulence (肠胃气涨), anorexia (食欲减退), weight loss, and chronic fatigue. This term should be reserved for those who actually have dysentery.

① Asymptomatic cyst passers: It is the common clinical form of intestinal amoebiasis. Gastrointestinal manifestations are not specific. It consists of colicky lower abdominal pain and increased frequency of bowel movements with loose watery diarrhoea. The condition is intermittent and chronic in nature.

② Symptomatic intestinal amoebiosis

Non-dysenteric colitis: It is a well recognised condition. It is usually chronic. Symptomatology consists of intermittent diarrhoea, abdominal pain, flatulence and loss of weight. These cases have less colonic inflammation and small amoebic ulcers, amoebae in their stool and associated antibodies in the serum of the patient. They respond well to treatment with anti-amoebic drugs.

Acute amoebic dysentery: It is the common form of invasive intestinal amoebiasis. The condition is characterised by the presence of blood and mucus in the stool, accompanied by

abdominal pain, tenderness, tender hepatomegaly (肝脾肿大) and rectal tenesmus. Fever is uncommon, present in less than one-third of the cases.

The condition can be differentiated from that of bacillary dysentery by the demonstration of RBCs, Charcot-Leyden crystals and haematophagous amoebic trophozoites in the amoebic dysenteric stool.

③Complications of symptomatic intestinal amoebiasis: Thick megacolon (巨结肠) occurs in less than 0.5 percent cases. It is resistant to treatment with anti amoebic drugs, hence requires colectomy.

Fuhninan amoebic colitis (阿米巴性结肠炎) has a high mortality and tends to occur more in pregnant women, malnourished persons and persons receiving corticosteroids (皮质类固醇). The onset is abrupt with high fever, widespread abdominal pain, leucocytosis (白细胞增多), profuse bloody ,mucosal diarrhoea with tenesmus (里急后重). The patients have necrotic involvement of their colon. Colonic perforation and haemorrhage frequently occur in this condition.

Amoeboma (阿米巴肿) is a pseudo-tumoral condition. Lesion may be single or multiple and occurs commonly in the colon, caecum or rectum. Anti-amoebic antibodies are present in the patient serum.

Complicated intestinal amoebiasis: This condition includes peritonitis, perianal ulceration, urogenital infection, colonic stricture, intussusception (肠套叠) and haemorrhage. These complications relatively are uncommon.

2) Extra-intestinal amoebiasis(肠外阿米巴病)

Clinical manifestations of extra-intestinal amoebiasis depends upon the organ involved. It can be Hepatic amoebiasis, pulmonary amoebiasis , cerebral amoebiasis, genitourinary amoebiasis, and splenic amoebiasis.

①Hepatic amoebiasis (肝阿米巴病) begins with the hepatic involvement (non suppurative amoebic hepatitis) and progress to form suppurative lesions in the liver (amoebic liver abscess).

Non-suppurative amoebic hepatitis(非化脓性阿米巴肝炎): This refers to a syndrome (综合症)of tender hepatomegaly, right upper quadrant pain, fever and leucocytosis in persons with amoebic dysentery. This results from non-specific peripheral inflammation of the liver, occurring during colitis.

Amoebic liver abscess(阿米巴肝脓肿): Onset is insidious. The condition is associated with fever, sweating, loss of weight and abdominal pain. Nearly, half of the patients presenting with acute manifestations have a single abscess situated most common only in the postero-superior surface of the right lobe of the liver.

Abdominal pain and tenderness in the right hypochondrium (季肋部) is the earliest important manifestation. It is caused by stretching of the liver capsule. Abdominal pain is frequently referred to the shoulder and is accompanied by a non-productive cough. On physical examination, the lower part of the liver is palpable below the costal margin and is tender. Point tenderness can be elicited in the postero-lateral part of the lower-right intercostal space. In less than half of the cases, hepatomegaly is usually present. In the base of the right lung, dullness and rales are common. Peritoneal signs and jaundice are unusual.

②Pulmonary amoebiasis(肺阿米巴病): It is the most common complication (并发症) of amoebic liver abscess and is caused by rupture of a superior right lobe abscess through the diaphragm (横隔膜) into the lung parenchyma. It occurs in 10 to 20 percent of patients. Cough, pleuritic pain and dyspnoea are the common clinical manifestations.

③Cerebral amoebiasis(脑阿米巴病): Amoebic brain abscess is very unusual. The abscess is single, small and is located in the cerebral hemisphere. It is difficult to diagnose the condition clinically.

④Genito-urinary amoebiasis(泌尿生殖系阿米巴病): It is a rare condition. Amoebiasis of the penis caused by *E. histolytica* is acquired during vaginal or anal intercourse. In females, recto vaginal fistula causes amoebic trophozoites spread to the genito urinary tract.

⑤Splenic amoebiasis(脾阿米巴病) is very unusual and is caused by transmission of amoebic trophozoites directly through an adherent splenic flexure of the intestine.

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, serodiagnosis, biochemical diagnosis, and radio-imaging diagnosis.

Parasitic diagnosis (病原诊断)

The specific diagnosis of intestinal amoebiasis is established by the demonstration of *E. histolytica* in the stool, rectal exudate and material collected from the base of rectal ulcers.

The stool is collected in wide-mouthed, chemically clean containers. Substances that interfere with stool examination should not be administered in an individual at least 10 days before the collection of stool. These include Kaolin, bismuth, barium sulphate, antimicrobial and anti-malarial drugs, liquid paraffin and anti-diarrhea agents. Since excretion of cysts in the stool is often intermittent, at least three consecutive specimens should be examined before excluding the diagnosis of amoebiasis.

Stool should be examined immediately within 15 minutes of the passage, for the detection of trophozoites. The cysts can be demonstrated even in the 3 days old formed stool specimen.

Stool is usually examined by microscopy, concentration and culture.

1) Microscopy: Trophozoites are demonstrated in the saline wet mount of fresh diarrhoeal stool. These are identified by their unidirectional motility with the help of finger-like pseudopodia. Permanent staining of the faecal smear by iron-haematoxylin or trichrome staining allows the best identification of haematophagous trophozoites of *E. histolytica*. Cysts can be demonstrated by iodine wet mount preparation of the diarrhoeal and formed stool. These cysts need to be differentiated from the cysts of other amoebae.

2) Concentration Concentration is a better method for the concentration of amoebic cysts in the stool. No method is available yet for concentration of trophozoites. The concentration method is useful when the numbers of amoebae in the stool are scanty.

3) Culture: Robinson's medium and NIH polyxenic culture media are frequently used for culture and isolation of the amoebae from the stool. Serum, bacterial flora and starch present in the media provide nutrients and growth factors for *E. histolytica*.

Stool culture is helpful especially in the cases of chronic and asymptomatic intestinal infections, excreting less number of cysts in the faeces.

Rectal exudate: The amoebic trophozoites are demonstrated in the exudate covering the rectal mucosa.

Rectal ulcer tissue: Trophozoites also are demonstrated, but less frequently, in the necrotic tissue at the base of rectal ulcer.

For amoebic liver abscess, demonstration of amoebic trophozoites in the aspirated pus by

microscopy or culture establishes the specific diagnosis of amoebic liver abscess. The parasitic diagnosis, however, is difficult. Trophozoites can only be demonstrated in the pus in less than 15% of cases of amoebic liver abscess.

Serodiagnosis.

1) Antibody Detection Amoebic antibodies frequently appear in the amoebiasis. Antibody detection is most useful in patients with extraintestinal disease, i.e., amebic liver abscess, when organisms are not generally found on stool examination. Indirect haemagglutination (IHA), indirect fluorescent antibody (IFA), enzyme-linked immunosorbent assay (ELISA), etc., are frequently used tests to detect the serum amoebic antibodies amoebiasis. However, these serological test are of no or little value in the diagnosis of asymptomatic cyst passers, as amoebic antibodies fail to appear in their sera.

2) Antigen Detection Antigen detection may be useful as an adjunct to microscopic diagnosis in detecting parasites and to distinguish between pathogenic and non-pathogenic infections. Recent studies indicate improved sensitivity and specificity of fecal antigen assays with the use of monoclonal antibodies which can distinguish between *E. histolytica* and *E. dispar* infections. At least one commercial kit is available which detects only pathogenic *E. histolytica* infection in stool; several kits are available which detect *E. histolytica* antigens in stool but do not exclude *E. dispar* infections.

Molecular diagnosis

In reference diagnosis laboratories, PCR is the method of choice for discriminating between the pathogenic species (*E. histolytica*) from the non-pathogenic species (*E. dispar*).

Radio diagnosis

Various imaging techniques have been used to demonstrate the presence of space occupying amoebic lesions in the liver and other organs. These include ultrasound, CT scan, magnetic resonance imaging (MRI) and GA scan. None of these methods however are absolutely specific. These can not differentiate amoebic liver abscess from those of pyogenic liver abscess and tumour.

Ultrasound is rapid, slightly sensitive than CT scan for the amoebic liver abscess. The CT scan is sensitive but not specific for amoebic liver abscess. MRI is sensitive like those of CT and ultrasound.

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*. 50 million cases of invasive amoebic diseases have been reported from the world . Every year, more than 100,000 persons die due to amoebic disease.

Source of transmission and infection

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 are susceptible for *E. histolytica*.

Transmission ways

Infection is transmitted from one person to another by following methods:

1) Faecal-oral route Amoebiasis is transmitted orally by ingestion of water, vegetable and food contaminated by faeces containing cysts. Water is contaminated by accidental leakage of sewage into treated water supplies. Water and food also are contaminated by unhygienic handling of food by food handlers such as cook. The infection is transmitted among the individuals with poor personal hygiene. In areas, where human faeces are used as fertilizers in the fields for cultivation of vegetables, crops, etc, the infection is transmitted by eating those vegetables raw.

2) Vectors Flies and cockroaches mechanically may transmit cysts from the faeces to the unprotected food and water.

3) Sexual contact *E. histolytica* is sexually transmitted among sexually promiscuous male homosexuals. The amoebae are transmitted by the sexual practice that allows faecal-oral contact.

E. histolytica is a leading cause of diarrhoea worldwide. It is an important cause of diarrhoea in persons with the acquired immunodeficiency syndrome(AIDS). Amoebiasis tends to be severe in pregnant and lactating mothers, and in children especially in neonates.

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.

Amoebae may be found in the lumen of the intestine, in the intestinal submucosa or in the extra-intestinal sites such as liver, lungs, etc. None of the amoebicidal drugs available now are effective against the amoebae found in all these sites. These amoebicidal drugs depending upon their sites of action can be grouped as follows

Luminal amoebicides: They act on the amoebic trophozoites present in the lumen of the bowel. They are ineffective against tissue amoebae. These include: a) diloxanide fluoride, b) diiodohydroxyquin and c) paromomycin.

Tissue amoebicides: They act on tissue amoebae present in different tissues. The tissue amoebicides which act on all the tissues include: metronidazole (灭滴灵), tinidazole (硝矾咪唑), emetine hydrochloride and 2-dehydroemetin. Amoebicides which act only on liver tissue is chloroquine (氯喹). Tetracycline (四环素) 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.

1) Individual prophylaxis (个人防护) 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.

2) 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 management of cases by an early and rapid detection and subsequent treatment of cases.

III PATHOGENIC FREE-LIVING AMOEBAE

Free-living amoebae are small, free-living amoebae, which under unknown conditions, become opportunistic pathogens in humans. These belong to two genera: *Naegleria* (耐格里属) and *Acanthamoeba* (棘阿米巴属). These free-living amoebae are widely distributed in the soil and water habitats throughout the world. Free-living amoebae are ubiquitous in nature, found commonly in the soil and water.

Naegleria fowleri (福氏耐格里阿米巴) and *Acanthamoeba spp* (棘阿米巴), commonly found in lakes, swimming pools, tap water, and heating and air conditioning units. While only one species of *Naegleria* is known to infect humans, several species of *Acanthamoeba* are implicated, including *A. culbertsoni* (卡氏棘阿米巴), *A. polyphaga*, *A. castellanii*, *A. astronyxis*, *A. hatchetti*, and *A. rhysodes*.

Naegleria fowleri causes **primary amoebic meningoencephalitis** (PAM, 原发性阿米巴脑膜脑炎). The condition is an acute, fulminant and rapidly fatal infection of the **central nervous system** (CNS, 中枢神经系统). Till now, more than 140 cases of PAM have been reported from different parts of the world.

Fowler and Carter in 1965, were the first to describe the free-living amoebae as the causative agents of primary amoebic meningoencephalitis in humans from South Australia. The following year, four cases were reported from USA, one from Florida by Bull and three from Texas by Patras and Andujar. Since then, the cases of PAM in humans have been reported almost from every part of the world. The term, primary amoebic meningoencephalitis was first coined by Bull to distinguish fatal human disease caused by the direct invasion of the CNS by free-living amoebae, from that caused by relatively rare invasion of the brain by enteric amoeba, *Entamoeba histolytica*. The latter secondarily invades the CNS from its primary site in the colon.

Acanthamoeba castellani, *Acanthamoeba culbertsoni*, and *Acanthamoeba astronyxis* can cause opportunistic **granulomatous amoebic encephalitis** (GAE, 肉牙肿性阿米巴性脑炎) and opportunistic infections of the lung and skin in the immunocompromised hosts including the AIDS or debilitated persons. In healthy persons, they cause **Acanthamoeba keratitis** (棘阿米巴角膜炎).

Naegleria fowleri (福氏耐格里阿米巴)

Naegleria are small, free-living amoebae widely distributed in the soil and fresh water. In man, these are present in the throat and nasal cavity.

MORPHOLOGY

The amoeba has 3 stages in its life cycle: trophozoite, a temporary flagellar stage known as amoeboflagellate and cyst.

Trophozoite

It is the vegetative or feeding stage of the amoeba (Fig. II – III – 1). It measures 10-15 μm in diameter and possesses a granular cytoplasm and a distinct ectoplasm. It is characterised by the presence of a prominent nucleus and a large endosome or karyosome surrounded by a halo. Trophozoite is actively motile with the help of a broadly rounded, granule-free projection that originates from the surface known as pseudopod. The latter helps to ingest bacteria, yeast cells and cellular debris; and may also serve as an organelle of attachment.

Giemsa or Wright-stained trophozoite usually shows a large karyosome and may or may not show any contractile vacuole.

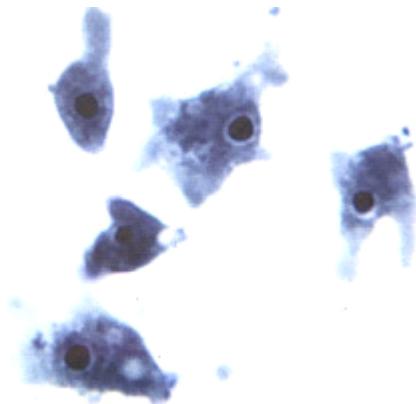


Fig II – III – 1 *Naegleria fowleri* trophozoites, cultured from cerebrospinal fluid. These cells have characteristically large nuclei with a large, dark staining karyosome. The amoebae are very active and extend and retract pseudopods. Trichrome stain. From a patient who died from primary amebic meningoencephalitis in Virginia. (Adapted from parasite image library of CDC, USA)

***Amoeboflagellate* (鞭毛型)**

It is pear-shaped with a flagellar apparatus at the broader end (Fig. 2). The flagellar apparatus consists of two terminal flagella, two basal bodies, microtubules and a single striated rootlet. The flagellated stage may exhibit a rapid forward movement or a slow spinning movement in circles.

Cyst

It is the resistant form of the parasite, which offers protection from desiccation, and shortage of food. It is round, measures 7 to 10 μm in diameter and is surrounded by a smooth double-layered

1 µm thick cyst wall (Fig. 3). It consists of a single nucleus and some cytoplasmic vacuoles, such as contractile vacuoles and food vacuoles. In stained preparations the vacuoles can only be demonstrated as fine granules but not the nucleus. Cysts usually are not seen in clinical specimens because the infection is so rapid and fatal that the patient dies before trophozoites encyst to the cysts.

LIFE CYCLE

Naegleria completes its life cycle through a cycle of asexual generation in man (Fig. II – III 4).

Naegleria fowleri is found in nature in warm water bodies as ameboid and amoeboflagellate trophozoites. Cysts also occur in nature, but not in human infections. Infection occurs during swimming or diving with the parasites gaining access, through the olfactory neuroepithelium, to the brain.

The trophozoites are neurotrophic (神经营养), they invade and penetrate the CNS, localise largely in the olfactory mucosa (嗅粘膜) and olfactory bulbs. The trophozoites enter the ventricular (心室的) system and reach the choroid (脉络膜) plexus. These then destroy the ependymal layer of the third, fourth and lateral ventricles, and produce acute ependymitis. They multiply by a process known as promitosis, during which an intact nuclear membrane, demonstrable by electron microscope, is present. Trophozoites only are found in the pathologic lesions in man.

Outside the human host, in non-nutrient media such as sterile distilled water or Page's saline, the trophozoites are transformed to amoeboflagellates usually within 2 hours. The amoeboflagellates are temporary, non-feeding and non-dividing forms of the parasite and have the potency to revert back to trophozoites within 24 hours. The trophozoites during unfavourable environmental conditions such as depletion of nutrition or even in the presence of drugs, round up and form cysts.

The cyst is the non-dividing resistant form of the parasite and when conditions become favourable, it excysts to trophozoite.

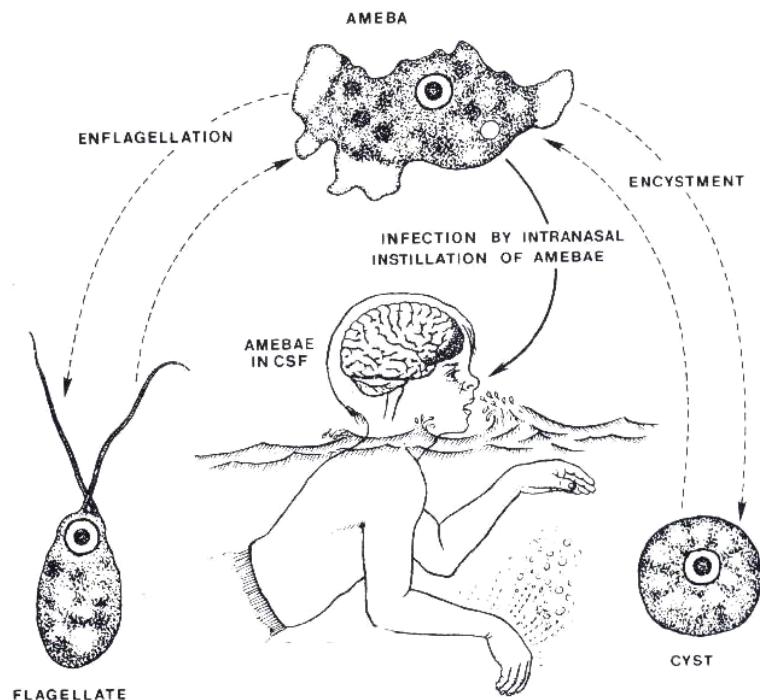


Fig II –III –4 Life cycle of *Naegleria fowleri* (adapted from E.K. Markell, 1999)

PATHOGENESIS AND CLINICAL MANIFESTATIONS

Trophozoites are neurotrophic (神经营养). Invasion of the CNS is facilitated by active phagocytosis of trophozoites by sub-lenticular cells of the olfactory neuro-epithelium (嗅神经上皮细胞).

The trophozoites invade the CNS and reach the subarachnoid space (蛛网膜下隙) and brain through the olfactory neuro-epithelium and the fascicles of the olfactory nerves. The subarachnoid space, which is highly vascularised facilitates the dissemination of amoebae to other areas of the CNS.

Cerebral oedema, congestion of leptomeningeal vessels, uncal and cerebellar tonsillar herniations, and acute rhinitis (鼻炎) are the characteristic macroscopic pathological lesions seen in primary amoebic meningoencephalitis (脑膜脑炎). The cerebral hemisphere is congested, haemorrhagic and necrotic. The olfactory mucosa and olfactory bulbs are the most affected areas. Only trophozoites are found in the lesions. Cysts are absent.

Incubation period ranges from a short 2 to 3 days to as long as 7 to 15 days.

Clinical symptoms of primary amoebic meningoencephalitis (PAM) are abrupt in onset and brief in duration. The condition shows an acute, fulminant course. The initial symptoms are characterised by a sudden onset of severe, persistent bitemporal (两颞) or bitemporal headache. These symptoms are followed by nausea, projectile vomiting, fever (from 38.2 to 40 °C) and signs of meningeal irritation and encephalitis.

Abnormalities in tastes (ageusia, 丧失味觉) or smells or generalised seizures may be noted in some instances. Drowsiness, convulsions (抽搐), photophobia (畏光) and coma may occur during the later stage of infection. The condition is fatal. Death occurs due to pulmonary oedema or cardio-respiratory arrest within a week of appearance of the first symptom.

Since the course of infection is fulminant and rapid, the patient with PAM dies usually within a short period of 5 to 10 days. Hence, detectable level of specific antibodies are not produced in the serum, during course of the disease. The role of cell mediated immunity (CMI) in resistance against *Naegleria* infection, which has been studied in guinea pigs, is also inconclusive.

DIAGNOSIS

Recent history of swimming in thermal or stagnant water (静水) or of contact with fresh water, mud or dust, 2 to 6 days prior to the onset of symptoms of meningeal irritation and the age of the patient (usually children and young adults) may suggest a possible diagnosis of primary amoebic meningoencephalitis (PAM).

The final diagnosis of the condition always is parasitic. The diagnosis can be made by microscopic examination of cerebrospinal fluid (CSF, 脑脊液). A wet mount may detect motile trophozoites, and a Giemsa-stained smear will show trophozoites with typical morphology.

The serological tests are not useful in the diagnosis of primary amoebic meningoencephalitis. Computerised axial tomography (CAT) recently is used to demonstrate pathological changes in the cerebral hemisphere.

EPIDEMIOLOGY

N. fowleri is a thermophilic free-living amoeba found world wide in the warm water lakes, spring, etc. Soil, polluted water, sewage, sludge, swimming pools and infected man harbouring *N. fowleri* as commensal in the nose and throat are tire sources and reservoirs of infection. Both

trophozoites and cysts are the infective stages. The infection is transmitted to man mainly by inhalation of dust and aspiration of water contaminated with both the cysts and trophozoites, and possibly by inhalation or aspiration of aerosols containing cysts.

Children and young adults are most commonly affected, particularly during summer months. Majority of human infections with *N. fowleri* have been associated with swimming in fresh water and a few cases with bathing in tap and hot water.

PREVENTION AND CONTROL

Majority of the cases of primary amoebic meningoencephalitis have proved to be fatal and various amoebicidal agents have been tried unsuccessfully. Till date, only 4 cases have survived *N. fowleri* infections.

All these cases were diagnosed and treated early. Three cases responded successfully to treatment with amphotericin (两性霉素) B intravenously (25 mg/day and subsequently 50 mg/day) and intrathecally (0.1 mg alternate day) and later intraventricularly in a dose of 0.1 mg on every other day.

A combination of intravenous and intrathecal amphotericin B, miconazole (双氯苯咪唑) and oral rifampicin (利福平) were successful in curing completely a 9 year old girl from PAM caused by *N. fowleri*.

Avoidance of contact with stagnant or thermal water (if *Naegleria* is detected in these source) may be the only method for prevention of the disease. Even hyperchlorination of swimming pool water is not protective against infection.

***Acanthamoeba* species (棘阿米巴)**

Acanthamoeba castellani, *A. culbertsoni*, and *A. astronyxis* can cause opportunistic granulomatous amoebic encephalitis (GAE, 肉芽肿性阿米巴脑炎) and opportunistic infections of the lung and skin in the immunocompromised hosts including the AIDS or debilitated persons. In healthy persons, they cause Acanthamoeba keratitis.

Acanthamoeba species live worldwide in the soil, salt water and fresh water. In man, they have been found to remain as commensals in the pharynx.

MORPHOLOGY

It has only two stages: trophozoite and cyst.

Trophozoite: It is slightly larger than that of *Naegleria* and is extremely variable in shape and size. Trophozoite is 10-40 µm in diameter. Cytoplasm is finely granular. It contains mitochondria and a single nucleus with a large dense central prominent nucleolus surrounded by a halo. The trophozoite is identified by the presence of distinctive, slender, spine-like projections of the plasma membrane.

Cyst: The cysts vary in shape and may be either polygonal, spherical or star-shaped. The cysts are double walled and measure 15 to 20 µm in diameter. The smooth inner wall of the cyst has pores or opercula at a number of points. The cysts contain a centrally located nucleus with a large dense karyosome surrounded by a clear halo as in the trophozoites.

LIFE CYCLE

Acanthamoeba spp. occur in the same environments of *Naegleria*, but are also found in soil and dust as well as more restricted liquid environments such as humidifiers and dialysis units. (They can also be cultured from the upper respiratory tract of some healthy individuals.) *Acanthamoeba spp.* do not have an amoeboflagellate form, and cysts can be found in human infections. Infections due to *Acanthamoeba spp.* occur more frequently in debilitated or chronically ill individuals, and reach the central nervous system by hematogenous dissemination from foci in the lungs, skin, or sinuses.

Man acquires infection by inhalation of aerosol or dust containing cysts and trophozoites and possibly, by direct invasion through broken skin (Fig. 5). The trophozoites reach the lower respiratory tract particularly the lungs. From the lungs, they invade the central nervous system (CNS) through the blood stream. The trophozoites multiply by binary fission. The nuclear membrane which is present in *Acanthamoeba* disappears while replicating. No such nuclear membranes are present in *Naegleria*. The growing trophozoites invade the posterior fossa, basal ganglia, base of the cerebral hemisphere and cerebellum.

PATHOGENESIS AND PATHOLOGY

Acanthamoeba species cause opportunistic infections in man. They enter the human host via respiratory tract through the aerosols and dusts. From the lung, they disseminate by haematogenous route to the brain and produce granulomatous amoebic encephalitis.

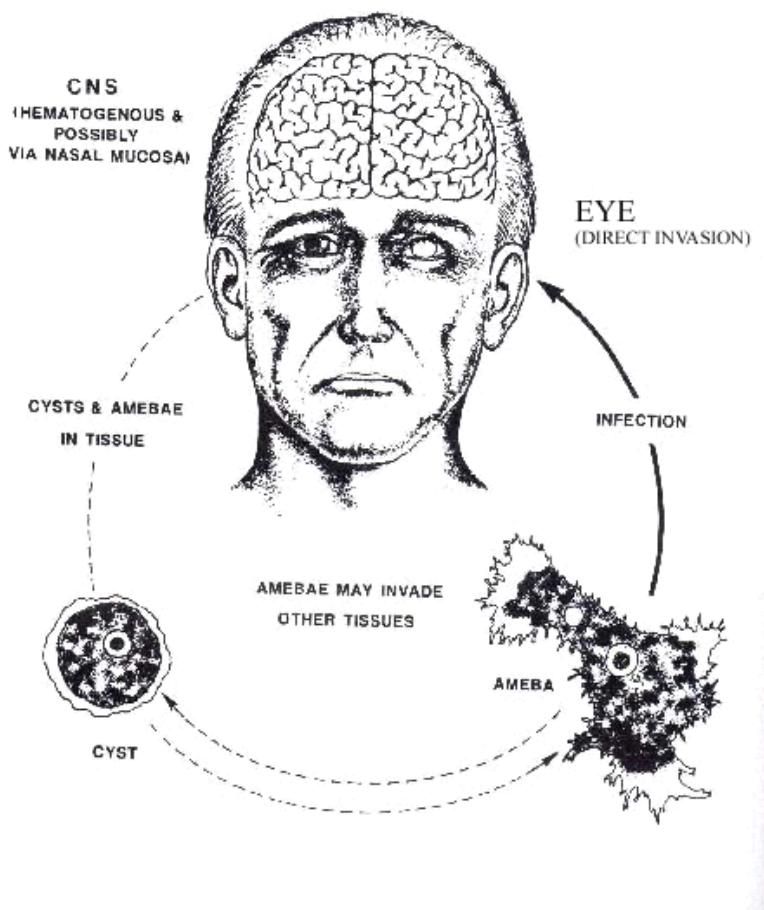


Fig II – III – 5 Life cycle of Acanthamoeba species (adapted from E.K. Markell, 1999)

The cerebral hemisphere in granulomatous amoebic encephalitis (GAE) is oedematous with focal cortical softening, haemorrhage and abscess. Bilateral unical or cerebellar tonsillar herniation may be present. Necrotising granulomatous lesions are mainly found in the cerebellum, midbrain and brain stem. Both trophozoites and cysts are found in these lesions.

In healthy persons, Acanthamoeba species cause keratitis. It is caused by the direct invasion of the amoebae through minor trauma in the cornea caused by the use of soft contact lenses. The amoebae enter the eye by the use of contaminated contact lens, cleansing solution or swimming contaminated water. Both amoebic cysts and trophozoites are found within the corneal stroma. It is usually associated with moderate granulomatous inflammation consisting of epithelial giant cells.

Granulomatous amoebic encephalitis (GAE) and Acanthamoeba keratitis are the two distinct clinical entities caused by Acanthamoeba. Granulomatous amoebic encephalitis (GAE) is usually seen in the immuno-compromised and debilitated persons. In contrast to PAM, GAE shows an insidious onset of symptoms with focal neurological symptoms that resemble clinical manifestations of simple or multiple space occupying lesions in the brain.

The signs and symptoms include mental anomalies, seizures, fever, hemiparesis (轻偏瘫), headache, meningism (假性脑脊膜炎) and visual anomalies. The disease worsens within a period of one to several weeks and ends in coma and finally death.

Acanthamoeba keratitis occurs in healthy individuals. It is generally associated with the use of contaminated contact lens. It is a chronic, progressive, ulcerative disease of the eye. Ulcers in the cornea (角膜) are painful and resistant to treatment with usual antifungal, antibacterial and antiviral agents.

The affected cornea shows a characteristic annular infiltration and congested conjunctiva, if not successfully treated, the disease progresses to corneal perforation which results in the blindness and corneal prolapse.

DIAGNOSIS

Granulomatous amoebic encephalitis (GAE) is rarely diagnosed before death. Most cases have been diagnosed post-mortem or shortly before death. Computerised axial tomography (CAT) may show multiple lucency, non-enhancing lesions in the cortex of the brain.

Laboratory diagnosis is made by demonstration of Acanthamoeba trophozoite and cyst in the brain biopsy specimen and in the cerebro-spinal fluid (CSF). Brain biopsy, frequently is the only way, for the specific diagnosis of the condition in more than 75% of cases. Trophozoites seldom are cultured or are demonstrated in the wet mount preparation of the CSF.

Acanthamoeba keratitis: Diagnosis of the condition is made by demonstration of the amoebae in the corneal scrapings and biopsy specimens by microscopy and culture. Wet mount preparation of the corneal scrapings shows motile trophozoites. Typical double-walled cysts and trophozoites can be demonstrated by staining them with haematoxylin-eosin trichrome, Wright, Giemsa and Picric acid-Schiff (PAS) stains. These also can be demonstrated by immunofluorescence method.

The amoebae may be cultured by inoculating contact lens or contact lens saline solution in non-

nutrient agar with Page's solution containing Escherichiacoli, Aero-bacter aerogenes or Gram-negative bacteria, at 37°C.

EPIDEMIOLOGY

Acanthamoeba infection frequently occurs in the immunocompromised hosts. More than 50 cases of granular amoebic encephalitis and 250 cases of amoebic keratitis have been reported from various parts of the world.

Soil, water and air are the sources of infection for GAE. Contaminated contact lens is the main source of infection for Acanthamoeba keratitis.

Both cysts and trophozoites are the infective stages. GAE is transmitted by inhalation of air, aerosol or dust containing both cysts and trophozoites of Acanthamoeba, and possibly through the broken skin. Acanthamoeba keratitis is acquired through eye trauma, and prolonged use of contact lenses.

GAE occurs predominantly in the immuno-compromised and debilitated persons with AIDS, liver disease, diabetes, skin ulcers and in the persons receiving kidney transplantation, and corticosteroid treatment.

Acanthamoeba keratitis also occurs in otherwise healthy persons. It is frequently seen in persons wearing contact lenses while swimming or using contaminated solutions to clean soft lenses.

PREVENTION AND CONTROL

No effective therapy is available for the treatment of GAE. Acanthamoeba are sensitive to sulphonamides (硫胺类), clotrimazole and polymyxin B, both in vitro and in vivo. Drug treatment of Acanthamoeba keratitis has been more successful than that of GAE. It has responded well to topical miconazole, propamidine isotheonate and antibiotics followed by keratoplasty.

The preventive measures to control granulomatous amoebic encephalitis is difficult as the amoebae are ubiquitous in air, soil and water. The amoebic keratitis, caused by the use of contact lens is preventable. It can be prevented by: 1) Proper cleaning of contact lenses by using commercial rather than home made saline solution. 2) Disinfection contact lenses preferable with a thermal system and 3) Not wearing lenses while swimming.

IV GENUS **LEISHMANIA**(利什曼属原虫)

The genus *Leishmania* was created by Ross in 1903 to include *Leishmania donovani* (杜氏利什曼原虫), the parasite causing Indian **kala-azar** (黑热病). Species belonging to this genus have two stages (amastigote, promastigote) in their life cycle. They require a vertebrate (脊椎动物) and insect host to complete the life cycle.

Leishmaniasis (利什曼病) is a **vector-borne disease** (虫媒病) that is transmitted by sandflies (白蛉) 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 3 species (*L. donovani*, *L. infantum*, and *L. chagasi*); the ***L. mexicana*** (墨西哥利什曼原虫) complex with 3 main species (*L. mexicana*, *L. amazonensis*, and *L. venezuelensis*); *L. tropica* (热带利什曼原虫); *L. major* (硕大利什曼原虫); *L. aethiopica*; and the subgenus (亚属) *Viannia* with 4 main species (*L. (V.) braziliensis* (巴西利什曼原虫), *L. (V.) guyanensis*, *L. (V.) panamensis*, and *L. (V.) peruviana*). The different species are morphologically indistinguishable, but they can be differentiated by isoenzyme analysis, molecular methods, or monoclonal antibodies.

Leishmania donovani (杜氏利什曼原虫)

Leishmama donovani causes **visceral leishmaniasis** (内脏利什曼病). 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.

The parasite was reported by both Leishman and Donovan 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 identified as a vector of the disease by Indian Kala-azar Commission (1931-1934).

L. donvani are obligate intracellular parasites of man and other mammalian hosts. They are always found as **amastigotes** (无鞭毛体) in the reticuloendothelial cells (网状内皮细胞) of the spleen, bone marrow, liver, intestinal mucosa and mesenteric lymphnodes (淋巴结).

MORPHOLOGY

The parasite exists in two forms: amastigote and promastigote.

Amastigote: Amastigotes are round in man and other vertebrate hosts. They are found inside monocytes (单核细胞), polymorphonuclear leucocytes (分叶白细胞) or endothelial cells (内皮细胞). They are small, round to oval bodies measuring 2.9-5.9 μm in length (Fig II - IV - 1). They are also known as **LD (Leishmania donovani) bodies** (利杜小体). 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 stained 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 (动基体) and extends to margin of the body. Vacuole, which is a clear unstained space lies alongside the axoneme.

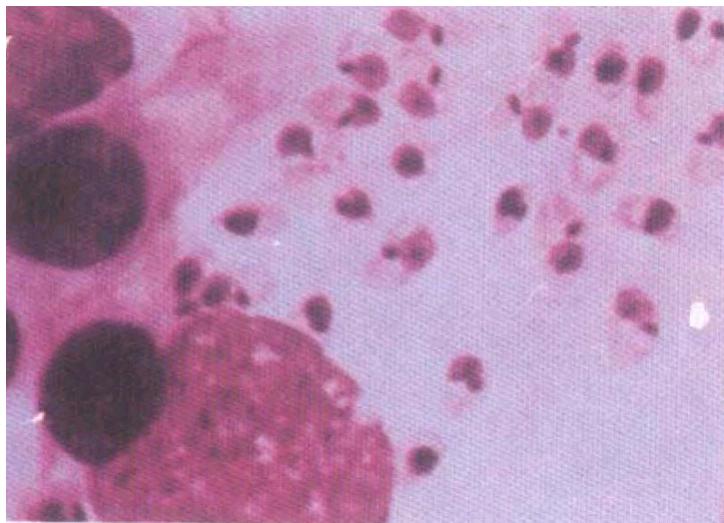


Fig II - IV - 1 *Leishmania donovani*. amastigote

Promastigote (前鞭毛体) (Fig II - IV - 2): Promastigotes are found in the digestive tract of sandfly (vector) 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 centre. The kinetoplast lies transversely near the anterior end. The flagellum is single, delicate and measures 15-28 μm . With Leishman stain, cytoplasm appears blue, the nucleus pink and the kinetoplast blight red.

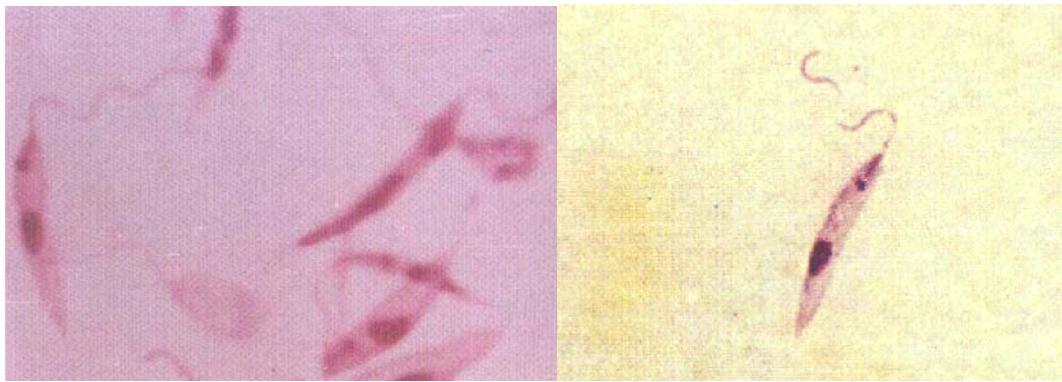


Fig II-IV-2 *Leishmania donovani* promastigote

LIFE CYCLE

L. donovani completes its life cycle in two different hosts (Fig II -IV-3): 1) Man and other mammals (e.g., dog), and 2) Sandfly of genus *Phlebotomus* and *Lutzomyia*.

The parasite is transmitted to man and other vertebrate hosts by the bite of blood-sucking female sandfly.

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 amastigotes. 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 (巨噬细胞) 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 (咽) and buccal cavity of sandfly. The sandflies which ingest fruit or plant juice after the first blood meal show heavy pharyngeal infection causing blockage (封阻) of the pharynx. Bite of the blocked sandfly transmits infection to

susceptible persons and the life cycle is repeated.

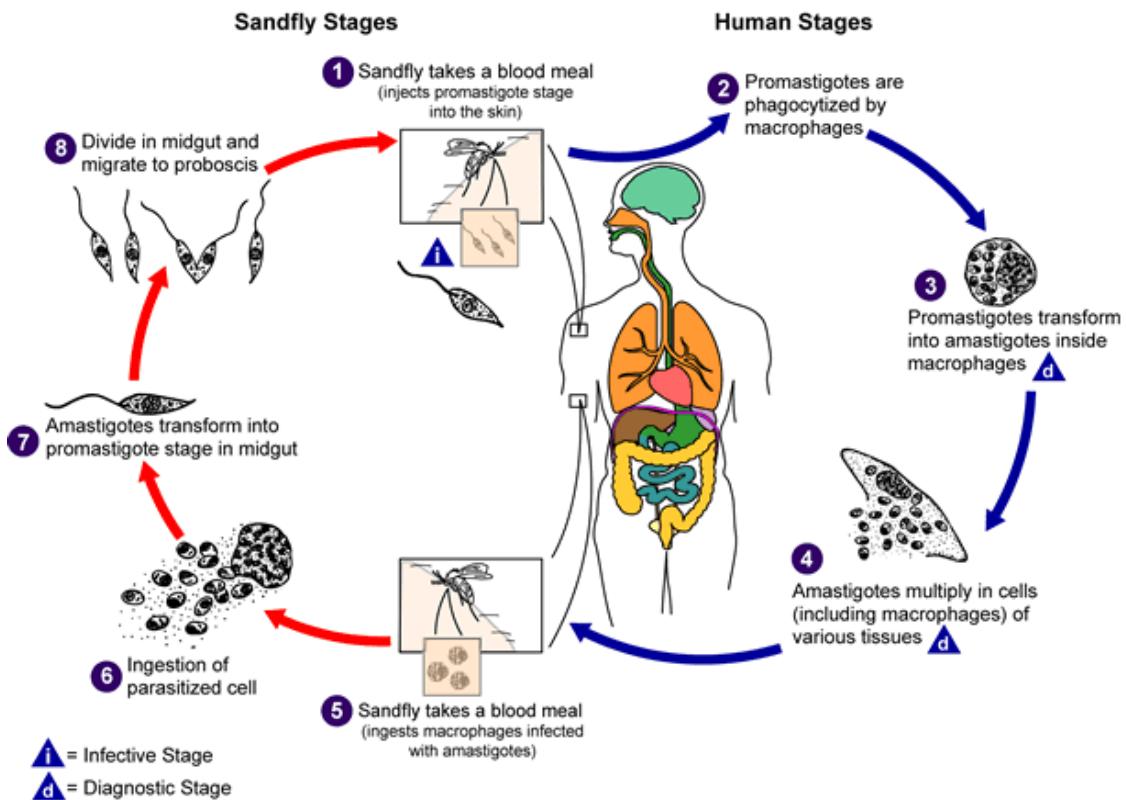


Fig II-IV-3 Life cycle of *Leishmania donovani* (Adapted from parasite image library of CDC, USA)

PATHOGENESIS AND CLINICAL MANIFESTATION

Bite of the female sandfly deposits promastigotes on the surface of the skin. The sandfly liberates biologically 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 haematopoietic tissue (造血组织) in the bone marrow. They progressively replace normal hepatocytes (肝细胞) in the liver.

Proliferation (增生) 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: **Spleen** is grossly enlarged, 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: **Liver** is enlarged with a sharp edge, soft consistency and smooth surface. The Kupffer cells are largely increased both in their size and number. They are filled with amastigotes. In contrast, hepatocytes do not contain any parasites. Atrophic areas, swelling and also 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. Haemopoietic tissue of the bone marrow are replaced by large numbers of parasitised macrophages. Plasma cells often are increased in number.

4) Lymph nodes: Lymph nodes are enlarged. In China and Mediterranean type of visceral leishmaniasis, amastigotes are demonstrated in the enlarged lymph nodes.

5) Kidney: 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 (心肌). Haematological (血液学的) changes occur. Typically, **anaemia** (贫血) is present in kala-azar. It is normocytic (正常红血球) and normochromic (正常学血色素). Anaemia is multifactorial (多因子的). It is caused by increased haemolysis (溶血), haemorrhage, haemodilution, replacement of bone marrow with parasitised macrophages and splenic sequestration of red cells. **Leucopenia** (白血球减少症) is well-marked. White blood cell count falls down to as low as $1100/\text{mm}^3$ of blood. Thrombocytopenia(血小板减少症) is caused by destruction of platelets (血小板).

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 (血球蛋白过多).

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**

(抗利什曼药). The intact cell mediated immunity confers protection against the infection.

Profound hyperglobulinaemia: Polyclonal lymphocyte activation causes profound hyperglobulinaemia. It is characterised by the production of a large volume of polyclonal non-specific immunoglobulins especially IgG and also specific anti-leishmanial antibodies. The complement is activated 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 **tumour necrosis factor** (肿瘤坏死因子) and interleukin (白细胞介素).

Clinical manifestation: Human leishmanial infections can result in 2 main forms of disease, cutaneous leishmaniasis and visceral leishmaniasis (kala-azar). The factors determining the form of disease include leishmanial species, geographic location, and immune response of the host.

1) Visceral leishmaniasis (内脏利什曼病): 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** (潜伏期) of visceral leishmaniasis is generally about 3 months. It may vary from as minimum as 3 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 (淋巴结病) is seen only in some cases of African kala-azar.

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

Leucopenia (white blood cell count as low as $1000/\text{mm}^3$) is a consistent feature.

Hypergammaglobulinaemia (血丙种球蛋白过多), circulating immune complexes and rheumatoid factors are present in sera of the most patients of kala-azar. Immune complex-

glomerulonephritis (血管球性肾炎) 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 hypopigmentation of the skin characteristically is seen in Indian patients giving the name kala-azar, which means black fever.

Peripheral oedema, epistaxis, gingival bleeding, petechiae and ecchymoses are the late manifestations.

Without treatment, death occurs within 3 to 20 months in 40 to 94% of adults and in 75 to 85% cases of children. Death often is due to superinfection bacterial pneumonia (肺炎), septicaemia (败血病), concurrent infections (tuberculosis, dysentery) or uncontrolled severe haemorrhage from the gastrointestinal tract and severe anaemia.

2) Cutaneous leishmaniasis(皮肤利什曼病): 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 (腋窝) if the sores are on the arm or hand).

3) 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.

4) Post kala-azar dermal leishmaniasis(PKDL)(皮肤型黑热病): 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, anaemia, 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.

1) 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 the 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 (凝血酶原) time more than five seconds longer than the normal or if the platelet count is below 40,000/mm³.

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 iliac 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.

2) Methods of examination:

① Direct microscopy: Direct microscopy of the splenic, bone marrow, liver or lymph node aspiration smears, fixed with methanol, then stained with Giemsa stain as for thin blood film; show L.D. bodies (amastigotes of *L. donovani*). L.D. bodies are usually found within macrophages. Some of the L.D. bodies can also be demonstrated free, released from the cells ruptured during making of the film. L.D. bodies can also be seen occasionally within non-nuclear cells in the stained smear of the buccal coat of the peripheral blood.

② Culture: Splenic and bone marrow aspiration, other tissues and buffy coats of the blood may show promastigotes in culture.

The specimen are inoculated in the water of condensation of NNN or any biphasic media, or into the fluid of liquid media and are incubated at 22-26 °C . In a positive culture, motile promastigotes can be demonstrated microscopically in a few days to 4 weeks.

③ Animal inoculation: Intraperitoneal inoculation of Chinese and golden hamster by clinical

specimens may reveal parasites. In a positive case, the amastigotes can be demonstrated in the stained impression smears of the spleen, collected from animals if they are dead or after killing them at 6 months.

Immunological diagnosis

Serological tests to demonstrate specific anti-leishmanial antibodies in the serum are specially useful in the diagnosis of early phase of visceral leishmaniasis.

Complement fixation test (CFT) 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-fluorescent** (IFA, 间接免疫荧光试验), **indirect haemagglutination** (IHA, 间接血凝试验), **enzyme-linked immunosorbent assay** (ELISA, 酶链免疫反应), 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 (DAT, 直接凝集试验), using trypsin treated Coomasie blue-stained promastigotes, has been developed recently as a simple test for use in poorly equipped laboratories.

Leishmanin skin test (Montenegro 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 1 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 leishmaniasis are zoonotic (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(人源型): 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 (犬源型): 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 (自然疫源型)** : 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 (爆发性的) visceral leishmaniasis have been described in most of the cases of AIDS. They respond poorly to anti-leishmanial 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 (五价锑剂). 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 (戊烷脒) isethionate or amphotericin B (两性霉素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 zoonotic 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.

Reference

THE LEISHMANIASES AND *LEISHMANIA/HIV* CO-INFECTIONS

Leishmania/HIV co-infection is emerging as an extremely serious, new disease and it is increasingly frequent. There are important clinical, diagnostic, chemotherapeutic, epidemiological and economic implications of this trend.

Although people are often bitten by sandflies infected with *Leishmania* protozoa, most do not develop the disease. However, among persons who are immunosuppressed (e.g. as a result of advanced HIV infections, immunosuppressive treatment for organ transplants, haematological malignancy, auto-immune diseases), cases quickly evolve to a full clinical presentation of severe leishmaniasis.

AIDS and VL(visceral leishmaniasis) are locked in a vicious circle of mutual reinforcement. On the one hand, VL quickly accelerates the onset of AIDS (with opportunistic diseases such as tuberculosis or pneumonia) and shortens the life expectancy of HIV-infected people. On the other hand, HIV spurs the spread of VL. AIDS increases the risk of VL by 100-1000 times in endemic areas. This duo of diseases produces cumulative deficiency of the immune response since *Leishmania* parasites and HIV destroy the same cells, exponentially increasing disease severity and consequences. VL is considered a major contributor to a fatal outcome in co-infected patients. Lately, however, use of tritherapy, where it is available, has improved the prognosis for *Leishmania/HIV* cases.

Leishmaniasis can be transmitted directly person to person through the sharing of needles, as is often the case among intravenous drug users. This group is the main population at risk for co-infection.

1. Areas of Co-infection

Cases of *Leishmania/HIV* co-infections are being reported more frequently in various parts of the world. It is anticipated that the number of *Leishmania/HIV* co-infections will continue to rise in

the coming years and there are indications that cases are no longer restricted to endemic areas. The overlapping geographical distribution of VL and AIDS is increasing due to two main factors: the spread of the AIDS pandemic in suburban and rural areas of the world, and the simultaneous spread of VL from rural to suburban areas.

Leishmania/HIV co-infections are considered a real threat, especially in south-western Europe. Of the first 1 700 cases of co-infection which have been reported to the World Health Organization (WHO) from 33 countries worldwide up to 1998, 1 440 cases were from the region: Spain (835); Italy (229); France (259); and Portugal (117). Of 965 cases retrospectively analyzed, 83.2% were males, 85.7% were young adults (20–40 years old) and 71.1% were intravenous drug users.

Most co-infections in the Americas are reported in Brazil, where the incidence of AIDS has risen from 0.8 cases per 100 000 inhabitants in 1986 to 10.5 cases per 100 000 inhabitants in 1997. As HIV transmission has spread into rural areas, VL has simultaneously become more urbanized specially in north-eastern Brazil increasing the risk of overlapping infection. The number of cases of *Leishmania/HIV* co-infection is expected to rise in Africa owing to the simultaneous spread of the two infectious diseases and their increasingly overlapping geographical distribution, complicated by mass migration, displacement, civil unrest, and war.

In general, the reported cases of *Leishmania/HIV* co-infection in Africa are a very modest estimation and would substantially increase if active surveillance were implemented throughout the continent. Ethiopia has a well-organized system of detection, management and reporting of co-infection. Kenya and Sudan began surveillance in 1998 and Morocco has also established a surveillance centre. In East Africa, cases of *Leishmania/HIV* co-infections have been reported in Djibouti (10), Ethiopia (74), Kenya (15), Malawi (1) and Sudan (3). West Africa has no official surveillance system yet, but several cases have been reported: Cameroon (1), Guinea Bissau (1), Mali (4) and Senegal (2). In North Africa, cases have been reported in Algeria (20) and Morocco (4).

2. Specific Problems

Leishmania/HIV co-infections impose specific difficulties in terms of diagnosis and treatment. The usual clinical features (fever, weight loss, liver and spleen enlargement, inflammation of the lymph nodes) are not always present. The clinical diagnosis can also be made difficult by associated diseases such as cryptosporidium, disseminated cryptococcosis, cytomegalovirus infection or mycobacterial infection.

The serological diagnosis is falsely negative in 42.6% of co-infected patients. HIV-positive patients have difficulty in producing antibodies against new infectious agents, especially at a late stage or during relapses. Consequently, there is a need to use two or more serological tests and antigens freshly prepared in the laboratory to increase sensitivity. Although multiple localizations are frequent (blood, skin, digestive tract, lungs, central nervous system), parasitological diagnosis can be difficult and has to be repeated to orient the treatment. Bone marrow aspirate (BMA) remains the safest and most sensitive technique, but spleen aspirate and liver biopsy are also used. When BMA cannot be performed, the search for *Leishmania* can be conducted in peripheral blood samples.

Treatment for co-infected patients is aimed at clinical and parasitological cures and prevention of relapses. Unfortunately, in such patients treatment failure, relapses due to drug resistance and drug toxicity are very common. In south-western Europe, follow-up studies using pentavalent antimonials, the same first-line drug used to treat classic leishmaniasis, show a positive response in 83% of cases. However, 52% of patients relapse within a period of one month to three years, with the number of relapses ranging from one to four.

The main alternative drugs include pentamidine, amphotericin B and amphotericin B encapsulated in liposomes. This encapsulation reduces the occurrence of side-effects, but relapses still occur and the drug remains extremely expensive.

3. The World Health Organization Response

Because of the anticipated substantial increase in *Leishmania*/HIV co-infections, they are among the priorities for WHO's Department of Communicable Disease Surveillance and Response (CSR).

In 1996, WHO established an initial surveillance system, comprised of 14 institutions in 10 countries. A standardized Case Report Form was elaborated and endorsed by the members of the network, and a Central International Registry was set up within WHO to centralize, process and disseminate information on co-infections.

In 1998, a new WHO/Joint United Nations Programme on HIV/AIDS (UNAIDS) initiative was launched which helped strengthen the surveillance network; it has been expanded to include 28 institutions, especially in East Africa and the Indian subcontinent (India, Nepal). All member institutions of the network report to WHO on an annual basis. A computerized Geographic

Information System (GIS) is used to map and monitor co-infections in a way that permits easy visualization and analysis of epidemiological data.

The evolution of *Leishmania*/HIV co-infection is being closely monitored by extending the geographic coverage of the surveillance network and by improving case reporting. WHO encourages active medical surveillance, especially in south-western Europe, of intravenous drug users, the main population at risk. Finally, because case notification of leishmaniasis is compulsory in only 40 of the 88 endemic countries, WHO strongly suggests the remaining 48 endemic countries follow suit.

V TRYpanosomes (锥虫)

Trypanosomes are hemoflagellate (血鞭毛虫) protozoa; they belong to the family Trypanosomatidae (锥虫科). Two distinctly forms of genus *Trypanosoma* occur in humans. They cause **African trypanosomiasis** (or African sleeping sickness, 非洲昏睡病) and **America trypanosomiasis** (美洲锥虫病) respectively.

The complex *Trypanosoma brucei* have two subspecies that are morphologically indistinguishable cause distinct disease patterns in humans: *T. b. gambiense* (冈比亚锥虫) causes West African sleeping sickness and *T. b. rhodesiense* (路得西锥虫) 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* (枯氏锥虫), causes America typansosmiasis (or Chagas' disease), a zoonotic disease that can be transmitted to humans by blood-sucking reduviid bugs.

TRYpanosoma bruceiCOMPLEX (布氏锥虫复合体)

Trypanosoma brucei complex causes African sleeping sickness. Originally, in the early years of 20 century, 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 closely related, and they were reduced to subspecies of *T. brucei*: *T. b. gambiense*, *T. b. 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 锥鞭毛体 , Fig II - V -1, 2): It found in man and other vertebrate. Trypomastigote 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, thick and stumpy trypomastigotes

1) Sender trypomastigote (细长型) : these forms are found in the blood during ascending parasitaemia. They measure 20-40 μm in length and 1.5-3.5 μm in breadth.

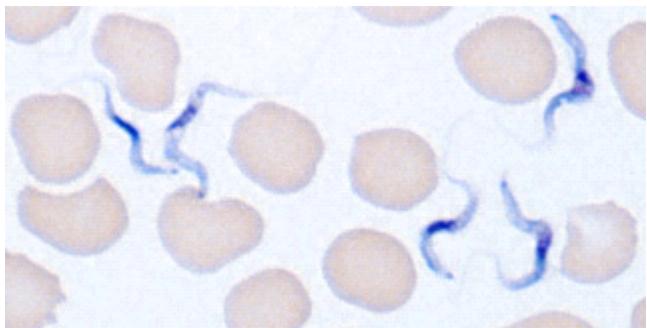
2) Stumpy trypomastigotes (粗短型) : they do not have any free flagella. These forms always are found in the blood during declining phase of parasitaemia. They measure 15-25 μm in length and 3.5 μm in breadth.

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.



Fig II - V -1 a blood smear from a patient with African trypanosomiasis show typical trypomastigote stages with a posterior kinetoplast, a centrally located nucleus, an undulating membrane, and an anterior flagellum. (Giemsa stain) (Adapted from parasite image library of CDC, USA)

Fig II - V -2 Blood smear from a patient with *T. b. rhodesiense*. (Giemsa stain, Adapted from parasite image library of CDC, USA)



Insect forms: It includes procyclic trypomastigotes, **epimastigotes** (上鞭毛体) and **metacyclic trypomastigotes** (循环后期锥鞭毛体). These forms are found in the salivary glands of **tsetse fly** (采采蝇). 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 (VAT) on their surface coat. These forms do not divide and are found free in the lumen of salivary glands. They are infective to humans.

LIFECYCLE (Fig II-V-3)

T. brucei complex their life cycle in vertebrate host and insect host. Vertebrate host include man and domestic animals. Insect host are **tsetse fly** (采采蝇) of *Glossina* species (*G. palpalis*, *G. morsitans*, *G. pallidipes*, etc.). Tsetse flies (Fig 4) are large bloodsucking Diptera (双翅目). 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. These flies inoculate metacyclic trypomastigotes (infective forms) 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 (结缔组织), 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 parasitaemia. 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.

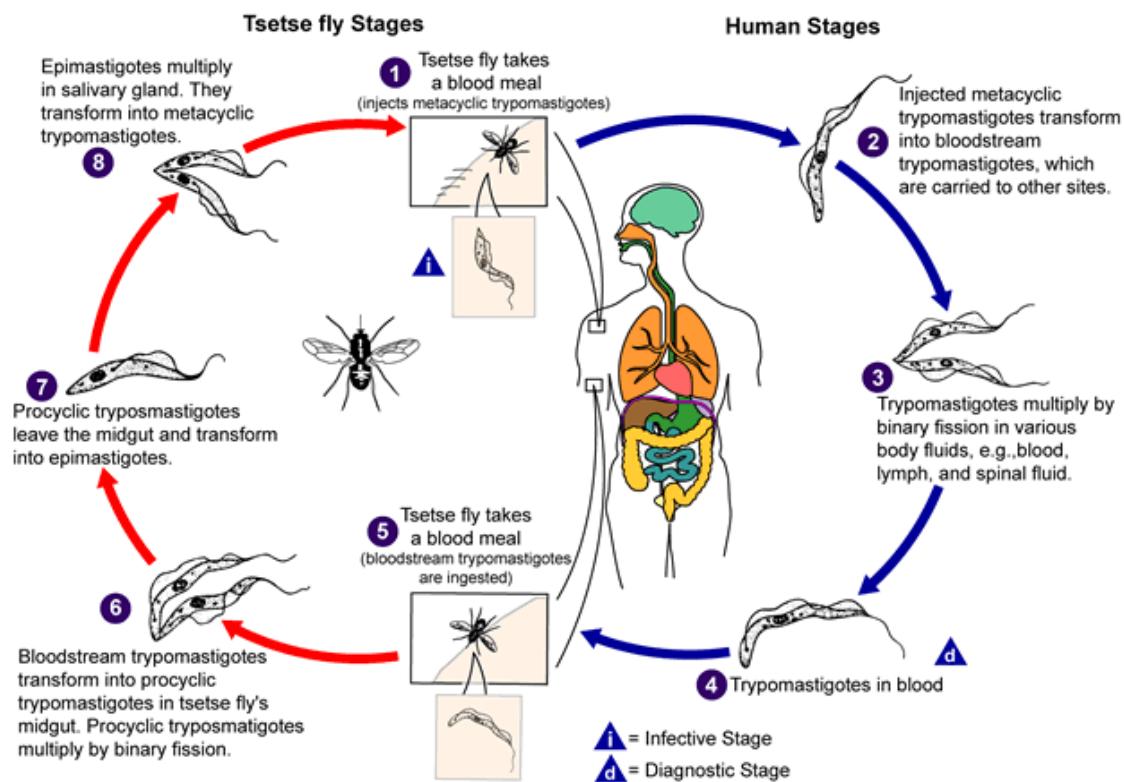


Fig II – V – 3 Life cycle of *Trypanosoma brucei* (Adapted from parasitic image library of CDC, USA)



Fig II – V – 4 Tsetse fly, *Glossina morsitans* feeding

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. brucei*.

rhodesiense

PATHOGENESIS AND CLINICAL MANIFESTATION

Infection occurs in 3 stages. A **trypanosomal chancre** (锥虫下疳) can develop on the site of inoculation. This is followed by a **haematolymphatic stage** (血淋巴期) with symptoms that include fever, lymphadenopathy, and pruritus. In the **meningoencephalitic stage** (脑膜脑炎期), 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. Trypanosomal 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 (血管舒张) and interstitial oedema. The chancre tissue is filled with parasites. A painful trypanosomal 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* (Fig 5).

Oedema, hepatosplenomegaly and tachycardia (心动过速) are other frequent findings.

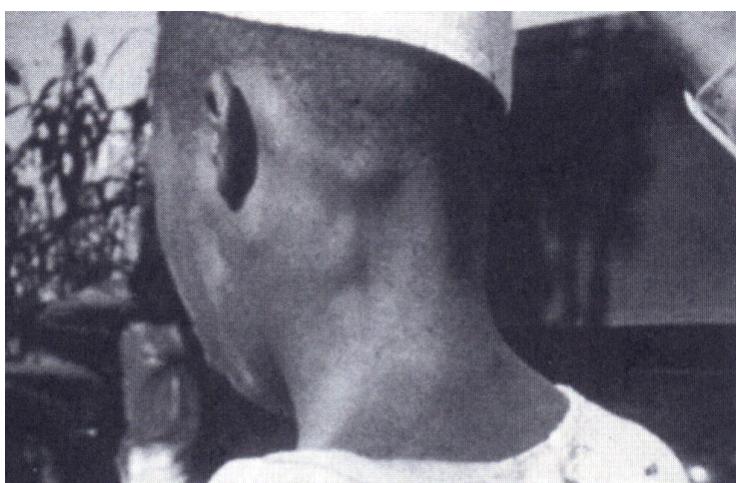


Fig II - V - 5. The swollen cervical glands constitute "Winterbottom sign". A classical diagnostic indication of infection with *T. gagamboense*

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

The infected host responds by mounting a cellular and humoral immune reaction. Immunoglobulin (IgM) is secreted into the CSF, and there is massive infiltration of lymphocytes into the membrane covering the brain, especially the arachnoid membrane (蛛网膜) and the pia mater. Since the pia mater surrounds all the blood vessels in the brain, its thickening and the lymphocytic infiltrate appear as characteristic “perivascular cuffing” within the brain substance. Among the infiltrating cells are “mulberry-like” bodies, the morula (or Mott) cells; these are plasma cells in the final stage of immunoglobulin secretion.

The outcome of the inflammatory process (meningoencephalitis) is brain damage leading to somnolence (嗜睡), coma and, unless treated, death in almost all cases. A few records exist of healthy carriers who, although infected and with trypanosomes in their blood, appear to remain well and do not develop the late stage of the disease.

There are differences between the clinical manifestations of East African and West African trypanosomiasis. In *T. b. rhodesiense* (East African trypanosomiasis), there is usually little obvious glandular involvement and Winterbottom's sign may not be present; weight loss is rapid, and CNS is involved early. Untreated persons usually die within 9 months to a year after onset of disease. The incubation period is commonly short. In *T. b. gambiense* (West African trypanosomiasis) chronic CNS disease develops

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 (or Field), 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 Buffy Coat (QBC) 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(血清转化) occurs after the onset of clinical symptoms and thus is of limited use. However, the Card Agglutination Trypanosomiasis Test (CATT) test is of value for epidemiologic 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 (羚羊) (bush buck and hartbeest) and domestic animal (cattle) 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(苏拉明) (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*(克氏锥虫)**

Trypanosoma cruzi, causes **Chagas disease** (恰加斯病), a zoonotic disease that can be transmitted to humans by blood-sucking reduviid bugs (锥蝽). Chagas disease (South American trypanosomiasis) 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(无鞭毛体): it is the non-flagellated, intracellular parasite found in man and other vertebrate host. Amastigote is a round or oval body measuring 2 to 4 μm in diameter. It has a nucleus, kinetoplast and an axoneme. Morphologically it resembles the amastigote of *Leishmania* species,

hence it is frequently called as leishmanial form. It multiplies in man in this stage only.

Trypanomastigote (锥鞭毛体): 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 11.7-30.4 μm in lengths and 0.7-5.9 μm 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. (Fig II-V-6)

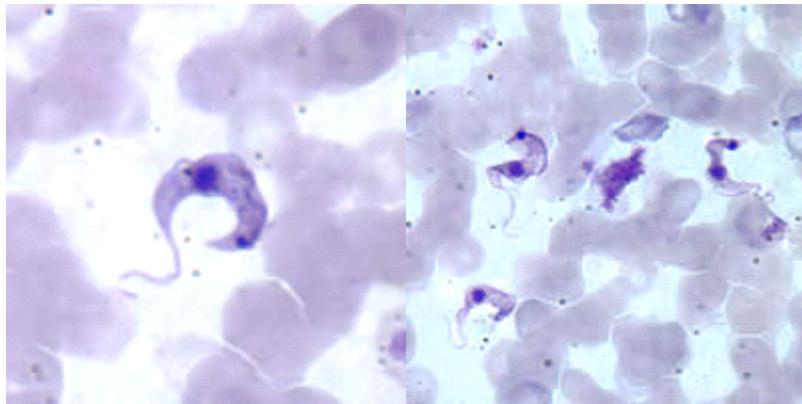


Fig II - V - 6 *Trypanosoma cruzi* in blood smears (Giemsa stain, Adapted from parasite image library of CDC, USA).

T. cruzi need two hosts to complete its life cycle. 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(锥猎蝽亚科) insect vector (or kissing bug) takes a blood meal and releases trypomastigotes in its feces near the site of the bite wound. Trypomastigotes enter the host through the wound or through intact mucosal membranes, such as the conjunctiva (结膜). Common triatomine vector species for trypanosomiasis belong to the genera *Triatoma*, *Rhodnius*, 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 (Fig7) Trypanosoma *cruzi* can also be transmitted through blood transfusions, organ transplantation, transplacentally, and in laboratory accidents.

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.

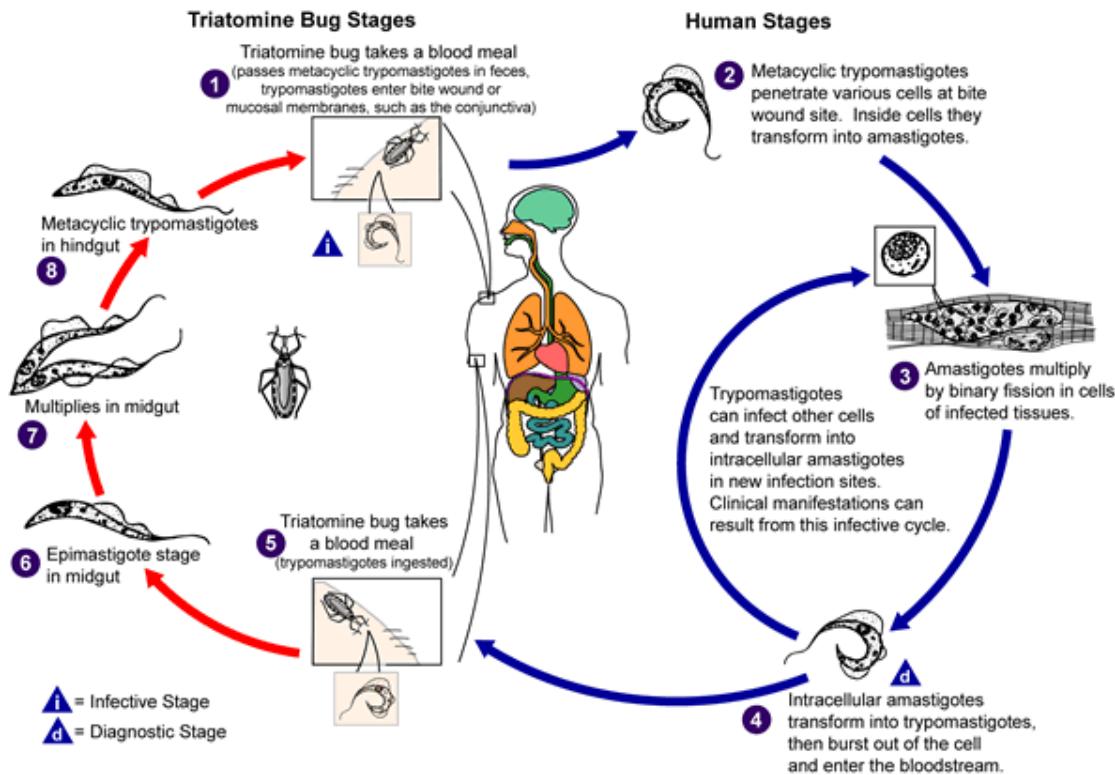


Fig II – V – 7 Life cycle of *Trypanosoma cruzi* (Adapted from parasite image library of CDC, USA)

A local lesion (chagoma, palpebral edema) can appear at the site of inoculation. Chagoma (恰加氏肿) 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 periocular 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 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*.

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 (pseudocysts, Fig) 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 (巨食管) or megacolon (巨结肠). 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.

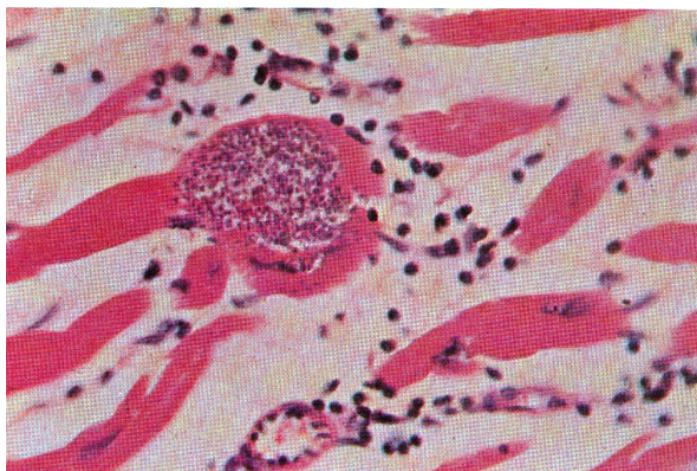


Fig II - V - 8 Amastigotes of *T. cruzi* lying in a pseudocyst in human cardiac muscle. H and E. × 1000. Enlarged by 9.6.

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 (e.g. NNN, LIT); and c) **xenodiagnosis** (病媒接种诊断法), 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 (栖息于森林的) 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.

PREVENTION 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 epidemiological 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.

VI ***GIARDIA LAMBLIA***(**藍氏贾第鞭毛虫**)

Giardia lamblia, a protozoan flagellate, inhabits the small intestine (duodenum and jejunum) 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.

Trophozoite: It is pear-shaped with broad rounded anterior end and a tapering posterior end (Fig.). It measures 9-21 μm in length and 5-15 μm in breadth and 2-4 μm in thick. Dorsal surface is convex (凸起) while ventral surface is concave (凹入). 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** (轴柱) and four pairs of flagella. Two **median bodies** (中体) are present on the axostyle at its origin.

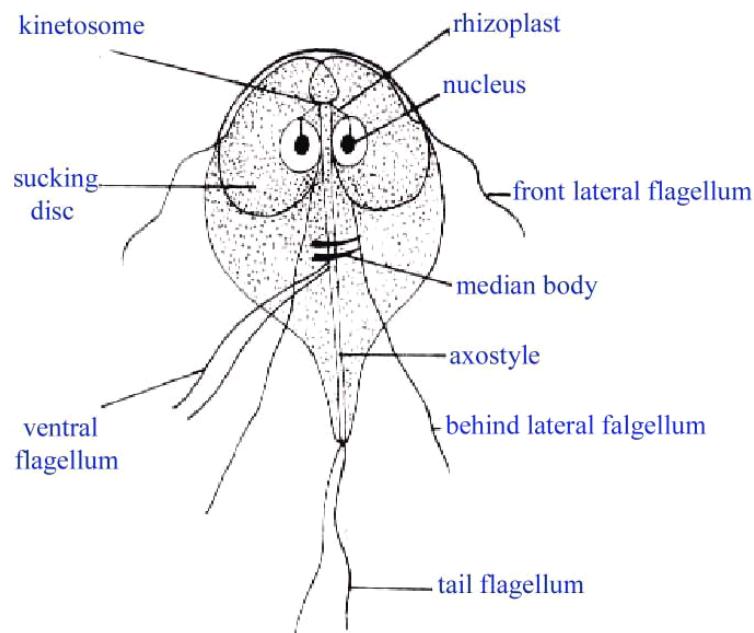


Fig II-VI-1 *Giardia lamblia* Trophozoite

Cytoplasm is uniform and finely granular. The trophozoites are motile due to the presence of four pairs of flagella.

Cyst: the oval cyst measuring 8-12 μm in length and 7-10 μm in breadth (Fig II-VI-2). 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 of 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 diagonally 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 II-VI-2)

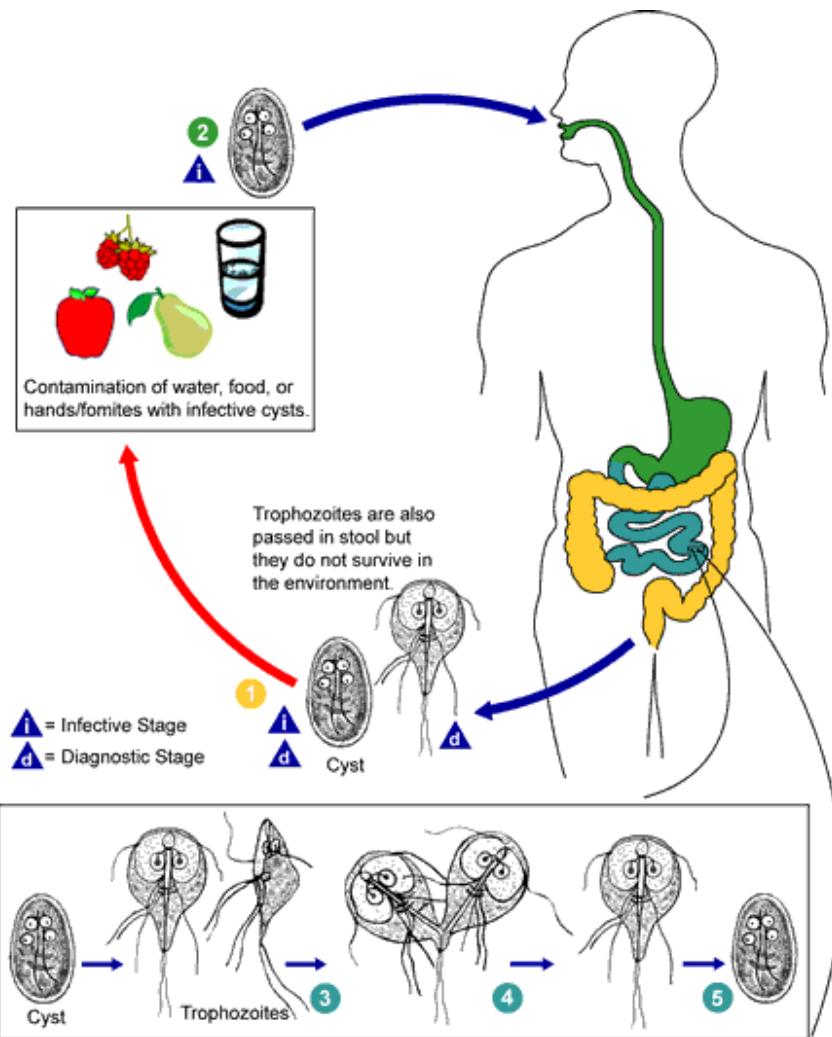


Fig II –VI–2 Life cycle of *Giardia lamblia* (Adapted from parasite image library of CDC, USA)

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 (hands or fomites). Cysts pass through the stomach and excyst to trophozoites in the duodenum within 30 minutes of ingestion, each cyst produces two tetranucleate (四核的) 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 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 (十二指肠) and upper ileum (回肠). The trophozoites may remain attached to the intestinal mucosa and rarely invades the submucosa.

As few as 10-25 cysts can cause giardiasis (贾第鞭毛虫病). Malabsorption (吸收障碍) 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 (组织病理学) of duodenum and jejunum (空肠) are highly variable and may range nearly from normal to markedly abnormal. Most commonly, there is shortening of microvilli (微绒毛) and elongation of crypts. The brush border of the absorptive cells are 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 (胃气涨), nausea (恶心), 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.

Pathogenic diagnosis

1) Fecal examination. Giardiasis is diagnosed by the identification of cysts or trophozoites in the feces, using direct mounts as well as concentration procedures. Repeated samplings may be necessary. In acute giardiasis, motile trophozoites are demonstrated in the direct wet mount of

liquid stool. The cysts are demonstrated in the semiformed stool. The stool specimens are examined either fresh or in case of delay. After preservation by formalin (福尔马林) or polyvinyl alcohol, and subsequent staining by trichrome (三色) or iron-haematoxylin method. Concentration of stool by formalin-either or zinc sulphate method increase the yield of parasites. In chronic giardiasis cysts often are excreted intermittently. Hence examination of at least three stool specimens collected at an interval of 2 days, helps in the detection of parasites

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:

① String test or Entero test (肠检胶囊法): It is a **gelatin capsule** (胶囊) which contains a nylon string at one end. The capsule is swallowed by the patient and the free end of the string is fixed at the mouth. In the stomach, the capsule is dissolved and the string remains in duodenum and jejunum. After overnight incubation, the string is removed, the bile stained mucus is collected on the glass side and examined microscopically for trophozoites.

② Duodenal aspiration(十二指肠引流): it is also collected to demonstrate trophozoites.

③ Jejunal biopsy (空肠活检): It is performed to demonstrate trophozoites but indicated only in very serious cases.

2). Immunological methods:

Alternate methods for detection include antigen detection tests by enzyme immunoassays, and detection of parasites by immunofluorescence. Enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody (IFA) are useful in seroepidemiological 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 epidemiological 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 cysts are the primary sources of infection.

Giardiasis is transmitted mainly by drinking fecally contaminated water and less frequently by eating contaminated food. It also can transmitted by direct person to person spread, it occurs most commonly in persons with poor sanitation and poor faecal oral hygiene. Occasionally, 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(灭滴灵) is the drug of choice. Metronidazole, tinidazole (替硝唑) 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 reinvoke 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, maintenance of food and personal hygiene, and health education.

VII TRICHOMONAS VAGINALIS (阴道毛滴虫)

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

MORPHOLOGY

Trichomonas vaginalis only exists in trophozoite stage. Cystic stage is absent. Trophozoite inhabit the vagina in female, the prostate (前列腺) and seminal vesicles in male and urethra (尿道) in both sexes.

The trophozoites of *Trichomonas*, measuring $14\text{-}17\mu\text{m} \times 5\text{-}15\mu\text{m}$ have a single nucleus, four anterior flagella and a single lateral flagellum attached to pellicle to form an **undulating membrane** (波动膜) (fig). 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 numbers of hydrogenosomes (氢化酶体) and sometimes viral particles.

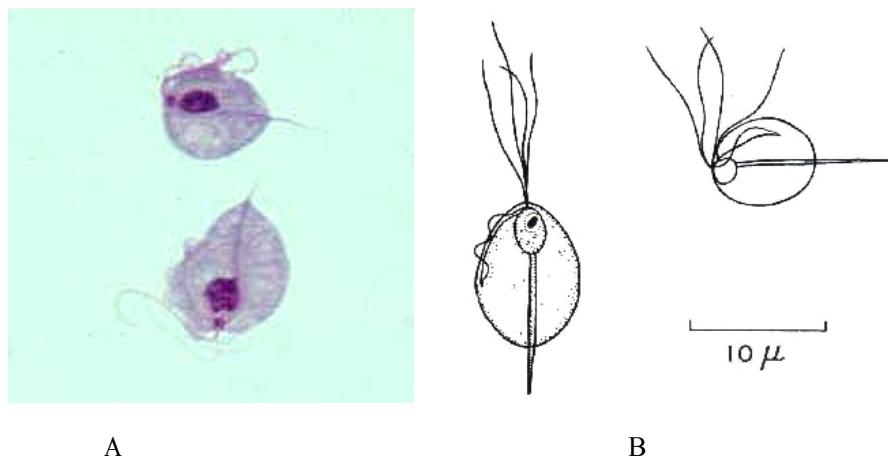


Fig II-VII-1 A: Two trophozoites of *Trichomonas vaginalis* obtained from in vitro culture. Smear was stained with Giemsa. B: Diagram of *T. vaginalis*

Trophozoite of *Trichomonas vaginalis* is facultative anaerobic (厌氧的). 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. It is completed in a single host either male or female (fig II-VII-2)

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 (脱皮) of the vaginal mucosa. Sometimes, it is associated with small blisters (水泡) or granules. The mucosa and superficial submucosa are infiltrated by lymphocytes, plasma cells and polymorphonuclear leucocytes.

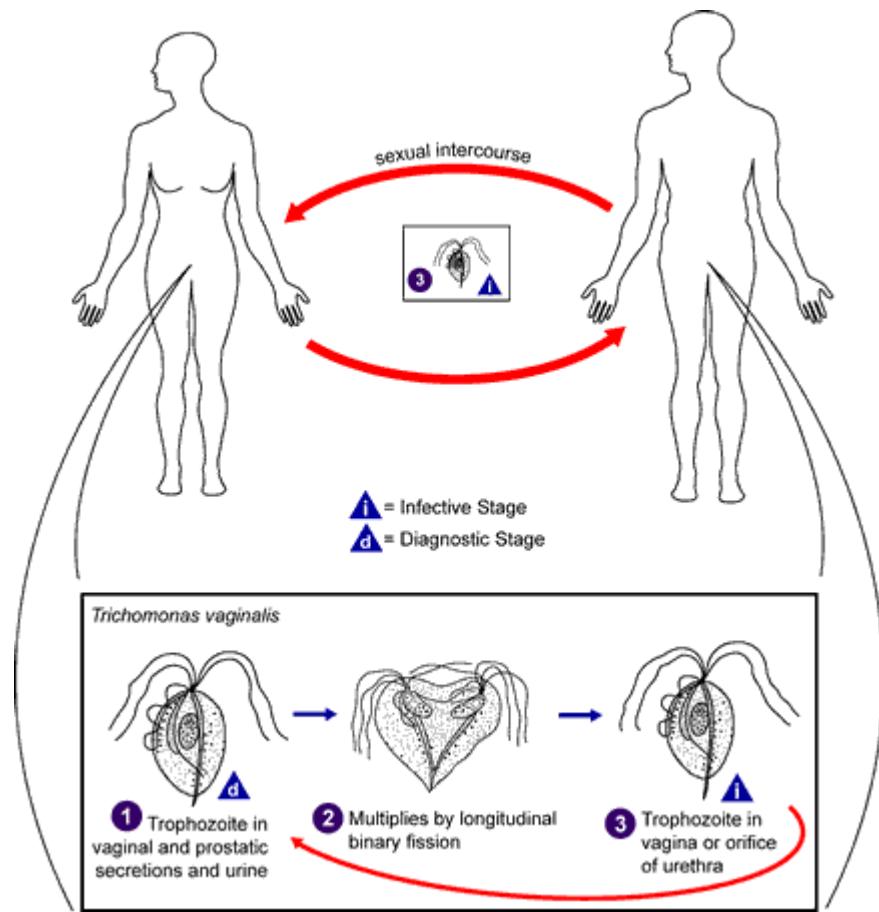


Fig II-VII-2 Life cycle of *Trichomonas vaginalis* (Adapted from parasite image library of CDC, USA)

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 (排尿困难). The acute stage may last for a week or month and often varies in intensity. It may become severe following menstruation. Vaginitis (阴道炎) with a purulent (含脓的) discharge is the prominent symptom, and can be accompanied by vulvar and cervical (子宫颈的) lesions, abdominal pain, dysuria and dyspareunia. The vaginal secretions are liquid, greenish or yellow and are present on the urethral orifice (尿道口), 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 (附睾炎), and prostatitis (前列腺炎) can occur.

Persistent or recurring 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 (频尿).

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

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(灭滴灵); therapy is usually highly successful. Tinidazole (硝矾咪唑), 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.

VIII PLASMODIUM

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*(恶性疟原虫), *P. vivax*(间日疟原虫), *P. ovale*(卵形疟原虫) and *P. malariae*(三日疟原虫). 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** (按蚊). 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, 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)

- 1) ***Exo-erythrocytic stage(红外期)***: During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates **sporozoites** (子孢子)into the human host.

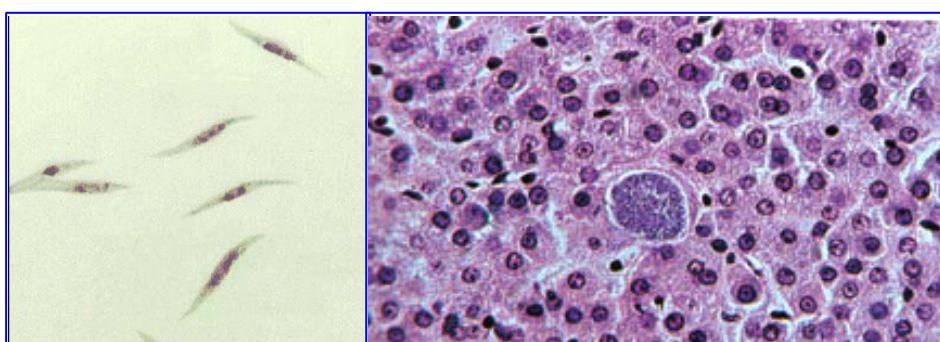


Fig II -VIII- 1 the malaria sporozoites

Fig II -VIII-2 Pre-erythrocytic schizont in liver

The **sporozoite** (fig II -VIII-1) a small, spindle-shaped (纺锤形) 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 (within 30 minutes) enter liver parenchyma cells. They then (unless hypnozoites, see below) begin a process of multiple divisions known as merogony (or schizogony, 裂体增殖). **Pre-erythrocytic schizont** (红外裂殖体) 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** (裂殖子) per meront (裂殖体) (Fig2). After the infected liver parenchyma cell is broken, the merozoites release from the liver cells, some of merozoites are phagocytized by host's macrophage (巨噬细胞) 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** (速发型子孢子) and **brady sporozoite** or hypnozoites(迟发型子孢子, 休眠子). The tachysporozoites develop into trophozoites and undergoes **EE schizogony** (红外期裂体增殖) 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 have subsided.

In *P. vivax*, hypnozoites (休眠子) are found inside the liver parenchyma (实质). These are single- nucleated parasites measuring 4-6 μ m in diameter and are the dormant (休眠) stages of the parasite. Relapse (复发) 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*.

② *Erythrocytic stages*(红细胞内期)

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 (网织红细胞) and erythrocytes (young and old). Erythrocytic stages such as **trophozoites, schizonts** and **gametocytes** are present.

① *Trophozoites* (滋养体): On entry into an erythrocyte, the merozoite again transforms into a trophozoite. 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** (环状体). The trophozoite is vacuolated, ring shaped, more or less amoeboid and uninucleate (单核的). As the trophozoite grows, its food vacuoles become less noticeable by light microscopy, but pigment granules of **hemozoin** (疟色素) 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 (1 μ m in diameter), which a very thin circle of cytoplasm; some appear to have two nuclei, 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 (2 μ m in diameter), and as the parasite grows the infected cell becomes enlarge and develop red-staining **Schüffner's dots** (薛氏点) 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: Ziemann's dots. Early trophozoite or ring forms of *P. ovale* are more similar to those of *P. malariae*. *P. ovale* ring forms are relatively 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.

② *Erythrocytic Schizonts* (红内期裂殖体) : The trophozoites multiply with division of

nucleus by mitosis, followed by a division of cytoplasm, to become mature schizonts. 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 host's cell ruptures, releasing parasite metabolic wastes and residual body, including **hemozoin** (疟色素). 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 reticuloendothelial 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.

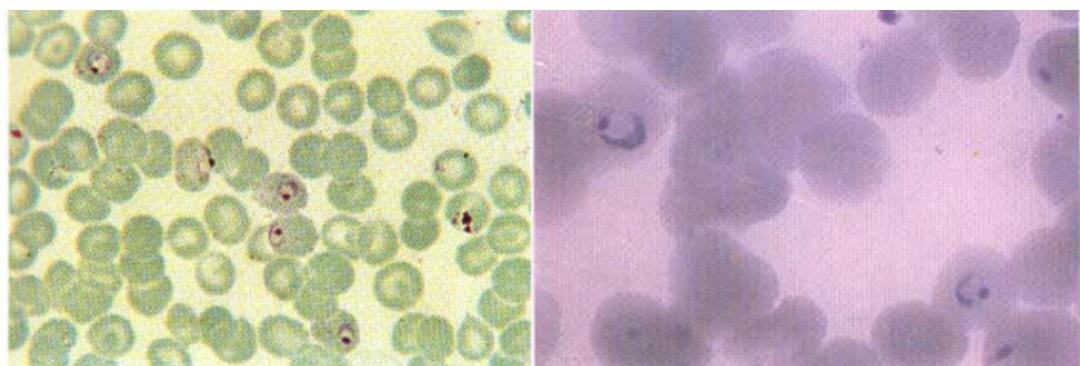


Fig II -VIII-3 *P.vivax* thin smear, showing early trophozoites. The infected red cells are enlarged and show some stippling.

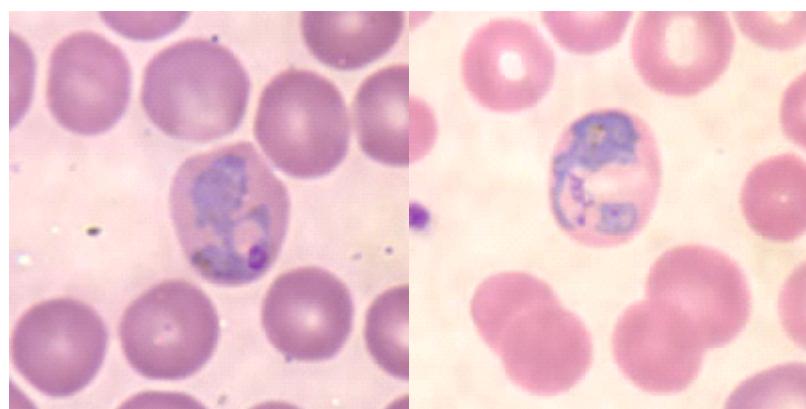


Fig II -VIII-4. *P. vivax* late trophozoites

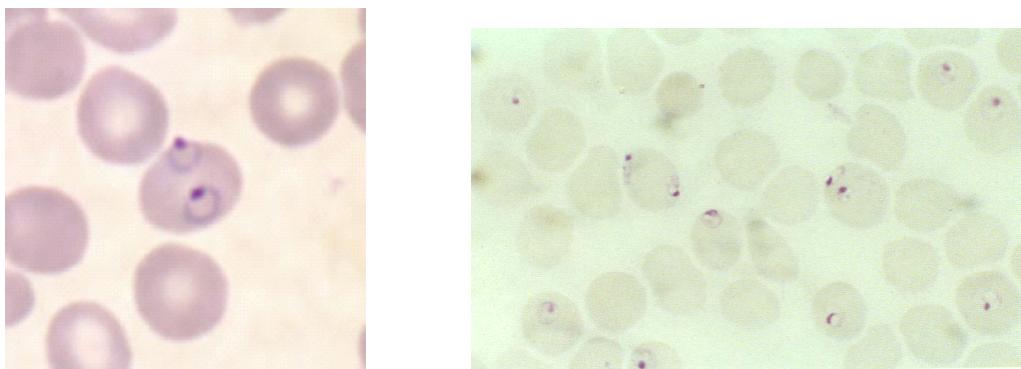


Fig II -VIII-6. *P. falciparum* early trophozoites

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.

③ Gametocyte (配子母细胞): After an indeterminate number of asexual generations, some merozoites enter erythrocytes and become macrogamonts (macrogametocytes, 大配子母细胞, 雌配子母细胞) and microgamonts (microgametocytes, 小配子母细胞, 雄配子母细胞). 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 (micro-) and female (macro-) 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. Comparison of Plasmodium species see table 1,2.

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. 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** (出丝). As the microgametocyte becomes extracellular (细胞外), within 10 to 12 minutes its nucleus divides repeatedly to form 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** (小配子). The stimulus for exflagellation is an increase in pH caused by escape of dissolved **carbon dioxide** (二氧化碳) 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** (合子) quickly elongates to become a motile **ookinete** (动合子). The ookinete is reminiscent of a sporozoite and merozoite in morphology. It is 10 to 12 μ m in length and has polar rings and **subpellicular microtubules** (表膜下微管) but no micronemes.

The ookinete penetrates the **peritrophic membrane** (围食膜) in the mosquito's gut. Migrates to the hemocoel (血腔) side of the gut, and begins its transformation into an **oocyst** (卵囊). The oocyst is covered by an electron-dense capsule and soon extends out into the insect's hemocoel.

Table II-VIII-1 Comparison of *Plasmodium* Species Which Cause Human Malaria

Plasmodium species	Stages found in blood	Appearance of Parasite
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<i>P. vivax</i>	Ring form	Large cytoplasm with occasional pseudopods; large chromatin dot
	Trophozoite	Large amoeboid cytoplasm; large chromatin; fine, yellowish-brown pigment
	Schizont	Large, may almost fill RBC; mature = 12 to 24 merozoites; yellowish-brown, coalesced pigment
	Gametocyte	Round to oval; compact; may almost fill RBC; chromatin compact, eccentric (macrogametocyte) or diffuse (microgametocyte); scattered brown pigment
<i>P. falciparum</i>	Ring form	Delicate cytoplasm; 1 to 2 small chromatin dots; occasional appliqué (accollé) forms
	Trophozoite	Seldom seen in peripheral blood; compact cytoplasm; dark pigment
	Schizont	Seldom seen in peripheral blood; mature = 8 to 24 small merozoites; dark pigment, clumped in one mass
	Gametocyte	Crescent or sausage shape; chromatin in a single mass (macrogametocyte) or diffuse (microgametocyte); dark pigment mass
<i>P. malariae</i>	Ring form	Sturdy cytoplasm; large chromatin
	Trophozoite	Compact cytoplasm; large chromatin; occasional band forms; coarse, dark-brown pigment
	Schizont	Mature = 6 to 12 merozoites with large nuclei, clustered around mass of coarse, dark-brown pigment; occasional rosettes
	Gametocyte	Round to oval; compact; may almost fill RBC; chromatin compact, eccentric (macrogametocyte) or more diffuse (microgametocyte); scattered brown pigment

<i>P. ovale</i>	Ring form	Sturdy cytoplasm; large chromatin
	Trophozoite	Compact with large chromatin; dark-brown pigment
	Schizont	Mature = 6 to 14 merozoites with large nuclei, clustered around mass of dark-brown pigment
	Gametocyte	Round to oval; compact; may almost fill RBC; chromatin compact, eccentric (macrogametocyte) or more diffuse (microgametocyte); scattered brown pigment

Table II -VIII-2 Comparisons of Erythrocyte Changes in *Plasmodium* Species Infection

Plasmodium species	Stages found in blood	Appearance of Erythrocyte (ABC)
<i>P. vivax</i>	Ring form	Normal to $1\frac{1}{4} \times$, round; occasionally fine Schüffner's dots; multiple infection of RBC not uncommon
	Trophozoite	Enlarged $1\frac{1}{2}$ to $2 \times$; may be distorted; fine Schüffner's dots
	Schizont	Enlarged $1\frac{1}{2}$ to $2 \times$; may be distorted; fine Schüffner's dots
	Gametocyte	Enlarged $1\frac{1}{2}$ to $2 \times$; may be distorted; fine Schüffner's dots
<i>P. falciparum</i>	Ring form	Normal; multiple infection of RBC more common than in other species
	Trophozoite	Normal; rarely, Maurer's clefts (under certain staining conditions)
	Schizont	Normal; rarely, Maurer's clefts (under certain staining conditions)
	Gametocyte	Distorted by parasite

<i>P. malariae</i>	Ring form	Normal to 3/4 ×
	Trophozoite	Normal to 3/4 ×; rarely, Ziemann's stippling (under certain staining conditions)
	Schizont	Normal to 3/4 ×; rarely, Ziemann's stippling (under certain staining conditions)
	Gametocyte	Normal to 3/4 ×; rarely, Ziemann's stippling (under certain staining conditions)
<i>P. ovale</i>	Ring form	Normal to 1 1/4 ×, round to oval; occasionally Schüffner's dots; occasionally fimbriated; multiple infection of RBC not uncommon
	Trophozoite	Normal to 1 1/4 ×; round to oval; some fimbriated; Schüffner's dots
	Schizont	Normal to 1 1/4 ×, round to oval, some fimbriated, Schüffner's dots
	Gametocyte	Normal to 1 1/4 ×; round to oval, some fimbriated; Schüffner's dots

The initial division of its nucleus is reductional; meiosis (减数分裂) takes place immediately after zygote formation as in other Sporozoea (孢子虫). The oocyst reorganizes internally into a number of haploid (单倍体) nucleated masses called sporoblasts (孢子母细胞), and the cytoplasm contains many ribosomes (核糖体), 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** (唾液腺), sporozoites enter its channels and can be injected into a new host at the next feeding.

Sporozoite development takes from 10 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.

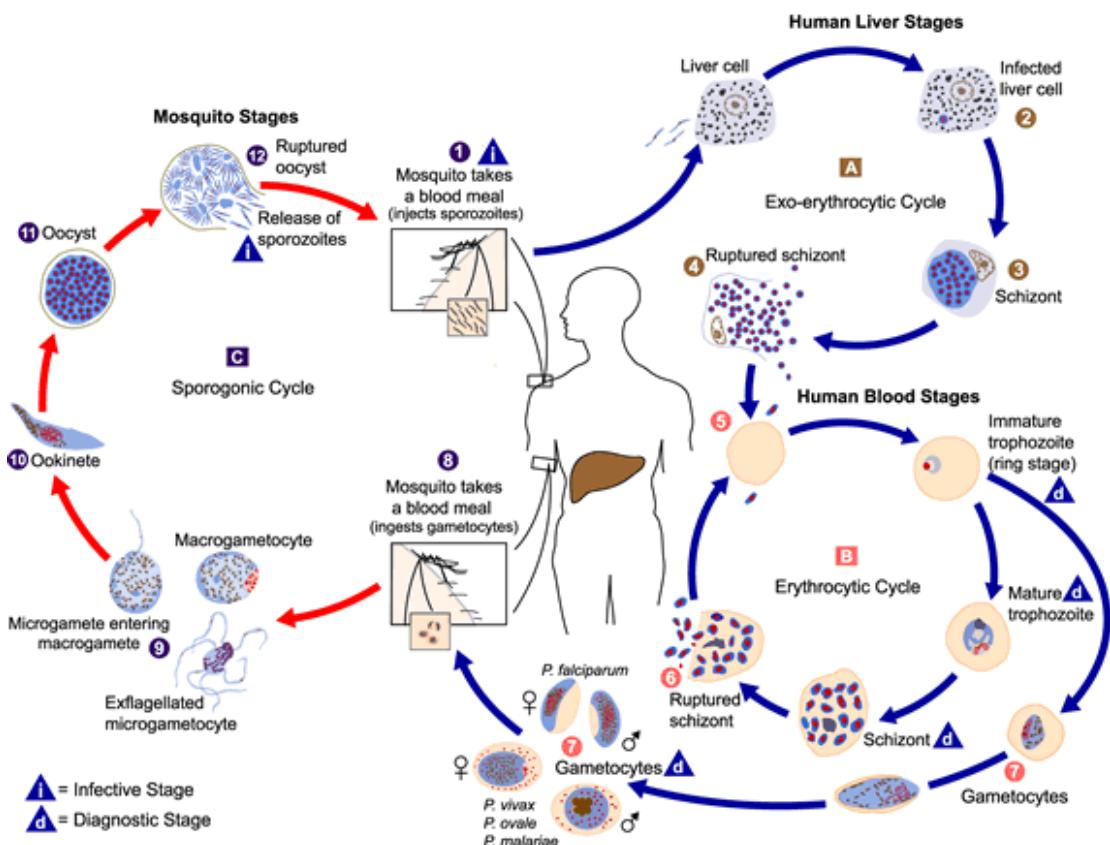


Fig II -VIII-7 The life cycle of Plasmodium (Adapted from parasite image library of CDC, USA)

METABOLISM OF PLASMODIUM SPECIES

Energy metabolism. The presence and importance of glycolysis (糖酵解) in the degradation of glucose by Plasmodium spp. are well established, although subsequent steps are unclear. This is complicated by the fact that malaria species from birds have recognizable mitochondria (线粒体), whereas unequivocal mitochondria have been demonstrated in very few species from mammals. The bird plasmodia apparently have a functional **tricarboxylic acid cycle** (三羧酸循环), but the existence of the complete cycle in the erythrocytic stages of the mammalian parasites is doubtful. Membranous structures in some of the mammalian species may represent mitochondria because of certain mitochondrial enzymes demonstrated in them cytochemically (NADH- and NADPH-dehydrogenases and cytochrome oxidase). Interestingly, the sporogonic stages of these organisms in the mosquito possess prominent, cristate mitochondria, reflecting perhaps a developmental change in metabolic pattern analogous to that observed in trypanosomes.

Treatment of the host with qinghaosu (青蒿素) leads to swelling of the mitochondria of *P. inui* (a mammalian species with prominent mitochondria) within 2.5 hours. Host mitochondria are unaffected. Similar reactions have been observed after primaquine treatment, leading to the suggestion that these drugs act via inhibition of mitochondrial metabolic reactions.

The erythrocytic forms of Plasmodium appear to be facultative anaerobes (厌氧生物), consuming oxygen when it is available. Infected red cells take up considerably more oxygen than do uninfected ones when incubated with various substrates. It has been suggested that Plasmodium uses oxygen for biosynthetic purposes, especially synthesis of nucleic acids. Also, a branched electron transport system has been proposed, analogous to that suggested for some helminthes, but a classical cytochrome system has not been demonstrated. Although the bird plasmodia have tristate mitochondria, they nevertheless depend heavily on glycolysis for energy. They convert four to six molecules of glucose to lactate for every one they oxidize completely. A limiting factor may be the parasite's inability to synthesize coenzyme A, which it must obtain from its host; this cofactor is necessary to introduce the two-carbon fragment into the tricarboxylic acid cycle.

The end products of glucose metabolism of the mammalian plasmodia are lactate (乳酸盐) and some volatile compounds, especially acetate and formate. The bird malaria parasites oxidize glucose more completely, producing some carbon dioxide and organic acids. Both bird and mammal plasmodia "fix" carbon dioxide into phosphoenolpyruvate, as do numerous other parasites. In plasmodia the carbon dioxide-fixation reaction can be catalyzed by either phosphoenolpyruvate carboxykinase or phosphoenolpyruvate carboxylase. Chloroquine and quinine inhibit both enzymes, possibly accounting for the antimalarial activity of these drugs. The significance of the carbon dioxide fixation is not clearly understood; it may be to reoxidize NADH produced in glycolysis, or its reactions may function to maintain levels of intermediates for use in other cycles.

The **pentose phosphate pathway** is an important and interesting metabolic pathway in Plasmodium. This path has several known functions in various systems, and its importance to plasmodia is probably twofold; to furnish pentoses (戊糖) from hexoses (己醣) for use in synthesis of nucleic acids (however, Plasmodium apparently lacks a full complement of enzymes

for nucleic acid synthesis, which will be discussed further) and to provide reducing power in the form of NADPH. The first steps in the path are the dehydrogenation (脱氢作用) and then hydrolysis (水解) of glucose 6-phosphate (六磷酸葡萄糖) to 6-phosphogluconate (六磷酸葡萄糖盐) by the enzymes glucose 6-phosphate & hydrogenase (G6PDH) and lactonase (内脂酶), and the next reactions are oxidation (氧化), decarboxylation (去碳酸基), and isomerization (异构化) of the 6-phosphogluconate to D-ribose-5-phosphate (a pentose) by an isomerase (异构酶) and 6-phosphogluconate dehydrogenase (6PGDH). Current evidence indicates that the plasmodia are entirely dependent on G6PDH and possibly 6PGDH and the entire pathway from the host cell. This dependency becomes even more interesting when it is observed that persons with a genetic deficiency in erythrocytic G6PDH, or favism (蚕豆病), are more resistant to malaria. Favism is a sex-linked trait in which ingestion of various substances such as aspirin (阿司匹林), the antimalarial drug primaquine (伯氨喹), sulfonamides (磺胺类药), or the broad bean *Vicia fava* brings on a hemolytic crisis in the female homozygote (纯合子) or male hemizygote. The gene is relatively frequent in blacks and some Mediterranean white people.⁴⁴ Over 5% of Southeast Asian refugees entering the United States have had a G6PDH deficiency. Since the trait is expressed as a mosaic, even heterozygotes (杂合子) have some red cells deficient in the enzyme. Therefore all conditions--heterozygous, homozygous, and homozygous are protected to some extent against *P.falciparum*. However, presence of the deficiency should be determined before treatment with primaquine to avoid a hemolytic crisis.

Digestive metabolism. That the parasites digest host hemoglobin, leaving the iron-containing residue (hemozoin), deserves further comment. The plasmodia depend heavily on this protein source; the trophozoites substantially reduce the hemoglobin content of the erythrocyte. The parasites ingest a portion of host cytosol via the cytostome, and the vesicle thus formed migrates to and joins the central food vacuole, where the hemoglobin is rapidly degraded. Chloroquine (氯喹) is a dibasic amine (a weak base) and increases the pH in the food vacuole to prevent the digestion of hemoglobin. Chloroquine is a very safe drug because it has no nonweak base effects on mammalian cells, but the basis of chloroquine resistance in *P. falciparum* is due to interference with the nonweak base mechanism. The explanation for the nonweak base effects is unknown. Mefloquine (甲氟喹) also affects the food vacuoles, and it is believed that quinine acts by a similar mechanism.

Resistance to *P. falciparum* by persons homozygous and heterozygous for sickle cell hemoglobin (HbS) may involve several mechanisms, partly involving feeding and digestion by the protozoa. The parasite develops normally in cells with HbS until those cells are sequestered in the tissues. Kept in this low oxygen environment for several hours, the cells have more of a tendency to sickle than cells that pass through at a normal rate. When sickling occurs, HbS forms filamentous aggregates. The filamentous aggregates actually pierce the Plasmodium, apparently releasing digestive enzymes that lyse both parasite and host cell. Furthermore, K⁺ leaks out of the sickled cell, depriving the parasite of this ion. Sickled cells also may block capillaries, further decreasing local oxygen concentration. Other workers have shown that sickling denatures hemoglobin and releases ferriprotoporphyrin IX (FP, hemin), which has membrane toxicity. They suggested that the FP lyses the parasites.

Synthetic metabolism. As a specialized parasite, Plasmodium appears to depend on its host cell for a variety of molecules other than the strictly nutritional ones. Specific requirements for maintenance of the parasites free of host cells are pyruvate, malate, NAD, ATP, CoA (辅酶 A), and folinic acid (亚叶酸). The inability of the organisms to synthesize CoA has been mentioned. They are unable to synthesize the purine ring de novo, thus requiring an exogenous source of purines (嘌呤) for DNA and RNA synthesis. The purine source seems to be hypoxanthine (次黃嘌呤) "salvaged" from the normal purine catabolism of the host cell? Several aspects of synthetic metabolism in Plasmodium have offered opportunities for attack with antimalarial drugs. Although plasmodia have cytoplasmic ribosomes of the eukaryotic type, several antibiotics that specifically inhibit prokaryotic (and mitochondrial) protein synthesis; for example, tetracycline and tetracycline derivatives, have a considerable antimalarial potency. It has been shown that tetracycline inhibits protein synthesis in *P. falciparum*, as well as growth in vitro. Antibiotics have only recently been used extensively in malaria therapy because they are effective less rapidly than conventional antimalarials and because of apprehensions relative to development of resistant bacteria.

PATHOGENESIS AND CLINICAL MANIFESTATIONS

The major clinical manifestations of malaria may be attributed to two general factors: (1) the host inflammatory response, which produces the characteristic chills and fever, as well as other related phenomena; and (2) 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 (潜伏期)

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 *P. 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 (疟疾发作)

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 typical 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°C. The teeth chatter (牙打战), 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, functioning at 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 parasites, 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.

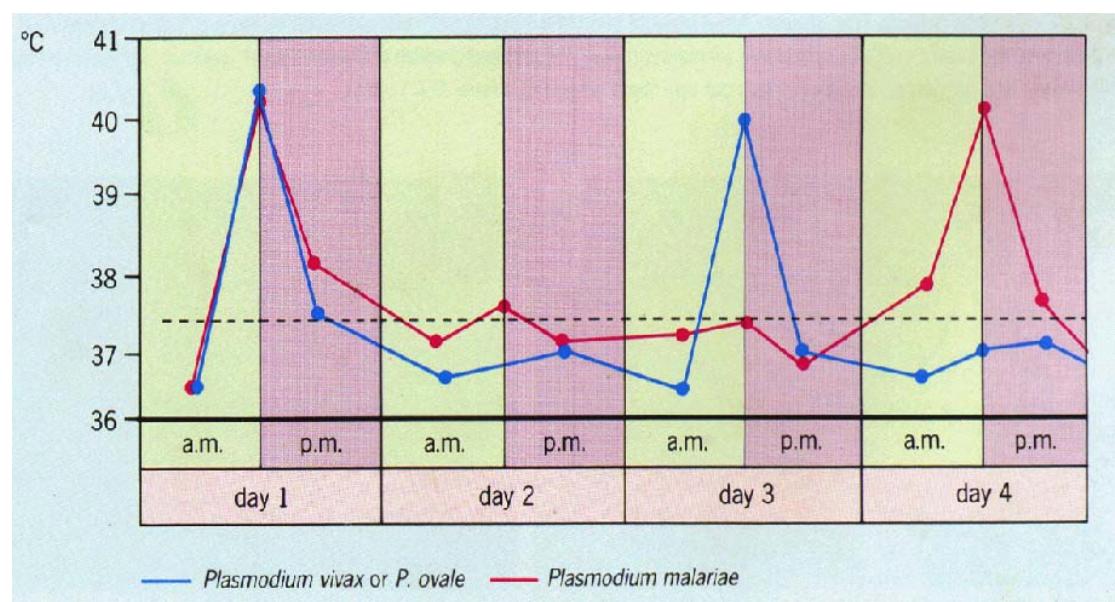


Fig II - VIII - 8 Temperature fluctuations in malaria patients: peaks of fever are related to the intraerythrocytic merogony cycle, occurring every 48 or 72 hours if the cycle is synchronized, as it often is. (Adapted from R Muller & JR Baker, 1990)

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.

***Relapse and Recrudescence in malarial infections* (复发和再燃)**

Since the advent of an antimalarial drug (quinine) in the sixteenth century, it has been noted that some persons, who have been treated and seemingly recovered, **relapse** back into the disease weeks, months, or even years after the apparent cure.

The discovery of preerythrocytic schizogony in the liver by Shortt and Garnham in 1948 seemed to have solved the mystery. It appeared most reasonable to assume that preerythrocytic merozoites simply reinjected other hepatocytes, with subsequent reinvasion of red blood cells. This would explain why relapse occurred after erythrocytic forms were eliminated by erythrocytic schizontocides, such as quinine and chloroquine.

However, not all species of *Plasmodium* cause relapse. Among the parasites of primates, only *P. vivax* and *P. ovale* of humans and *P. cynomolgi*, *P. fieldi*, and *P. simiovale* of simians cause true relapse. If preerythrocytic merozoites reinvaded hepatocytes, then relapse should occur in all species.

Two populations of exoerythrocytic forms have now been shown. One develops rapidly into schizonts, as previously described, but the other remains dormant as hypnozoites ("sleeping animalcules"). These have been demonstrated for *P. vivax*, *P. ovale*, and *P. cynomolgi*, but they have not been found in any species that does not cause relapse. How long the hypnozoite can remain capable of initiating schizogony and what triggers it to do so are unknown. Primaquine has been shown to be an effective hypnozoiticide.

Recrudescence (再燃) was long thought that *P. malariae*, a dangerous species in humans, also exhibited relapse, but it has been shown that this species can remain in the blood for years, possibly for the lifetime of the host, without showing signs of disease and then suddenly can initiate a clinical condition. This is more correctly known as a recrudescence, since preerythrocytic stages are not involved. The danger of transmission of this parasite in blood transfusion is evident. Treatment of this species with primaquine is unnecessary.

***Anemia* (贫血)**

The main causes of the anemia are destruction of both parasitized and nonparasitized erythrocytes, inability of the body to recycle the iron bound in the insoluble hemozoin, and an

inadequate erythropoietic response of the bone marrow. Why such large numbers of nonparasitized red cells are destroyed is still not understood, but some evidence has indicated autoimmune (自身免疫) hemolysis (溶血). Other reports have suggested increased phagocytosis of erythrocytes by the reticuloendothelial system. The defective bone marrow response may be due in part to limitation in iron supply and in falciparum malaria it may be due to blockage (封阻) of the capillaries by parasitized erythrocytes. Destruction of erythrocytes leads to an increase in blood bilirubin (胆红素), a breakdown product of hemoglobin. When excretion cannot keep up with formation of bilirubin, jaundice (黄疸) yellows the skin. The hemozoin is taken up by circulating leukocytes and is deposited in the reticuloendothelial system. In severe cases the viscera, especially the liver, spleen, and brain, become blackish or slaty as the result of pigment deposition.

Complications of malaria (疟疾并发症)

Falciparum malaria (恶性疟) is always serious, and sometimes severe complications are produced. The most common of these is **cerebral malaria (脑型疟)**, 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°C, and psychotic (精神病的) symptoms or convulsions (抽搐), 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.

Tropical splenomegaly syndrome (TSS, 热带综合巨脾症) recently is known as hyper reactive malarial splenomegaly. It occurs in some persons living in endemic areas of Africa, Indonesia and New Guinea. The condition is characterized by massive splenomegaly (脾肿大), a moderately enlarged liver with hepatic sinusoidal lymphocytosis and markedly elevated serum IgM malarial antibodies. It is also characterized by absence of parasites in the peripheral blood. The condition shows a favourable response to treatment with antimalarial chemotherapy with a decrease in the spleen size and reversal of the

pathological changes in liver.

A combination of other severe manifestations leads to a condition known as **algid malaria**. This is associated with a bacterial infection of the blood (septicemia) with toxemia and massive gastrointestinal hemorrhage. There is a circulatory collapse with markedly low blood pressure. The skin is cold and clammy; peripheral veins are constricted.

The direct cause of these severe complications has traditionally been cited as a "plugging" of the capillaries in the affected organs by clots. Some evidence has suggested that the conditions are caused by a manifestation of the inflammatory response: an increase in vascular permeability, with accompanying water and protein lost from the blood to the tissues, leading to circulatory stasis and hypoxia. However, recent investigations have led to the conclusion that symptoms of cerebral malaria are not due to edema but rather that the dysfunction is a consequence of stagnant hypoxia caused by adherence of the parasitized erythrocytes to the endothelium of cerebral venules and capillaries. Edema seen at autopsy is probably a condition that developed at the death of the patient. The symptoms of algid malaria appear to result from circulatory stasis in the gastrointestinal tract due to the same mechanism.

Blackwater fever is another grave condition associated with falciparum malaria, but its clinical picture is distinct from the foregoing. It is an acute, massive lysis of erythrocytes, marked by high levels of free hemoglobin and its breakdown products in the blood and urine and by renal insufficiency. Because of the presence of hemoglobin and its products in the urine, the fluid is quite dark, hence the name of the condition. A prostrating fever, jaundice, and persistent vomiting occur. Renal failure is usually the immediate cause of death. Damage to the kidney is now thought to result from renal anoxia, reducing efficiency of the glomerular filtration and tubular resorption, the massive hemolysis is not directly attributable to the parasites; the organisms frequently cannot be demonstrated. However, the condition is almost always associated with areas of *P. falciparum* hyperendemicity, found in persons with prior falciparum malaria, and very frequently with irregular or inadequate treatment for the infection. Inadequate suppressive or therapeutic doses of quinine most often have been implicated, but many cases have been reported following treatment with

quinacrine and pamaquine and can occur in persons who have not been treated at all. It is now believed that blackwater fever is an autoimmune phenomenon and is triggered by some stimulus that results in release of large amounts of antibodies, which act as hemolysins, into the circulation. Mortality is 20% to 50%. The incidence of blackwater fever has declined in recent years, perhaps due to the use of drugs other than quinine for prophylaxis.

Hypoglycemia (reduced concentration of blood glucose , 血糖过低症) is a common symptom in falciparum malaria. It is usually found in women with uncomplicated or severe malaria who are pregnant or have recently delivered, as well as other cases of severe falciparum malaria, Coma produced by hypoglycemia has commonly been misdiagnosed as cerebral malaria. This condition is usually associated with quinine treatment.

Congenital malaria (先天性疟疾)

It is a recognized entity in malaria. It is caused by transmission of erythrocytic asexual forms of the parasite through the placenta, when the latter is injured. Infection does not take place either by EE forms or sporozoites. Congenital malaria is usually acquired during parturition (分娩). The conditions are known to occur more frequently in non-immune infected mothers than in highly immune mothers, with intense parasitisation of the placenta (胎盘). It is relatively a rare condition seen only in highly endemic areas.

Transfusion malaria (输入性疟疾)

Transfusion of infected blood and the use of contaminated needle of the intravenous drug addicts can cause transfusion malaria. Pre-erythrocytic development is absent, incubation period is short. Clinically, it behaves like naturally acquired infection. Relapse does not occur.

IMMUNITY.

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

Natural immunity (先天性免疫)

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 haemoglobin molecules, seen in certain disorders. Factors that can contribute to genetic resistance are certain heritable anemias: sickle cell, favism (蚕豆病), and thalassemia (地中海贫血症). 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 (谷氨酸残基) in the amino acid sequence of hemoglobin is replaced by a valine (缬氨酸), 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 phosphate dehydrogenase (G6PD) deficiency trait is a genetic deficiency trait believed to confer some protection 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 (等位基因), *Fy^a* and *Fy^b*, recognized by their different

antigen. The Fy/Fy genotype is common in African and in American black people (40% or more) and rare in white people (about 0.1%). It has been shown that Fy^a and Fy^b are receptors for *P. vivax* and *P. knowlesi*; 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 tropical Africa.

Acquired immunity(获得性免疫)

Specific acquired immunity in malaria involves both humoral and cellular immunities. The specific immunity restricts the level of parasitemia (寄生虫血症) 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** (免疫逃避).

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 passively 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 protection against malaria. The cellular mechanism is mainly of non-specific type. In acute *P. falciparum* infection, a positive correlation has been found between natural

killer (NK) and resistance to malaria.

Activated macrophages (巨嗜细胞) may phagocytose (吞噬) and induce extra-cellular killing of target cells (靶细胞). 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 pathological 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 immuno-suppressive therapy.

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

Premunition(带虫免疫) and immune evasion

The development of some protective immunity is evident in malaria, and we will consider only briefly some of the practical effects. Relapses and recrudescences may be associated with lowered antibody titers or increased ability of the parasite to deal with the antibody, but they may depend on genetic differences in sporozoite populations. Symptoms in a relapse are usually less severe than those in the primary attack, but the level of parasitemia is higher. After the primary attack and between relapses, the patient may have a tolerance to the effects of the organisms and in fact may have as high a circulating parasitemia level as during the primary attack, although remaining asymptomatic. Such tolerant carriers are very important in the epidemiology of the disease. The protective immunity is primarily a **premunition** (带虫免疫), that is, resistance to superinfection. It is effective only as long as a small, residual population of parasites is present; if the person is completely cured, susceptibility returns. Thus, in highly endemic areas, infants are protected by maternal antibodies, and young children are at greatest risk after weaning. The immunity of children who survive a first attack will be continuously stimulated by the bites of infected mosquitoes as long as the children live in the malarious area. Nonimmune adults are highly susceptible. Immunity is species specific and to some degree strain specific so

that a person may risk a new infection by migrating from one malarious area to another. Falciparum malaria is unmitigated in its seventy to a person who is immune to vivax malaria.

The premunition of malarial parasites shows that the malarial parasites can produce effective immunical reaction. But some malarial parasites can exist in an immunocompetent host; they can coexist with host's protective antibody. This preference is called **immune evasion**.

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

Microscopic identification is the method most frequently used to demonstrate an active infection.

1) Collection of blood. Peripheral blood should be collected before starting treatment with antimalarials. It can be collected any time during the fever. Timing of collection of the blood is less important although a high density of malaria parasites appear in circulation during paroxysm. More important is the frequent of examination of blood smear. Smears should be examined at least twice daily until parasites are detected.

2) Microscopy examination. both thick and thin smears are prepared from the peripheral blood. They are stained with one of the Giemsa or Wrights' stain. Thick smear is used for detecting parasites, quantitating parasitaemia and demonstrating malaria pigments. It not used for species diagnosis. Thick smears have the advantage of concentrating the parasites and therefore increase the sensitivity of diagnosis. Once parasites are detected in the thick blood smear, thin blood smear are examined for marking a species diagnosis.

Thin smear is used for detecting parasites and for determining the species of the infecting parasite. The thin smears are air-dried rapidly, fixed in methanol and stained. The red blood cells in the tail

end of the smear are examined under oil-immersion for the parasite.

***Quantified buffy coat (QBC) technique* (血沉棕黃層定量分析法)**

The detection of malaria parasites using the quantified buffy coat (QBC) 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.

1) Detection of an antigen (histidine rich protein-2, HRP-2) associated with malaria parasites (especially *P.falciparum* and *P.vivax*). The detection of HRP-2 and of pLDH forms the basis for diagnostic kits that have been, and continue to be evaluated. Consensus information, when available, will be reviewed.

2) Detection of antibodies Malaria antibody detection can be performed using various techniques. For the clinical laboratory, the most practical approach is the indirect fluorescent antibody (IFA) test. This test, with malaria parasites as antigens, detects most sensitively antibody responses to a wide range of plasmodial antigens.

The IFA procedure can be used to determine if a patient has been infected with *Plasmodium*. Because of the time required for development of antibody and also the persistence of antibodies, serologic testing is not practical for routine diagnosis of malaria. However, serology may be useful for screening blood donors involved in cases of transfusion-induced malaria when the donor's parasitemia may be below the detectable level of blood film examination, testing a patient with a febrile illness who is suspected of having malaria and from whom repeated blood smears are negative

Species-specific testing is available for the four human species: *P.falciparum*, *P.vivax*, *P.malariae*, and *P.ovale*. Cross-reactions often occur between *Plasmodium* species and *Babesia* species. Blood stage *Plasmodium* species schizonts (meronts) are used as antigen. The patient's serum is exposed to the organisms; homologous antibody, if present, attaches to the antigen, forming an antigen-antibody (Ag-Ab) complex. Fluorescein-labeled anti-human antibody is then added, which attaches to the patient's malaria-specific antibody. When examined with a

fluorescence microscope, a positive reaction is when the parasites appear fluorescent yellow.

Molecular diagnosis

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/ μl 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 (聚合酶链反应, PCR) 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 (see map, Fig) 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 *P. 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.)

A variety of interrelated factors contribute to the level of natural transmission of the disease in a given area.

1) Reservoir-the prevalence of the infection in humans, and in some cases other primates, with high enough levels of parasitemia to infect mosquitoes; this would include persons with symptomatic disease and tolerant individuals

2) Vector-suitability of the local anophelines as hosts; their breeding, flight, and resting behavior; feeding preferences; and abundance. Of the approximately 390 species of Anopheles, some are more suitable hosts for Plasmodium than are others. Of those, which are good hosts, some prefer animal blood other than human; therefore transmission may be influenced by the proximity with which humans live to other animals. The preferred breeding and resting places are very important. Some species breed only in fresh water, others in brackish; some like standing water around human habitations, such as puddles, or trash that collects water, such as bottles and broken coconut shells. Water, vegetation, and amount of shade are important, as are whether the species enters dwellings and rests there after feeding and whether the species flies some distance from breeding areas. Anopheles spp. exhibits an astonishing variety of such preferences. *Anopheles sinensis*, *A. anthropophagus*, *A. dirus* and *A. minimus* are important vector in China.

3). **Susceptible population:** that mean new hosts--availability of nonimmune hosts

4). **Local climatic conditions**

5). Local geographical and hydrographical (水文地理) conditions and human activities that determine availability of mosquito breeding areas

One must thoroughly study and understand all these factors before understand a malaria

control program with any hope of success. Sometimes deliberate government policy exacerbates transmission of malaria.

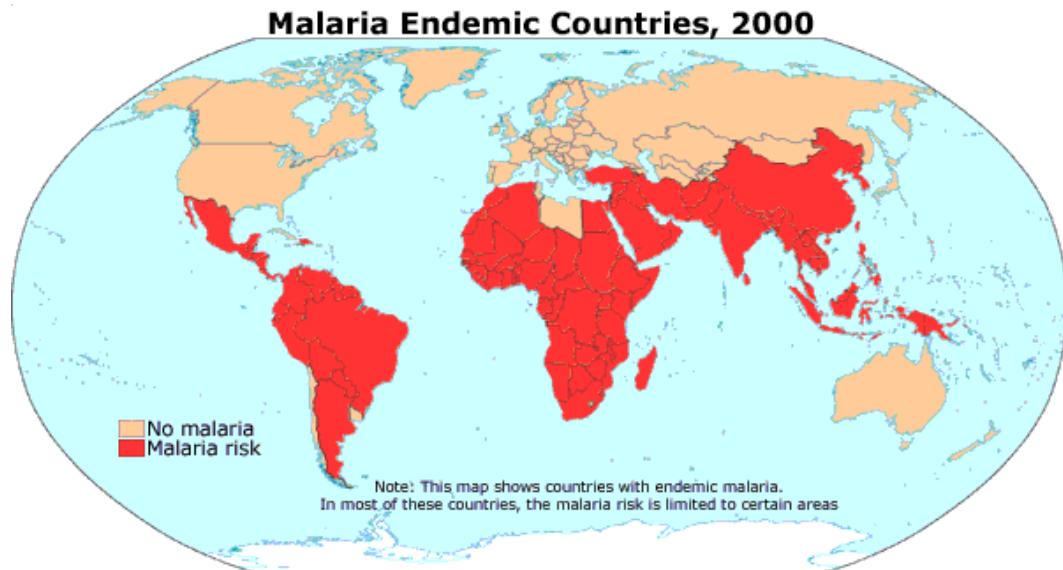


Fig II-VIII-9 Distribution of malaria in world (adapted from WHO report, 2000)

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. Centuries ago the Chinese used extracts of certain plants, such as chang shan and shun qi (the roots and leaves of *Dichroa febrifuga*, family Saxifragaceae) and qing hao (the annual *Artemisia annua*, family Compositae), that actually had antimalarial properties. In the meantime, Europeans were medically powerless and depended on absurd and superstitious remedies until quinine (奎宁) was discovered in the sixteenth century. Extracts of bark from Peruvian trees had been used with varying success to treat malaria, but alkaloids from the bark of the Peruvian tree *Cinchona ledgeriana* proved to be dependable and effective. The most widely used of these alkaloids has been quinine. The alkaloid of *D. febrifuga*, febrifugine (退热碱), is now considered too toxic for human use, but the terpene from *A. annua*, called qinghaosu (artemisinin), has been recently "rediscovered" and promises to be a valuable drug.

Only two synthetic antimalarials were discovered before World War II. Japanese capture of cinchona plantations early in the war created severe quinine shortage in the United States, stimulating a burst of investigation that produced a number of important drugs. The most important of these was chloroquine(氯喹).

Subsequently a number of valuable drugs have been developed, including primaquine, mefloquine, pyrimethamine, proguanil, sulfonamides such as sulfadoxine, 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 breeding places when possible or practical, introduction of mosquito predators such as the mosquito-eating fish *Gambusia affinis*, and judicious use of insecticides. The efficacy and economy of DDT have been a boon to such efforts in underdeveloped countries. Although we now seem to be aware of the supposed environmental dangers of DDT, we consider these dangers preferable and minor compared with the miseries of malaria. Unfortunately, reports of DDT-resistant strains of *Anopheles* are increasing, and this phase of the battle will become more difficult in coming years. For exterminating susceptible *Anopheles* spp. that enter dwellings and rest there after feeding, spraying the insides of houses with residual insecticides can be effective and cheap, without incurring any environmental penalty. Unfortunately, some *Anopheles* rest in houses only briefly before or after feeding, and sufficient quantities of DDT are becoming difficult to obtain on the world market.

Prevention of mosquito bite

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

* Further Reading —*Drug resistance in malaria infection*

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?

There have been no reports of clinical resistance to the artemisinin drugs so far although artemisinin-resistant strains of *P. falciparum* (Inselberg, 1985) and *P. yoelii* (Peters, *et al.*, 1993) have been developed in the laboratory. Clinical isolates and laboratory stains have been shown to vary in their sensitivities to these drugs but there is no evidence that this is related to clinical failure (Basco and Le Bras. 1993; Wongsrichanalai, *et al.*, 1997).

Laboratory studies have also shown that strains resistant to mefloquine appear to be less sensitive to artemisinin. There have been reports of reduced susceptibility of falciparum infections to artemisinin in Yunnan Province in China in border areas with the Lao PDR and Myanmar where there is migration, the out reach of health services has decreased and self treatment has increased (WHO, 1997).

There is no doubt that resistance to artemisinin will arise but it is impossible to predict where and when.

IX *TOXOPLASMA GONDII*(刚地弓形虫)

Toxoplasma gondii Nicolle & Manceaux, 1908 is a protozoan parasite that infects most species of **warm-blooded animals** (温血动物), including humans, causing the disease

toxoplasmosis (弓形虫病). The parasite probably is the only protozoan, whose 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 (*Ctenodactylus gundi*), a small rodent of North Africa. It was named as *Toxplasma*, due to crescent shape of its tachyzoite (速殖子). 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 cause the disease toxoplasmosis, especially in the immunocompetent hosts or in the immuno- compromised hosts. *T. gondii* is an important **opportunistic protozoan** (机会致病原虫).

MORPHOLOGY

There are five forms in *T. gondii* life cycle: trophozoite (tachyzoite, 速殖子), tissue cyst (bradyzoite, 缓殖子), schizont (裂殖体), gametocyte (配子体) and oocyst (卵囊). Tachyzoites, tissue cysts and oocysts are important stages seen during the life cycle of the parasite, all these stages are infectious to man.

Trophozoite (tachyzoite)(Fig 1,2): it is oval to crescent-shaped with a pointed anterior end and a rounded posterior end. It measures 4-7 μm in lengths and 2-4 μm 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 process known as endodyogeny, or by binary fission or schizogony. Tachyzoites divide until they fill the host cell, which then liberates them, and they reinvoke (or ingested by) other macrophages, repeating the process. The cell which contains them, when it becomes merely a bag full of tachyzoites, is called a pseudocyst (假包囊) (Fig1)



Fig II - IX -1 *T. gondii* tachyzoite and pseudocyst

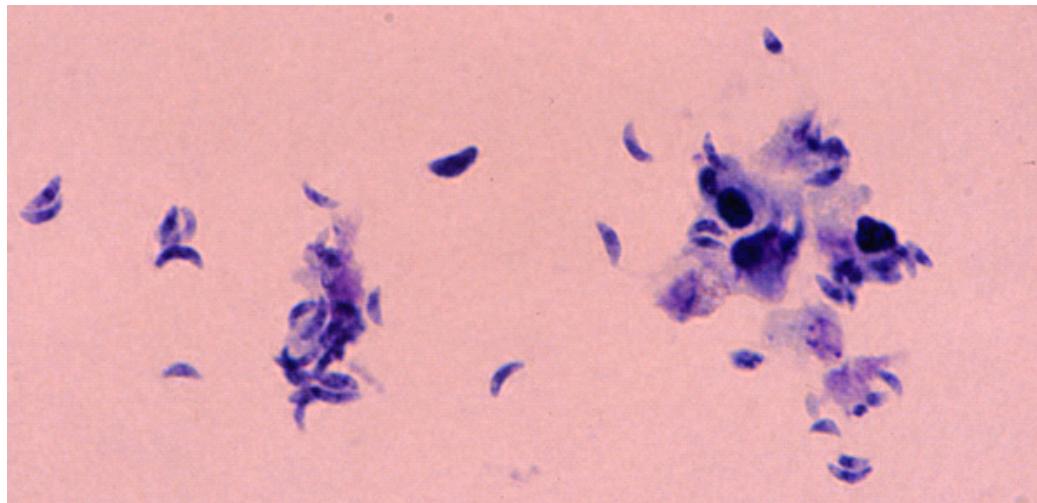


Fig II - IX - 2 *Toxoplasma gondii* in the bronchoalveolar lavage (BAL) material from an HIV infected patient. Numerous trophozoites (tachyzoites) can be seen, which are typically crescent shaped with a prominent, centrally placed nucleus. Most of the tachyzoites are free, some are still associated with bronchopulmonary cells

Tissue cyst: it is spherical and may vary in size from 5 to 100 μm 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 muscles. An eosinophilic cyst wall surrounds each cyst. The cyst contain hundreds of bradyzoite (缓殖子) or cystozoites. Bradyzoites multiply slowly.

Oocyst (卵囊): This stage is only present in cat and other felines but not in humans. It is oval and measures 10-12 μm in diameter. Each cyst is surrounded by a thick resistant wall which encloses a spheroplast (原生质球). The oocyst is liberated from the intestinal epithelial cell while still immature; it complete 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 (孢子囊). The contents of each sporocyst then divide once more to produce two infective sporozoites. Once mature, the oocyst may infected any warm-blooded animal which swallows it.

LIFECYCLE

Toxoplasma gondii needs two hosts to complete its life cycle. The definitive hosts are domestic cat and other members of the family Felidae (猫科) such as bob cats, ocelots, Bengal tigers, mountain lion, etc. The sexual multiplication or gametogony (the intestinal cycle) 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 intermediate hosts

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 reinvoke (or ingested by) 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

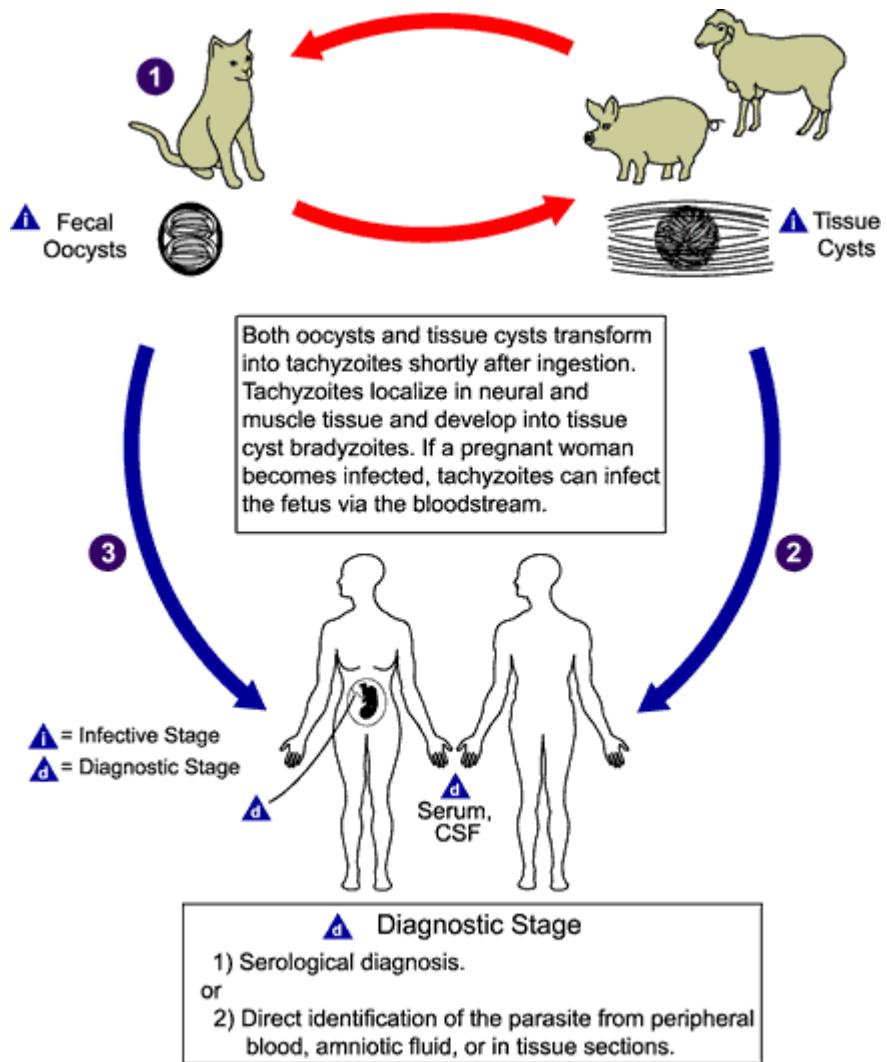


Fig II-IX-3 Life cycle of *Toxoplasma gondii* (Adapted from parasite image library of CDC, USA)

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 forming 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 (or tachyzoites) 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.

Develop in definitive hosts

Members of the cat family (Felidae) 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 reinvoke 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 21 days in cat's faeces. Millions of oocysts are excreted in the faeces daily, up to 3 weeks. The oocysts sporulate (形成孢子) 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.

Surprisingly the extra-intestinal cycle, which is seen in man and other non-feline hosts, also occurs in cat itself, possible by direct invasion of lymphatics or lymph nodes by tachyzoites produced in its own intestine.

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 (免疫缺陷) 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 recrudescence 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. These organs show areas of necrosis without inflammatory cells and the presence of tachyzoites and cysts.

Toxoplasmosis in man occurs as congenital, acquired, ocular infections in the immunocompetent hosts or an infection in the immunocompromised host.¹

Congenital toxoplasmosis(先天性弓形虫病)

Congenital toxoplasmosis results from an acute primary infection acquired by the mother during pregnancy. Transplacental (经胎盘的) 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 (chorioretinitis), hydrocephalus (脑积水), convulsions (抽搐), intracerebral calcifications (脑石灰化), etc.) may result. Infection of the foetus (胎儿), during last trimester of pregnancy, is more likely to be mild or asymptomatic at birth. Asymptomatic infection at birth, however, may manifest as several sequelae 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 (后遗症) in the infant, prompt and accurate diagnosis is important.

Acquired toxoplasmosis(获得性弓形虫病)

Acquired infection with Toxoplasma in immunocompetent (免疫活性的) persons is generally an asymptomatic infection. However, 10% to 20% of patients with acute infection may develop cervical lymphadenopathy (淋巴节病) 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 (CNS) disease but may have chorioretinitis (脉络视网膜炎), or pneumonitis (肺炎). In patients with AIDS, toxoplasmic encephalitis (脑炎) 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.

Ocular toxoplasmosis(眼弓形虫病)

Acute infection of eye begins as single or multiple foci of necrosis (坏疽) of retina (视网膜) with severe inflammation and exudation into the vitreous (玻璃体). Granulomatous inflammation of choroids (脉络膜) occurs secondary to necrotising retinitis (视网膜炎). 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 consequence of congenital infection. Bilateral central chorioretinitis and vitreous exudates are the typical ocular manifestations of congenital toxoplasmosis 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.

Infection in 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 (恶性肿瘤); or persons receiving organ transplantations (器官移植) 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 outcome of the condition.

IMMUNITY

Development of both the antibody and cell-mediated (CMI) 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 infection. 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

Pathogenic diagnosis:

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. The mice should be tested for the presence of *Toxoplasma* organisms in the peritoneal fluid 6 to 10 days post inoculation; if no organisms are found, serology can be performed on the animals 4 to 6 weeks post inoculation.

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 (IIF), indirect haemagglutination (IHA) and direct agglutination, latex agglutination and enzyme-linked immunosorbent assay (ELISA) are most frequently used tests. Many pf these have been marketed commercially as diagnostistic tests for toxoplasmosis. The semipurified or completely purified tachyzoites obtained from mice peritoneum are used as antigens in a variety of these assays.

Sabin-Feldman dye test was the first serological method to be described by Sabin and Feldman (1948) to detect circulating antibodies in toxoplasmosis. In this test, live tachyzoites are incubated with the accessory factor and test serum. Subsequently, alcoholic solution of alkaline methylene blue (pH 11) is added to the reaction mixture and re-incubated. The live tachyzoites are obtained from the peritoneal exudates of mice. The accessory factor is complement in nature and the normal human serum is the routine source of accessory factor. If the test serum contains *T. gondii* antibodies, live tachyzoites are inactivated and killed as a result of complement-mediated lysis. Hence, tachyzoites appear thin, distorted and colourless even in the presence of alkaline blue. If more than 50 percent of free tachyzoites first take up the stain and cytoplasm is colourless, the test is considered to be positive. The presence of 90-100 percent tachyzoite, deeply swollen and stained blue by alkaline methylene blue shows the test to be negative. It denotes the absence of *Toxoplasma* antibodies in the serum.

Dye test is a highly sensitive and specific test with no false positives reported so far in the literature. Since the test requires live tachyzoites and relatively is more cumbersome, it is now used only in a reference laboratory as a reference serological test, against which new immunoassays developed in toxoplasmosis are evaluated.

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 aqueous 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 (85%) 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 (NHANES III) between 1988 and 1994 was found to be 22%, with seroprevalence among women of childbearing age (15 to 45 years) of 10% to 15%. In China, 509 cases of recognizable disease result from 1964 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 (zoonotic 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 foetus, by the tachyzoites 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. Adequate cooking kill all the cysts in the meat. Fruits and vegetables that may be contaminated with oocysts should be washed adequately before eating.

X CRYPTOSPORIDIUM (隱孢子虫)

Cryptosporidium is a coccidian (球虫), which causes infection of the intestinal tract, particularly the small intestinal tract, particularly the small intestine. Once thought to be a non-pathogenic, this coccidian has been recognised recently as a cause of diarrhoea in man.

Numerous species of *Cryptosporidium* are known to affect amphibians, fish, birds and mammals, but *Cryptosporidium parvum* is the only species known to cause infection in man.

The parasite was first described by Tyzzer in 1907, in the peptic glands of a laboratory mouse. He suggested its present name *Cryptosporidium*. When first described, the organism was thought to be non-pathogenic and only 15 reports of *Cryptosporidium* infections in animals were recorded prior to 1975.

The first description of infection in man was reported in a three year old healthy girl in USA as late as 1976. Since then, the infection has been frequently diagnosed in patients with acquired immune deficiency syndrome (AIDS) and others receiving immuno-suppressive therapy.

MOPHOLOGY

The parasite shows six distinct morphological forms during its life cycle: oocyst, sporozoite, trophozoite, meront, microgamont, and macrogamont.

Oocyst: *Cryptosporidium* oocyst is the smallest coccidian known to cause infection in man. It is colourless, spherical to oval and measures 4.5 to 6 μm in diameter (Fig1). It does not stain with iodine and is acid-fast. The cyst is surrounded by a 50nm thin cyst wall. The latter consists of an electroluscent middle zone surrounded by two electron dense layers.

Each oocyst contains up to four slender bow-shaped sporozoites and many small granules. The oocysts are excreted in small numbers in the faeces. The number of oocysts excreted bears no relationship with the severity of illness. The oocysts which sporulate inside the host are of two types: Thick-walled and Thin-walled. The thick-walled sporulating oocysts are infectious to susceptible human hosts, whereas thin-walled oocysts always cause autoinfection ill the same host only.

Sporozoite: The sporozoite is slender, crescent-shaped and measures 1.5 to 1.75 μm in diameter. The anterior end is pointed but the posterior end that contains a prominent nucleus is rounded. The sporozoites numbering four remain always parallel to each other within an oocyst and are released only after partial digestion of the oocyst.

Trophozoite: It is the intracellular transitional form of the parasite. It is round or oval and measures 2 to 2.5 μm in diameter. Each trophozoite consists of a large nucleus (1 to 1.3 μm in diameter) with or without a conspicuous nucleolus (Fig2.). Unlike in the sporozoites and

merozoites, the apical complex is not present in the trophozoite.

Meront: It is of two types: type I and type II meronts. These two forms morphologically are indistinguishable from each other. They are crescent-shaped and measure 1 to 5 μ m in diameter showing rounded anterior and posterior ends (Fig2.).

Microgamont(小配子): Microgamonts are the male sexual forms. These are wedge-shaped, 0.2 to 0.7 μ m in length and are covered by a double layered membrane. Each microgamont contains a large compact nucleus and a polar ring. A single microgamont gives 1 to 4 microgametocytes.

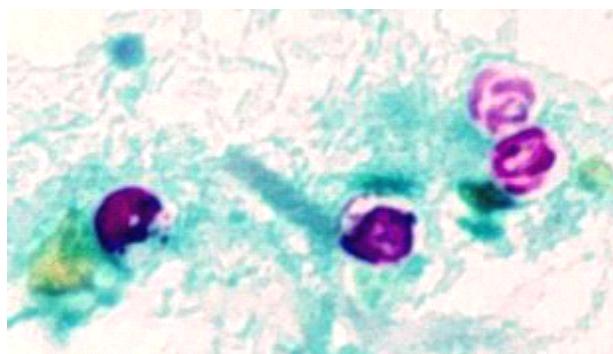


Fig II - X -1 Oocysts of *Cryptosporidium parvum* stained by the acid-fast method. Against a blue-green background, the oocysts stand out in a bright red stain. Sporozoites are visible inside the two oocysts to the right

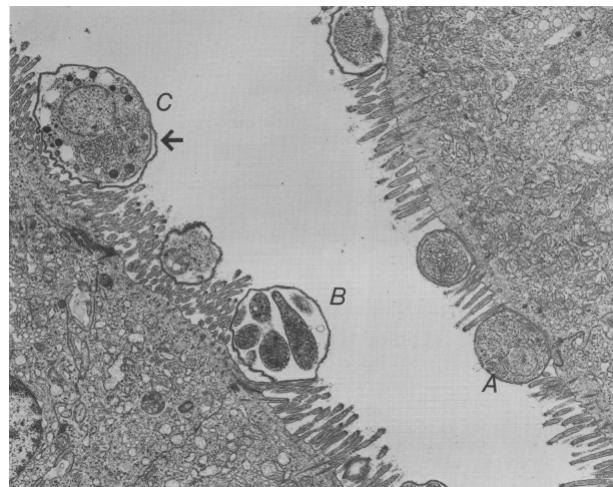


Fig II - X -2 Transmission electron micrographs of Cryptosporidium of sheep. Trophozoite (A), meront (B), and macrogamete-like stage(C) among microvilli at surface of epithelial cells, each stage intracellular and surrounded by host cell membrane (arrow)

Macrogamont(大配子): Macrogamonts are the female sexual forms. These are spherical, measure 3 to 5 μ m and are covered by a double layer membrane. Each macrogamont consists of a single large nucleus and endoplasmic reticulum. The old macrogamonts characteristically contain dense polysaccharide granules.

LIFE CYCLE

Cryptosporidium completes its life cycle through the stages of asexual generation (schizogony) and sexual generation (gametogony) in a single host (Fig. II-X-3). All these stages of the parasite are truly intracellular and are being surrounded by a host cell membrane, which is extra-cytoplasmic.

Man acquires infection on ingestion of food or drink contaminated with the faeces, containing sporulated thick-walled oocysts of Cryptosporidium. On ingestion, the infective sporozoites after being released from the oocysts in the small intestine, invade the epithelial cells in which they parasitise.

Inside the epithelial cells, the sporozoites subsequently differentiate into intracellular trophozoites. These trophozoites multiply asexually by nuclear division to produce two types of meronts, type I and type II (sexual generation or schizogony). Each type I meront produces six to eight type I merozoites, which develop into type II meronts. These in turn produce four merozoites each, which are known as type II merozoites. Some of the type II merozoites invade new host cells and initiate sexual replication (gametogony). Inside the host cells, they differentiate either into female (macrogamont) or male (microgametocyte) forms. Each microgametocyte produces 16 sperm-like microgametes, which fertilize the macrogamonts resulting in the formation of oocysts (zygote). Four sporozoites are formed inside each oocyst *in situ*.

The sporulating oocysts are of two types, thin or thick-walled. The thin-walled oocysts release the sporozoites inside the lumen of the intestine and cause auto-infection in the same host by repeating the cycle of schizogony and gametogony. The thick-walled oocysts excreted in the faeces are infective to other human hosts. The cysts under favourable conditions remain viable and infectious relatively for a long time. These cysts, when taken up by other susceptible human hosts, cause infection and the cycle are repeated.

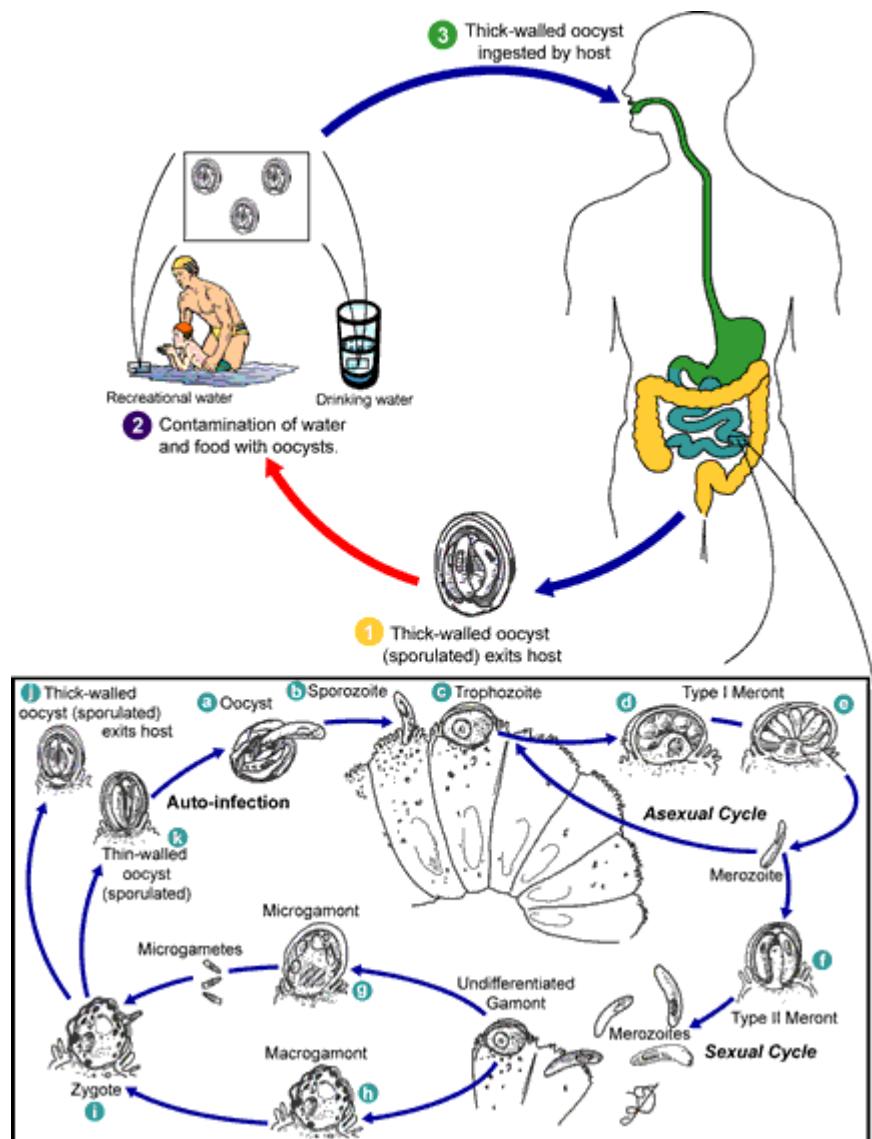


Fig II-X-3 The life cycle of *Cryptosporidium* (Adapted from parasite image library of CDC, USA)

PATHOGENESIS AND SYMPTOMS

The parasite inhabits the intestinal tract. It is found attached to the surface epithelial cells of villi or crypts of the small intestine. The organisms have also been found but less frequently in the stomach, appendix, colon, rectum and even pulmonary tree of the small intestine.

Infection begins with the firm attachment of *Cryptosporidium* to the mucosal surface of the intestine followed by invasion of epithelial cells. The specific mechanism by which the parasite causes illness in man is not known. The cholera-like voluminous watery diarrhea, seen in *Cryptosporidium* infection in the immunosuppressed hosts including, those with the AIDS is

possibly caused by a toxin. Reduction in the mucosal surface and decrease in the mucosal enzymes frequently seen in this condition also may contribute to pathophysiology of osmotic diarrhea by lowering the absorbing capacity of the small intestine.

Bacterial fermentation of sugars and fatty acids of the unabsorbed nutrients present in the lumen of the intestine, cause offensive and foul smelling stool, characteristically seen in *Cryptosporidium* diarrhea.

Cryptosporidium is found attached to the brush border of the small intestine particularly the jejunum. In the immunocompromised hosts, the parasites are also found in the uncommon sites such as pharynx, oesophagus, stomach, gall bladder, ileum, colon or rectum. They appear as small, basophilic round structures, staining readily with Giemsa and haematoxylin eosin stain. They are arranged in a row or clusters, along the border of the epithelial cells alone or in association with other intestinal parasites such as *Giardia intestinalis*.

Blunting and loss of villi, lengthening of the crypts and infiltration of lamina propria by lymphocytes, polymorphonuclear cells and plasma cell are the pathological changes of the intestinal tract, in cryptosporidiosis.

Incubation period ranges from 2 to 14 days. The prepatent period (time between infection and oocysts shedding) ranges from 5 to 21 days in man. The patent period (duration of oocysts shedding) may last for more than 30 days in an immunocompetent host.

The clinical manifestations of *Cryptosporidium* infection vary depending upon the immune status of the host.

Cryptosporidiosis in immunocompetent host: It is a mild infection in normally healthy patients. The duration of symptoms is relatively short and recovery is complete. The condition rarely is fatal.

Flue-like illness with watery diarrhea, malaise, nausea, fever and crampy abdominal pain are the characteristic features of the condition in immunocompetent hosts. Diarrhea is foul smelling with 2 to 10 motions per day, beginning on the first or second day of the illness. In some other cases, it is accompanied by prostration and weight loss even up to 10%. The oocysts may continue to be excreted in the faeces of the cases, twice as long, an average, as they had diarrhea.

Cryptosporidiosis in immunocompromised host: Cryptosporidiosis is a serious condition in

patients with depressed immunity due to AIDS, congenital hypogammaglobulinaemia or severe combined immunodeficiency syndrome, patients receiving immunosuppressive drugs such as corticosteroids and cyclophosphamide, and persons with severe malnutrition.

Cryptosporidium produces a cholera-like watery or mucus diarrhea in these groups of patients. Diarrhea relatively is more severe, profuse and watery with as many as 70 stools per day and loss of body fluids even up to 17 litres/day. Diarrhoeic stool may contain mucus but rarely blood or leucocytes. The main duration of diarrhea is 20 weeks with variability between 1 to 48 weeks. The prolonged diarrhea may lead to significant weight loss.

Low grade fever (39~C), nausea, vomiting and crampy abdominal pain are other but less frequent symptoms of the condition. Occasionally, non-specific symptoms such as malaise, myalgia and headache may be present. In some of the immnunosuppressed patients, *Cryptosporidium* affects the entire gastro-intestinal tract including the gall bladder, bile duct and pancreas and even pharynx and bronchial tree.

Cryptosporidium infection is of long duration and death is the frequent outcome of the infection in AIDS and AIDS related diseases. A few cases of spontaneous recovery have also been reported. The patients of reversible immune deficiencies show recovery from the infection when the cause of immune suppression is removed.

DIAGNOSIS

Clinical diagnosis of cryptosporidiosis is difficult as the condition clinically mimics giardiasis, isosporiasis and a few other infections caused by enteropathogens.

The absence of blood, pus cells, Charcot-Leydencrystals in the faeces may rule out amoebiasis, isosporiasis and bacillary dysentery and suggest the possibility of cryptosporidiosis.

The laboratory diagnosis of cryptosporidiosis is aided by parasitic and serologic methods.

Pathogenic diagnosis

The specific diagnosis of the condition is made by identification of oocysts in the faeces and less frequently the non-faecal specimens by microscopy and direct fluorescent antibody test.

1) Microscopy examination: The microscopic examination of direct faecal smear (wet smear and stained smear preparations) is adequate tot demonstration of oocysts in the acute cases shedding a large number of oocysts in their faeces.

2) Acid-fast staining methods: Acid-fast staining methods, with or without stool concentration, are most frequently used in clinical laboratories. A large number of staining procedures have been employed to demonstrate acid-fast oocysts in the faecal smears. The hot Ziehl-Neelsen carbol fuchsin staining method, a modification of acid-fast staining, is most frequently used to detect red-stained oocysts in the faeces.

Red-stained oocysts also can be demonstrated in the sputum, bronchial washings and duodenal or jejunal aspirations by acid- fast staining method.

3) Direct fluorescent antibody examination: It is a specific method for accurate identification of Cryptosporidium in the faeces. It is particularly useful to diagnose those doubtful cases, which are negative by faecal smear examinations.

Histopathological diagnosis

This is based on the demonstration of the developmental stages of the parasite (cysts 2 to 5 um in diameter, arranged in single or clusters in the intestinal mucosa) in the biopsy specimen from the jejunum and occasionally from the rectum. The invasiveness of the procedure and need for immediate processing of the specimen to avoid autolysis are the inherent disadvantages of the method.

Serodiagnosis

The indirect fluorescent antibody (IFA) and enzymelinked immunosorbent assay (ELISA), using purified oocysts as antigens have been used to detect circulating antibodies specific to Cryptosporidium in the serum. These antibodies appear in about six to eight weeks after onset of the infection. At the moment, these tests are carried out only in few laboratories to diagnose cryptosporidiosis.

EPIDEMIOLOGY

Cryptosporidium infection in the immunocompetent hosts has been described in more than 26 countries. The condition is worldwide with a prevalence of 0.6-20 percent in western countries and 4-20 percent in developing countries. In the AIDS, the condition has been described with a prevalence of 3-4 percent in the United States and same to 50 percent in Africa and Haiti. In China, the condition has been described from 19 provinces with a vary prevalence.

Reservoir, source of infection

Man is the key reservoir of infection. Livestocks such as cattle and pet animals (cat, dog) are other reservoirs. Human and animal faeces containing thick-walled oocysts are the important sources of infection.

Transmission: *Cryptosporidium* infection can be transmitted to man in the following ways:

1) Person-to-person transmission: This infection takes place by faecal-oral route through ingestion of sporulated thick-walled oocysts excreted in the human faeces, by drinking contaminated water. Rarely, it may be acquired by ingestion of milk or food contaminated with oocysts.

2) Zoonotic transmission : The infection is transmitted from the live stock, cattle or pet animals (cat, dog) either directly by ingestion of the oocysts derived from the faeces of these animals or indirectly by close contact with these animals.

3) Auto-infection. This is caused by sporozoites released from the thin walled oocysts inside lumen of the intestine. It is primarily responsible for persistence of infection in the infected host.

4) Other mode of infection

Rarely, the infection can be transmitted by aerosols, sexual contact and possibly by accidental laboratory infection.

Children between 1 to 5 years of age are at greater risk to the infection. Cryptosporidiosis has been detected more frequently in the urban children. In the rural areas where breast feeding is more common, the infection has been detected less frequently in the children below 1 year of age. The disease, which appears on increase in the last decade, tends to be more common during the warm rainy and humid months of the year.

Cryptosporidium oocysts show extreme resistance to the destruction by level of free chlorine present in potable water.

PREVENTION AND CONTROL

Cryptosporidium infection in the immunocompetent hosts is self-limiting and requires supportive treatment to prevent dehydration.

Infections in the immunosuppressed hosts with severe diarrhea and symptoms of malabsorption require supportive therapy with replacement of fluid, electrolytes and nutrients.

Antidiarrhoeal agents are of no value. For persons with AIDS, anti-retroviral therapy, which improves immune status, will also decrease or eliminate infection. Paromomycin (巴龙霉素) is approved for treatment.

The reduction or elimination of oocysts from the environment forms the mainstay of control of cryptosporidiosis but is difficult.

Freezing and heating at 65°C for 30 minutes kill all the oocysts. The care to avoid contamination of food and water with faecal oocysts prevent transmission of infection to man. Hand washing, use of gloves and improved personal hygiene will minimise risk of acquiring the infection in a hospital.

XI **PNEUMOCYSTIS CARINII**(卡氏肺孢子虫)

Pneumocystis carinii is a pathogen of uncertain taxonomic status. The capability to produce spores and cysts, places *Pneumocystis* (肺孢子虫属) within either of the two protistan groups, fungi or protozoa.

Pneumocystis carinii causes **interstitial plasma cell pneumonia** (间质性浆细胞肺炎), 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 (1912) described the organism in rats and they were the first to identify the organism as a distinct aetiological 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 aetiological agent of interstitial plasma cell pneumonia by Van der Meet and Brug (1942). Since then, there is an increased report of *P. carinii* infection, especially associated with the acquired immuno-deficiency syndrome (AIDS).

MORPHOLOGY

Three morphologically distinct stages are recognized: trophozoite or trophic form, pre-cyst and cyst.

Trophozoite(Fig II - XI-1): Trophozoites or trophic forms are always present in large numbers. 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.

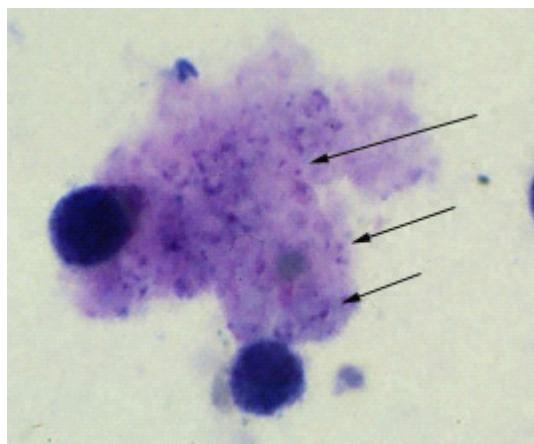
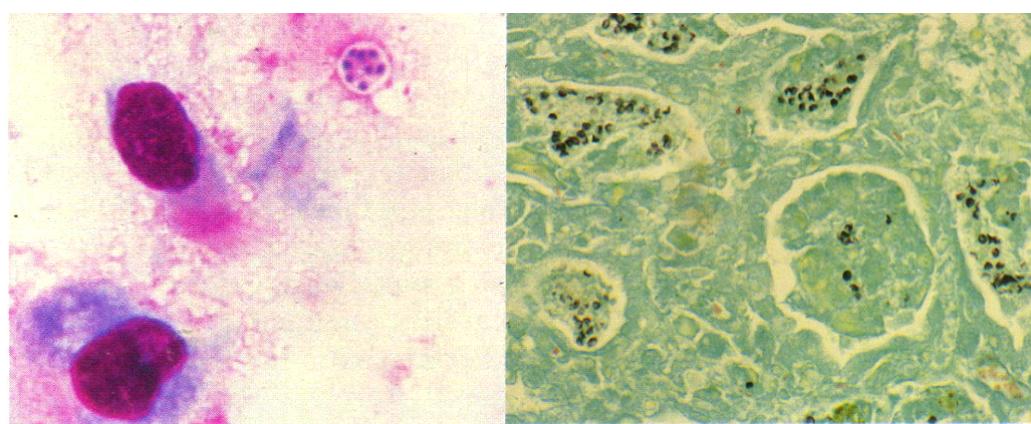


Fig II - XI - 1 *Pneumocystis carinii* trophozoites in bronchoalveolar lavage (BAL) material. Giemsa stain. The trophozoites are small (size: 1 to 5 μm), and only their nuclei, stained purple, are visible (arrows). AIDS patient seen in Atlanta, Georgia. (Adapted from parasite image library of CDC, USA)

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 (PAS) and silver methenamine stain clearly the cell wall. It is difficult to demonstrate this stage in the tissue.

Cyst(Fig2): It is spherical. 5-8 μm in diameter and is surrounded by a 70-140 nm thin cell wall. A mature cyst consists up to eight daughter forms or extra-cystic bodies called sporozoites. The sporozoites are spherical, crescent-shaped, measure 1-1.5 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

Fig. II - XI - 2 Cyst of *Pneumocystis carinii* (A. Giemsa stain. B. silver methenamine stain)

LIFE CYCLE (Fig II - XI - 3)

P. carinii occurs as a saprophyte (腐生物) 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. It is based on the morphologic studies of the lung sections obtained from the rat and on the parasites grown in culture.

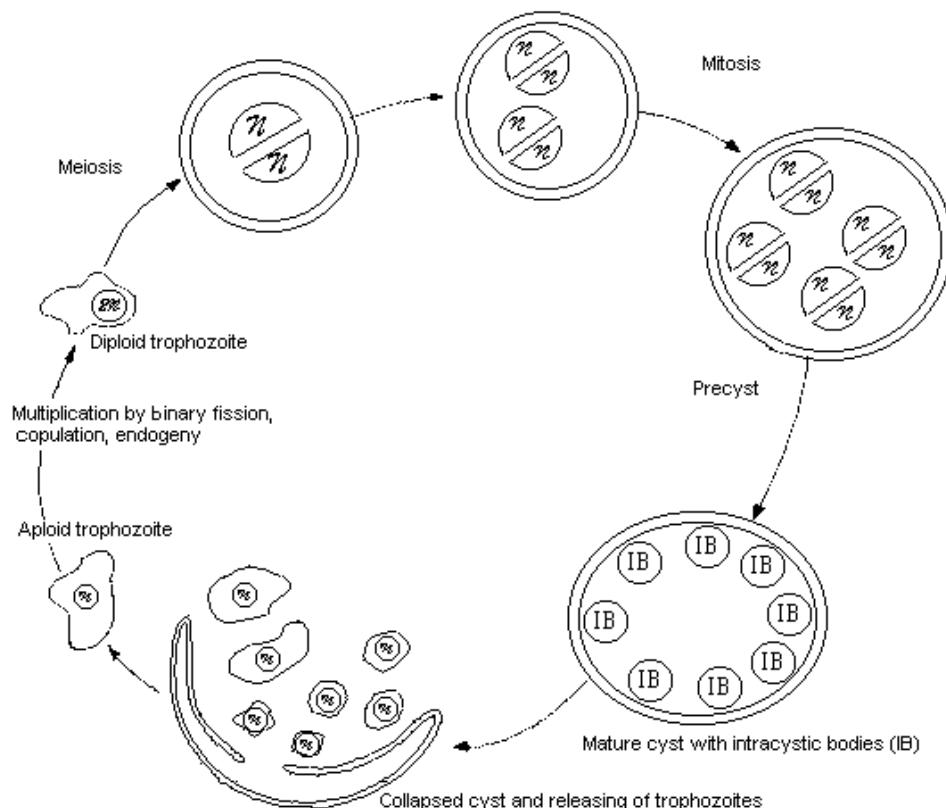


Fig II - XI - 3 Life cycle of *Pneumocystis carinii*

In man, the life cycle of *Pneumocystis* is extra-cellular and occurs in the lung alveoli. Intracellular 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 (内牙殖).

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 (脱囊) of cysts or encystation (成囊) 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.

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 intra-membranous particles.

The lungs, in pneumocystosis (肺孢子虫病) in humans are consolidated and appear reddish grey at necropsy (尸检). Histologic studies of the lung shows 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 immunosuppressed 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 pneumoeytosis (流行型或婴儿肺孢子虫病): This occurs in premature, malnourished (营养不良) and debilitated (虚弱) infants. Incubation period varies from 1 to 2 months. The symptoms of *P. carinii* pneumonia (PCP) include dyspnea (呼吸困难), non-productive cough (干咳), 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(散发型肺孢子虫病): *P. carinii* produces sporadic pneumocystosis in immunocomprornised children and adults with acquired immunodeficiency syndrome (AIDS), 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 (<3%) 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.

Pathogenic diagnosis

The specific diagnosis is based on identification of *P. carinii* in bronchopulmonary (支气管肺的) secretions obtained as induced sputum or bronchoalveolar lavage (BAL, 支气管灌洗液) 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 (such as Giemsa) 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 (IFA), complement fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA) 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 (CIEP-) and latex agglutination test (LAT) 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. Air-borne 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. carinii* 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(戊烷脒), trimethoprim (甲氧苄氨嘧啶) and sulfamethoxazole (新诺明) 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 (5 mg/kg per day) and sulphamethoxazole (25 mg/kg per day).

Section III TREMATODES (吸虫)

I. INTRODUCTION

Trematodes are members of phylum platyhelminthes(扁形动物门), which also includes the cestodes or tapeworms. The parasites are known as flukes. All trematodes parasitic to humans belong to Class Trematoda, subclass Digenea (复殖亚纲) .

The digenetic (世代交替的) 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 mollusc (软体动物) or, very rarely, an annelid (环节动物) . 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.

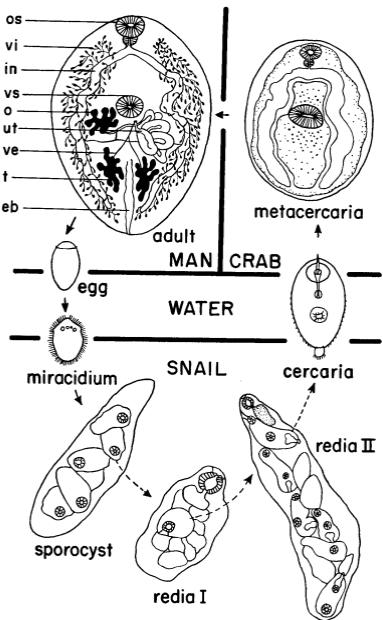
SPECIES

Digentic trematodes constitute one of the largest groups of platyhelminths (扁形动物) , 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.

复殖目 Digenea	后睾科 Opisthorchiidae	支睾属 Clonorchis	华支睾吸虫 C. sinensis	肝胆管 bile duct
	片形科 Fasciolidae	姜片属 Fasciolopsis	布氏姜片虫 F. buski	小肠 small intestine
		片形属 Fasciola	肝片吸虫 P. hepatica	肝胆管 bile duct
	并殖科 Paragonimidae	并殖属 Paragonimus	卫氏并殖吸虫 P. westermani	肝(脑等) live, brain etc
		狸殖属 Pagumogonimus	斯氏狸殖吸虫 P. skjabinii	皮下或肝 subcutaneous or live etc
	裂体科 Schistosomatidae	裂体属 Schistosoma	日本血吸虫 S. japonicum	门脉系统 portal-mesenteric vascular system
	棘口科 Echinostomatidae	棘隙属 Echinocasmus	日本棘吸虫 E. japonicus	小肠 small intestine
	异形科 Heterophyidae	异形属 Heterophyes	异形异形吸虫 H. heterophyes	肠管 intestine

LIFE CYCLE

Typical Life Cycle include sexual generation (有性生殖) and asexual generation(无性生殖) (Fig III- I -1).



1. Asexual generation in intermediate host(gastropod 腹足类 and peleypod 斧足类): Ovum 虫卵; Miracidium 毛蚴; Sporocyst 包蚴; Redia 雷蚴; Cercaria 尾蚴; Metacercaria 囊蚴
2. Sexual generation in definitive host (Mammalian host): Juveniles(larva); Adult worm

Fig III- I -1 An example of trematode life cycle

MORPHOLOGY

Despite superficial differences, the morphology of the various groups of digenetic trematodes is basically uniform. They characteristically flat, leaf like, hermaphrodite (雌雄同体) organisms except for the schistosomes which have a boat shaped male and a cylindrical female. One or more muscular suckers (吸盘) are always present on the ventral surface(usually possess a powerful oral sucker that surrounds the mouth, and most also have a midventral acetabulum (腹吸盘) or ventral sucker (腹吸盘) . Reproductive system is highly developed. Excretory and nervous are present.

The eggs of trematodes are operculated (有盖的) except for schistosomes.

Tegument (体壁组织) : The tegument of the adult worm is now recognized as a dynamic, cellular structure. Under light microscopy, it appears as a generally homogenous layer about 7-16 μ m thick. The tegument is a syncytium(合胞体), i.e., a multinucleated tissue with no cell boundaries. The outer zone of this syncytium is external plasma membrane, a surface coat, or glycocalyx (糖萼) , that varies in thickness according to species. It's function include absorption of exogenous molecules, shielding(protection) from hostile environmental influences and sense. Surface invaginations, the number and extent of which also vary according to species, serve to increase tegumental surface area, much like microvilli (微绒毛) on the surface of human intestinal cells.

Digestive system: It consists of mouth(oral sucker, prepharynx, pharynx, esophagus and intestine which divided into two cecums (肠管) .

Reproductive system: Most trematodes are hermaphroditic (雌雄同体的) except schistosoma. Male reproductive system consists of testis, vas efferens (输出管), vas deferens (输精管), seminal vesicale (储精囊), prostatic gland (前列腺), ejaculatory duct (射精管) or cirrus (阴茎), and cirrus pouch (阴茎囊) etc. Female reproductive system consists of ovary (卵巢), oviduct (输卵管), ootype, (卵模) Mehlis' gland (梅氏腺), seminal receptacle (受精囊), Laurer's canal (劳氏管), vitellaria (卵黄腺), vitelline duct (卵黄管), common vitelline duct (总卵黄管), vitellaria reservoir (卵黄囊), uterus (子宫) and metraterm (子宫末端) etc.

Excretory system It consists of flame cells(焰细胞), collecting tutules (集合管) and excretory bladder(排泄囊). These flame cells provide the basis for the identification of the species.
(Fig III- I -2)

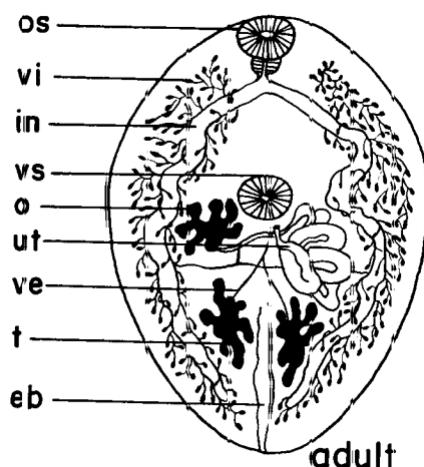


Fig III- I -2 An example of a adult trematode morphological structure

(os) oral sucker; (vi) vitellaria; (in) intestine; (vs) ventral sucker.; (o) ovary; (ut) uterus; (ve) vas efferens; (t) testis; (ab) excretory

II *CLONORCHIS SINENSIS* (华支睾吸虫)

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, measuring about long 10~25mm, wide 3~5 mm. It is relatively a small fluke. The tegument lacks spines(棘), Oral sucker is larger than ventral sucker(acetabulum). 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. Testes.

Mature egg The eggs are flask-shaped, operculated and relatively smaller in size, and measure $29 \times 17\mu\text{m}$. They are yellow-brown(bile stained), containing a well-developed miracidium, and possessing a small knob at the posterior end giving an appearance of a electric bulb..

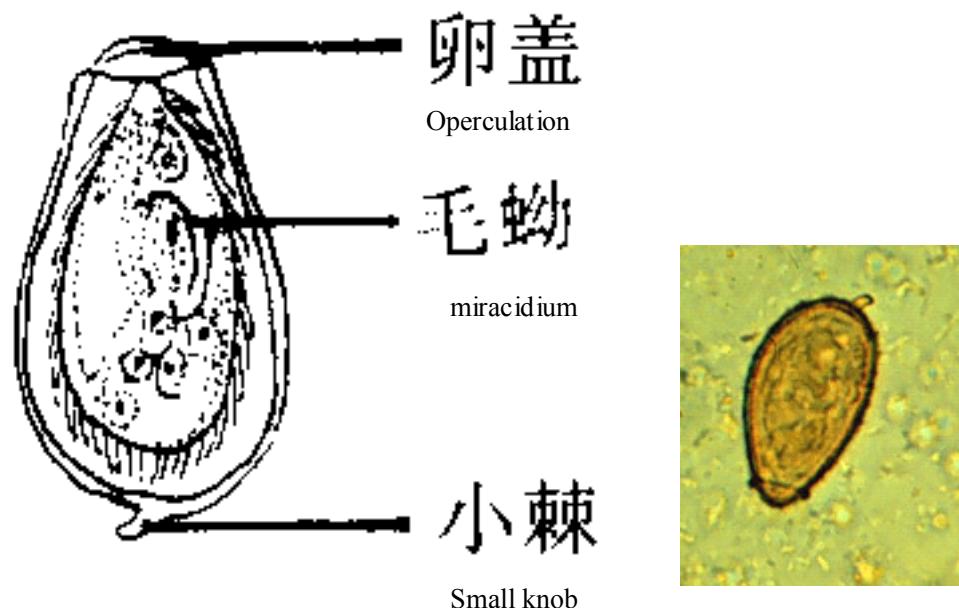


Fig III-II-1 Egg of *Clonorchis sinensis*

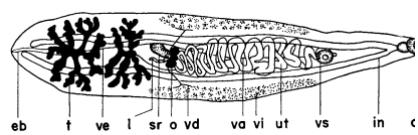


Fig III-II-2 Adult of *Clonorchis sinensis*, (ab) excretory; (in) intestine; (l) Laurer's canal; (o) ovary; (os) oral sucker; (sr) seminal receptacle; (vd) testis; (ut) uterus; (va) vas deferens; (vd) vitelline duct; (ve) vas efferens; (vi) vitellaria; (vs) ventral sucker.

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 undercooked fish or crustaceans (甲壳类动物) with metacercaria (囊蚴), definitive host will be infected. The young flukes excyst in 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 develop. Mammalian, i.e. cats, dogs and rates etc are important reservoir hosts.

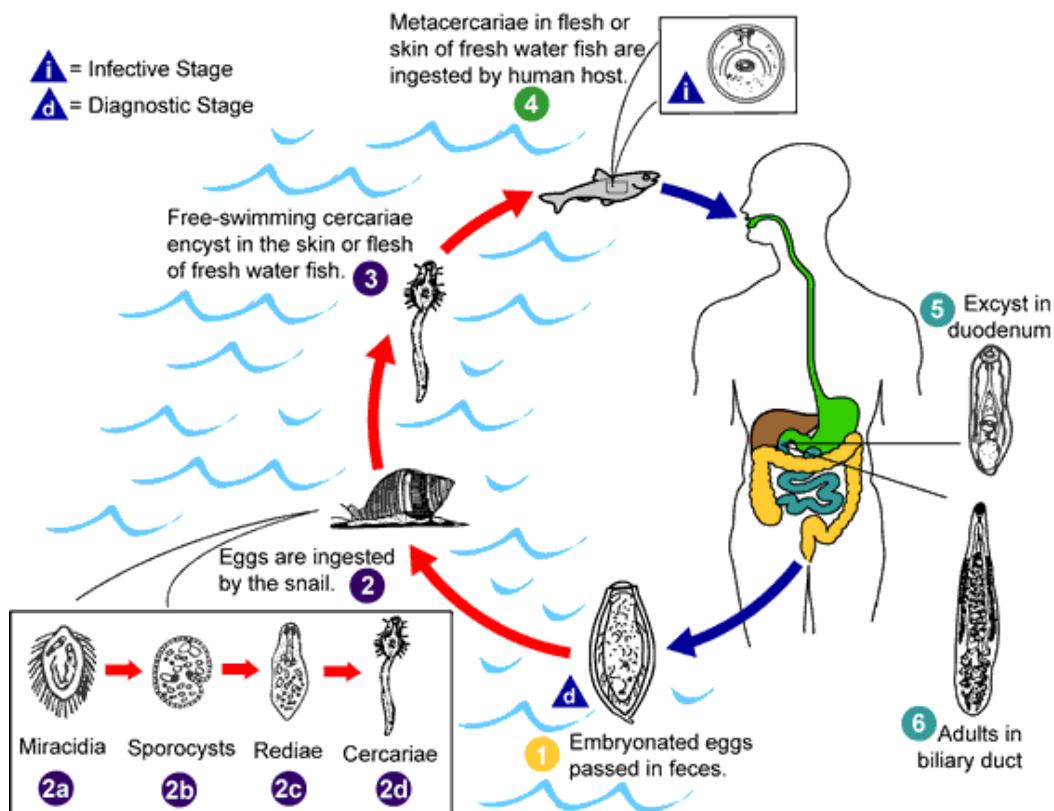


Fig III-II-3 Life cycle of *Clonorchis sinensis* (from Parasite image library of CDC, USA)

In the first intermediate host Eggs are hatched (孵出) into miracidium after being eaten by a suitable snail, then develop into a sporocyst (胞蚴); sporocyst transforms into redia (雷蚴); redia produce cercariae (尾蚴) 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 (metacercaria).

First Intermediate host: fresh-water snails eg. *Parafossarulus striatulus*(纹沼螺), *Alocinma longicornis*(长角涵螺), *Bithynia fuchsianus*(赤豆螺).

Second intermediate host: fresh-water fish i.e. *Ctenopharyngodon idellus*(草鱼), *Pseudorosbora parva*(麦穗鱼) et al.

PATHOGENESIS AND CLINICAL MANIFESTATION

The basic pathogenesis of the infection is erosion(糜烂) of the epithelium(上皮) 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(胆囊), 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 is 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(肝实质) are 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(胰腺炎). Cholangiocarcinoma(胆管癌) 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 examination* 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 十二指肠引流) is recommended also.

2) *Immunological tests* The intradermal test(IDT), 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. In our country, the infection cases were found in 24 provinces except Qinghai, Ningxia, Xinjiang, Neimenggu and Tibet etc. The prevalences of the infection in population of endemic areas varied from 1% to 30%. In Guangdong, the prevalence of the infection in population were up to 16.7%. it was estimated there was 4.7 million infected persons in China.

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.

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

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

PREVENTION AND CONTROL

Preventive measurement Metaceriae 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 °C, metaceriae in 1 mm slice of fish will be killed within 1 second; 75°C for 3 second; 70°C for 6 second and 60°C 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 (吡喹酮) has been reported to be effective for clonorchiasis. The recommended dosage is 75 mg per kilogram(kg) 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.

III *FASCIOLOPSIS BUSKI* (布氏姜片虫)

Fasciolopsis buski(*F.buski*) is the largest intestinal fluke, which can cause Fasciolopsis. In 1873, the first case of *Fasciolopsis* was found in Guangzhou by Dr. Kerr. Recently the 200 thousand infected persons was estimated in China.

MORPHOLOGY

Adult worm It is fleshy, reddish-beef-colored, elongate, and ovoid in shape. It measures 20~75mm in long, 8~20mm in breadth and 0.5~3mm in thickness, its body covered by spines. There are two sucker, ventral and oral. The ventral sucker is located close to oral sucker, as 4~5 time as oral sucker. Two branched ceca like wave pass to the posterior of the worm, two branched testes are located in the posterior half of worm, the ovary is also branched and lies in the midline anterior to the testes. Uterus is located between ovary and acetabulum

Egg It measure $130 \sim 140\mu\text{m} \times 80 \sim 85\mu\text{m}$ in size, which is the largest egg of helminths. It is pale yellowish, thin shell with a small operculum at one end, and contains one undeveloped embryonic cell (卵细胞) as well as 20~40 vitelline cells (卵黄细胞).



Fig III-III-1 The egg of *Fasciolopsis buski*

LIFE CYCLE

The life cycle include adult worm, egg, miracidium, sporocyst, mother rediae, daughter rediae, cercariae, metacercariae stage etc.

Human and pig etc are the definitive host of *F. buski*. Adult worm lives normally in the small intestine and to some extent in the stomach of definitive host.

Fertilized eggs passed into fresh water with stool, mature to miracidium and hatch at $26^\circ\text{C} \sim 32^\circ\text{C}$. Then miracidium enter several species snails and finish the development of sporocyst, mother rediae, daughter rediae, cercariae. *Hippeutis cantori* (尖口圆扁螺), *Polypylis hemisphaerula* (半球多脉扁螺), *Gyraulus convexiusculus* (凸旋螺), *Hippeutis umbilicalis* (大齐圆扁螺) are intermediate hosts. After 1~2 months in intermediate host, mature cercariae escape from snail and encyst into metacercariae on underwater vegetation, which including cultivated water chestnut and water caltrop etc.

If metacercariae are swallowed, worms excyst in the small intestine, become attached to the nearby intestinal wall, grow and mature in about 3 months without further migration.

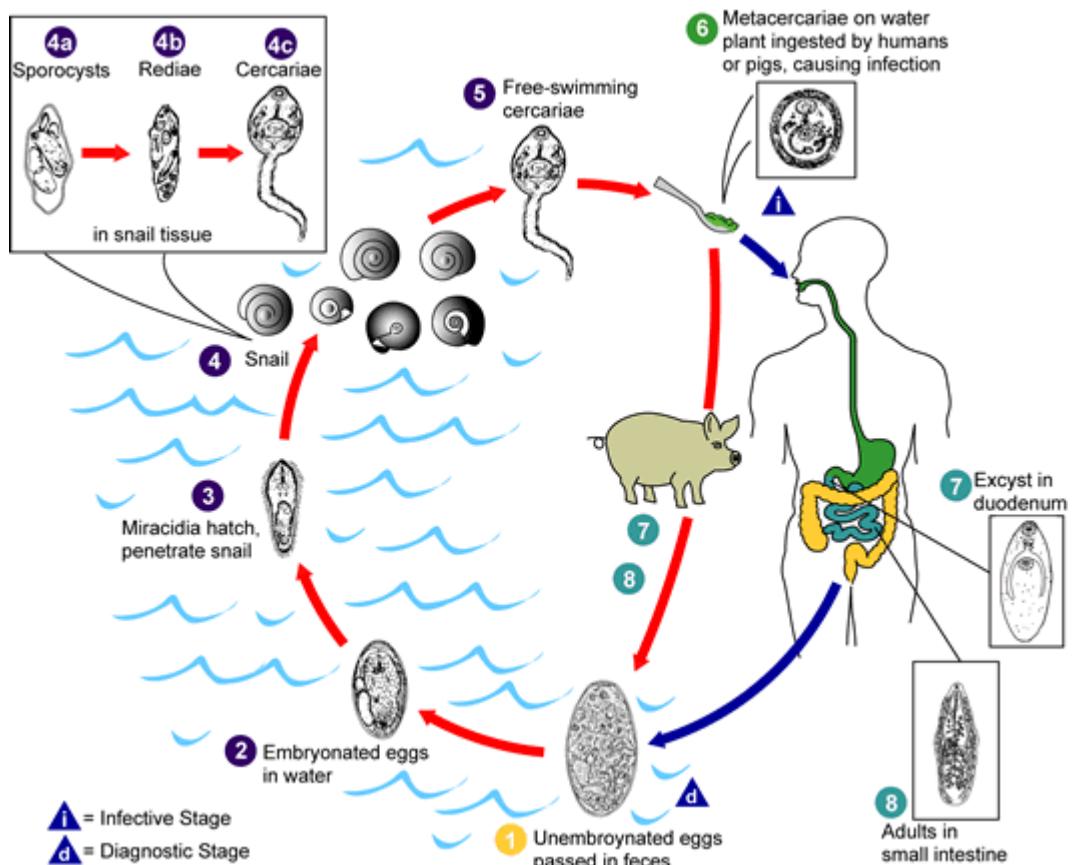


Fig III-III-2 Life cycle of *Flasciolopsis buski* (from Parasite image library of CDC, USA)

PATHOGENESIS AND CLINICAL MANIFESTATION

Because *F. buski* is larger fluke with stronger sucker, the mechanical irritation or damage is more serious than other flukes. In heavy infection, the flukes bodies covered the walls of intestinal and block the passage of food and interfere with normal digestive juice secretions. Its toxic substances can cause the hypersensitivity. Inflammation at the site of attachment provokes excess mucous secretion, which is a typical symptom of infection. Hemorrhage, ulceration (溃疡), and abscess of the intestinal wall result from long-standing infections.

Most of the infections are light and asymptomatic. In heavy infections, the main symptom is diarrhea with hunger pains, simulating peptic ulcer (消化性溃疡). In infected children these clinical manifestation such as weight loss, edema and anaemia (贫血) are found.

DIAGNOSIS

Specific diagnosis can be made by demonstrating the characteristic eggs and/or adult flukes in the feces. The eggs must be distinguished from those of other species such as *Echinostoma sp.*,

Fasciola hepatica and *F.gigantica*.

EPIDEMIOLOGY

Distribution The infection of *F.buski* is endemic in China, Thailand, Laos, Bangladesh, and India etc. In China, the endemic areas are found in 19 provinces such as Liaoning, Shanghai, Jiangsu, Zhejiang, Anfei, Fujian, Jiangxi, Shandong, Henan, Hunan, Hubei, Guangdong, Guangxi, Hainan, Sichuan, Guizhou, Gansu, Taiwan, and Shanxi etc.

Epidemic characters The worm of *F. buski* seems to be restricted to definitive host, which are only human, pigs and wild pigs. Meanwhile, fasciolopsiasis (姜片虫病) seems to be restricted to areas where people raise water plants and pigs, and to populations that commonly eat freshwater plants.

Human are infected by eating raw stems, leaves, and pods of water plants.

PREVENTION AND CONTROL

Water plants should be cooked or grown in ponds that are not contaminated with human or pig feces. Molluscicides could be used to eradicate the snail vectors.

The drug of choice is praziquantel (吡喹酮), a single dose of 15mg/kg after supper before going to bed. It is very effective.

IV ***PARAGONIMUS WESTERMANI* (卫氏并殖吸虫)**

More than 30 species of trematodes (flukes) 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 (咯血) or pulmonary paragonimiasis in man, it is also a parasite of carnivores. In 1878, *P. westermani* was first described from tigers. In 1880, the infection in human were found in Taiwan of China. Details of the life cycle were worked out by Yokogawa and Kobayashi during 1917 to 1921.

MORPHOLOGY

Adult worm (hermaphrodite 雌雄同体) It is thick, fleshy and when freshly passed it is reddish brown, in colour. 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 are **two 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 (未分裂) ovum surrounded by more than 10 vitelline cells.

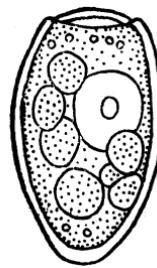


Fig III-IV-1The egg of *Paragonimus westermani*

LIFE CYCLE

The life cycle is completed in three different hosts, one definitive host and two intermediate host. 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, a

crustacean such as a crab (蟹) or crayfish (蜘蛛), where they encyst and become metacercariae.

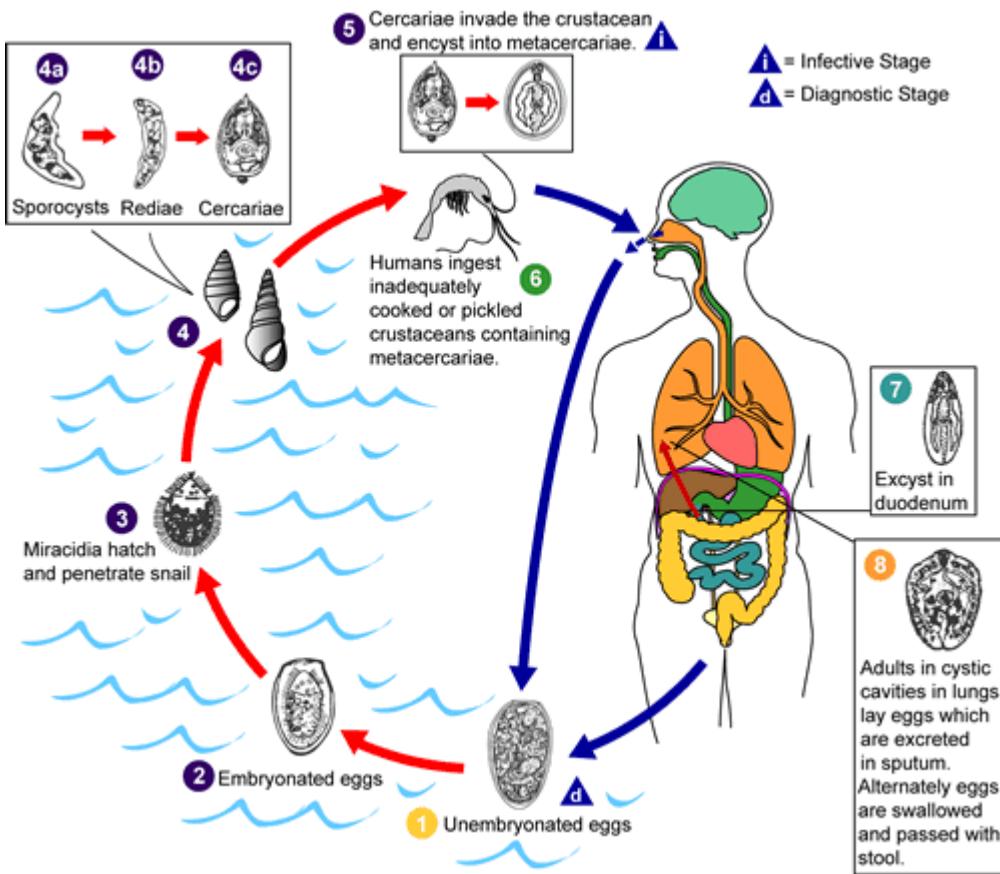


Fig III-IV-1 Life cycle of *Paragonimus westermani* (from Parasite image library of CDC, USA)

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 (产卵) 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** (损伤) 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** (肉芽肿) ,and **Fibrous scar** (纤维瘢痕) .*

1) Abscess The migration of flukes caused the destruction and **hemorrhage** of tissues; after that **neutrophilic** and **eosinophilic leukocytes infiltrate** (浸润) 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 cyst**".

3) Fibrous scar When the worm(s) die 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(larvae) 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 caused **ectopic lesions**, **worm cysts**, or **abscesses** in these other organs. he 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.

① Pulmonary paragonimiasis The symptoms **include** chronic cough, chest discomfort, blood-tinged sputum or rusty-brown sputum(containing eggs).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.

② 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.

③ **Liver paragonimiasis** Disorder of liver function, large liver, and liver pain are common symptoms of liver paragonimiasis.

④ **Cutaneous paragonimiasis** Some patients with lightly infection don't appear symptoms, but serological examination usually show 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 (IDT) 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%; **Dot-ELISA** for detecting circulating antigens(CAg) is considered to be useful for evaluating the effect of treatment.

X-ray examination of chest /CT In pulmonary paragonimiasis, chest x-ray shows radioopaque shadows in the middle and lower segments of the lung. CT scan of the chest, abdomen and head shows a cystic cavity. These radiological examinations are very useful especially in the areas where paragonimiasis also co-exists with tuberculosis. The interpretation of the radiological finding may not be always easy.

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

In our country, Paragonimiasis used to be in **23 provinces**, including Shandong, Jiangsu, Anfei, Jiangxi, Zhejiang, Fujian, Guangdong, Henan, Hubei, Hunan, Sichuan, Guizhou, Guangxi, Yunan,

Hainan, Taiwan, Gansu, Shanxi, Hebei, Liaoning, Jilin, Heilongjiang etc. The distribution of endemic areas is spot and most of endemic areas is located in mountain areas. Through control, Paragonimiasis have been controlled or eliminated in most endemic areas except a few endemic areas in Northern part of China. In these survival endemic areas, there are a few cases of Paragonimiasis. If a new endemic spot is found, the epidemic situations easy to be controlled.

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(wild pig) etc. It was reported that the disease possible is transmitted by ingestion of infected raw meat from the wild boars containing immature parasite.

3) Natural focus(自然疫源地) and parasitic zoonosis(人兽共患病) In some forest and desert the parasitic zoonoses transmit among vertebrate, which areas is called natural endemic focus(自然疫源地). In Kuandian county of Liaoning, infected dog is major reservoir, there non-human host play more important role in transmission. So paragonimiasis is a typical zoonosis.

4) First intermediate hosts: *Melaniidae* (黑螺科), fresh water snail, including *Semisulcospira libertina* (放逸短沟蜷) etc **Second intermediate hosts:** freshwater crabs and crayfish, such as *Potamon spp* (溪蟹), *Sinop otamon spp* (华溪蟹) and *Cambaroides spp* (蝲蛄).

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

- 2) ingesting raw or uncooked crab or crayfish containing metacercariae
- 3) ingesting the meat of a paratenic host containing immature flukes
- 4)** 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.

*** Compare**

	<i>P. westermani</i>	<i>C. Sinesis</i>	<i>F. buski</i>
Definitive host	human	human	human
Reservoir host	tiger, dog etc	dog, cat etc	pig
Paratenic host	boar etc	no	no
Life cycle:			
Egg	sputum/feces	feces	feces
Miracidium	water	snail	water
Sporocyst	snail	snail	snail
Rediae		snail	
Mother rediae	snail		snail
Daughter rediae	snail		snail
Cercariae	snail/water	snail/water	snail/water
Metacercariae(cyst)	crab/crayfish	fish/shrimp	water vegetation
Larvae	migration	no migration	no migration
Adult worm	lungs/other organs	bile ducts	intestine

V *SCHISTOSOMA JAPONICUM* (日本血吸虫)

INTRODUCTION

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 (复殖的) dioecious (雌雄异体的) trematods. The three main species infecting humans are *Schistosoma haematobium* (埃及血吸虫), *S. japonicum* (日本血吸虫), and *S. mansoni* (曼氏血吸虫). Two other species, more localized geographically, are *S. mekongi* (湄公血吸虫) and *S. intercalatum* (间插血吸虫). *S. japonicum* is the most pathogenic (致病性的) of all the human schistosome species. It causes schistosomiasis japonica

or Oriental schistosomiasis in human. Schistosomiasis japonica was first described by a physician Fujii(1847). The eggs were demonstrated in the faeces by Fujinami(1904). 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(1912-1913) 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(about 2100 years ago).

The discovery history of other human schistosomiasis are as follows:

- 1) *S.haematobium* eggs were found in the kidneys of twentieth-dynasty Egyptian mummies(1250-1000 B.C)
- 2) The first Europeans to record contact with *S.haematobium* were surgeons with Napoleon's army in Egypt(1799-1801).
- 3) In 1858(or 1852), Weinland proposed the name *Schistosoma haematobium*.
- 4) In 1905, Sir Patrick Manson found another species of worm, and was named *S. mansoni* by Sanbon(1907);

MORPHOLOGY

Adult Worm The adult worm of *S.japonicum* have elongate and slender bodies, and is sexual dimorphism(dioecious). 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 0.5-0.55 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* (抱雌沟) . 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(average50 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 (小刺) or delicate spine without operculum . There is a *mature eggs in the feces with miracidium* in the egg.

It is about 99 μ m in long and 35 μ m wide, the body like veritable spinning ball with cilia (纤

毛).

Cercaria Cercaria is consist of a body, a tail and a pair of furcae(分叉). The body is 100-150 μ m long, tail 140-160 μ m long, and furca 50-70 μ m 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.

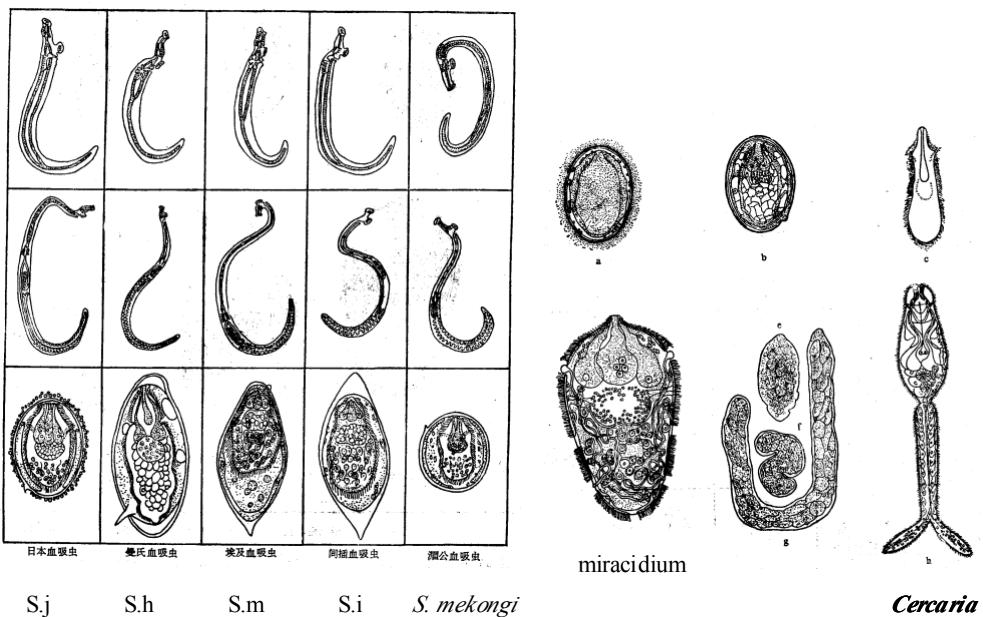


Fig III-V-1 Different development stages of *Schistosoma*

LIFE CYCLE

Life cycle include adult worm, egg, miracidium, sporocyst(mother and daughter sporocyst), cercariae, and schistosomula (童虫) 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* 湖北钉螺 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 (异位的) site such as the lungs, testis, kidney etc. **After** mating, the females are laying eggs. The worm work 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 (孵化) of miracidium in freshwater **After** mature eggs with miracidium enter water, the miracidium will be hatched out. The factors related to hatching include temperature of water(25-30 °C), osmotic pressure, PH value(7.5-7.8) 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(penetrate) 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 redelial 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(15-35 °C) and sun light etc. **Cercariae is the infection stage.** They are active swimmers, but may have motionless from the surface of the water. **The 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.

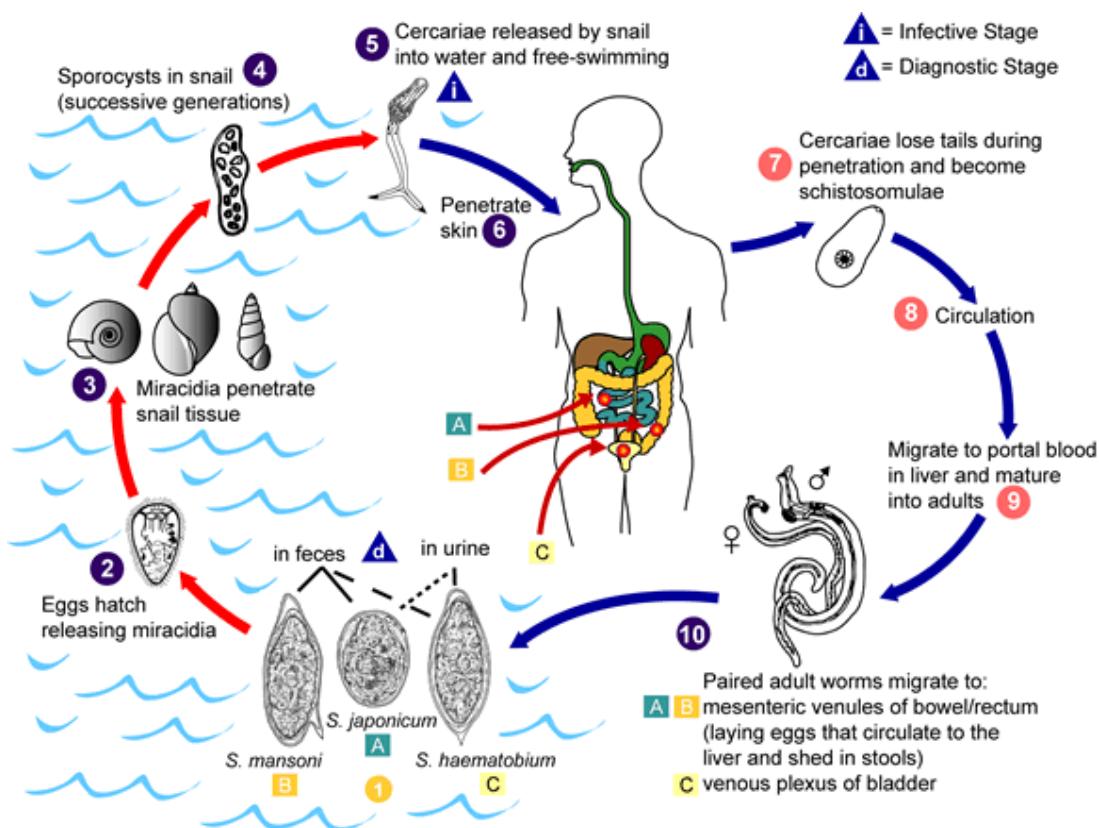


Fig III–V–2 Life cycle of *Schistosoma* (from Parasite image library of CDC, USA)

Migration and inhabiting

When a cercaria has penetrated the skin, it becomes a schistosomules (Once the organism penetrate 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 enter 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

Eggs 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 cause 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 **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 are 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 are plasmacyte(浆细胞). Meanwhile, antigen-antibody complex response named **Hoepppli 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 mediated."** 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**, than the permanent fibro-changes due to schistosoma infection was established. Late in the disease, periportal pipistem fibrosis and fibrosis in intestinal walls may result in a **clinical picture of cirrhosis**, including **portal hypertension (门脉高压) and splenomegaly (脾肿大)**. **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 is usually observed in persons entering the endemic area for the first time.

Chronic stage **In** 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(diarrhoea), hepato-splenomegaly, anemia and emaciation etc.

Late stage **In** general, some chronic cases with heavy infection will become **advanced cases** of schistosomiasis(late stage cases) 5 years after infection. **Advanced** cases has three types, **splenomegaly (large spleen), ascites and dwarfism.** **The common clinical finding** are *hepato-splenomegaly, ascites, portal hypertension, abdominal collateral vein dilatation and oesophagogastric varices* (食道及胃血管曲张). Blood loss due to bleeding of oesophagogastric

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 used for diagnosis. Circulating antigens(**Cag**) are useful for diagnosis, including **GAA**(gut associated antigens), **MAA**(membrane-associated antigens) and **SEA**(soluble egg antigens).

Concomitant immunity (伴随免疫) "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 phenomena** in helminthes infection. In schistosome infection, the mechanism of concomitant immunity is related to "**immune evasion (免疫逃避)**" of schistosome.

The ways of immune evasion are as follows:

1) Masquerade (伪装) by the uptake of 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 (分子模拟) *Schistosomes* were capable of synthesizing antigens that cross-reaction with host molecules.

3) Shedding of schistosome surface antigens *Shedding* of schistosomula antigens can

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 epidemiological 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, platelets, 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, epidemiological studies showed that humans can **slowly develop an acquired resistance to reinfection**, which immunity is **age-depend**. 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 thick 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(IDT) Antigen: adult worm antigen is used for IDT commonly, the sensitivity is more than 95 %, false positive rate is about 2 %. The test can be done 2 weeks after infection, so it possess the values for early diagnosis(or for new infection) 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.

(1) Circumoval precipitin test(COPT)

antigens: viable eggs

specimen: sera

sensitivity: 97.3%

false positive rate: 3.1%

(2) Indirect haemagglutination test(IHA)

antigens: soluble egg antigen(SEA)

specimen: sera

sensitivity: 92.3-100%

false positive: 2%

③ ***Enzyme-linked immunosorbent assay(ELISA)***

antigens: SEA/AWA(adult worm antigen)

specimen: sera

sensitivity: 95-100%

false positive rate: 2.5%

3) Antigen detection **Unlike** antibody detection which can not distinguish between

active and past infections, the detection of circulating schistosome antigens(**CAg**) may be a promising approach to the diagnosis of a current infection and the evaluation of drug treatment.

methods: dot-ELISA and sandwich ELISA(double-antibody method)

antibodies: monoclonal antibody(McAb)

sensitivity: 84.5%(29.0-85.0 %)

false positive rate: 3.1% (data from Qu Lizhu 1992)

the negative rate a half year after treatment was still 50%

* ***It is considered that the methods still need to be improved or modified.***

CHEMOTHERAPY

Praziquantel is a antischistosomal agent with high efficacy and low toxicity. A single dose of **40 mg/kg (50mg/kg)** is applied for treating chronic cases or mass treatment in our country. WHO recommends a dosage of **60mg/kg in 2 divided** doses give with a 4-h interval on the same day. A total of **120 mg/kg in 4-6 days** for treating acute cases. Up to now, no deaths due to the drug have been reported in more than thousands individuals treated in our country.

EPIDEMIOLOGY

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

Distribution In our country schistosomiasis used to be in **391(381)** counties of **12**

provinces in the south of China, including Hubei, Hunan, Jiangxi, Anhui, Jiangsu, Shanghai, Zhejiang, Fujian, Guangdong, Yunnan, Guangxi, and Sichuan. **79 million** people resided in endemic areas and **14.8 billion m²** snail-ridden areas. **After** more than 40-year control, schistosomiasis has been eliminated in 5 provinces/municipality/autonomous region, including **Shanghai(1985), Guangdong (1985), Fujian, Guangxi, and Zhejiang(1995)**. **Among** 391 endemic counties, of **222 counties** have eliminated schistosomiasis, and of **56** controlled transmission of schistosomiasis.

The current epidemic situation There are still **113** endemic counties still in China, including *marshy type* endemic areas (Hubei, Hunan, Jiangxi, Anhui and Jiangsu) and *high mountains* endemic areas (Sichuan and Yunnan). Up to 1995, infected individuals was estimated to be **865084(4.89 % prevalence)**. **Therefore, schistosomiasis is still considered to be a public health problem in China.**

Types of endemic areas There are three types of endemic areas, including **Wwater-net regions of plain 水网型** (e.g., Shanghai, Jiangsu and Zhejiang), **marshy regions 湖沼型** (e.g., Hubei, Hunan, Jiangxi, and Anhui), and **mountainous and hilly regions 山丘型** (e.g., Sichuan, Yunnan, Guangdong, Guangxi, and Fujian). **In marshy regions** including Poyang lake region, Tongtin lake region and beaches of Yangtze river from Hubei to Jiangsu. There are vast snail-infested areas, which areas is about 82% total snail-infested areas in China.

Epidemic features

1) Main reservoirs of infection *Sjaponica* 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 buffaloes are major reservoirs**.

2) Way to infection *Contacting infected water containing cercaria is only the way(route) 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 clothes; **for playing** e.g. bathing.

3) Susceptible population **Humans** is a susceptible host of *Sjaponicum*. **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 *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 The 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

Control objectives In China, the objective for schistosomiasis control is to **control morbidity** due to schistosomiasis and **to interrupt**, in some areas, **its transmission**. The specific objectives of the technical strategy are to: a) to **reduce morbidity** in areas of the high endemicity (>15 % prevalence); b) to **control morbidity** in areas of medium endemicity(areas with prevalence >3% and <15%) and c) **control morbidity, and transmission**(where appropriate), 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 mollusciding 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(蒿甲醚) and Artesunate(青蒿琥酯) which are artemisinin(Qin-hao-su)'s derivatives are recommended to take.

CERCARIAL DERMATITIS (尾蚴性皮炎)

Cercarial dermatitis is called "**rice-field dermatitis (稻田皮炎)**" in our country, and named "**swimmer's itch (游泳痒)**" 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 larva 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 (毛毕属)** and **Orientobilharzia (东毕属)**. *Trichobilharzia* include *T.paoi* (包氏毛毕吸虫), *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 contact the water containing the cercaria in rice fields or pools, the cercaria can penetrate the skin and cause cercarial dermatitis.*

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

Cercarial dermatitis is prevalent in Jiling, Liaoling, Shanghai, Jiangsu, Fujian, Guangdong, Hunan, Sichuan etc. **Infected cattle and ducks are major reservoirs.** *Contact the water-body containing the cercariae is common way to acquire infection.* The transmission seasons are usually from May to July in Liaoling, or from March to Oct. in Sichuan.

Treatment of cercarial dermatitis is symptomatic, and **avoiding exposure** to contaminated waters is the only effective control measure.

Section IV TAPEWORM(绦虫)

I. INTRODUCTION

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

MORPHOLOGY

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

Adult worm Adult worm is flat, long, white or milk white in color. It consists of **scolex** (头节), **neck** (颈节), and **strobilus** (链体). 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** (节片). 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 (吸盘), grooves (吸槽), hooks (小钩), spines (小刺), glands, or combinations of these.

The scolex of *Cyclophyllidea* (圆叶目) is like a ball, in which there are four circular-suckers with rostellum (顶突). The scolex of *Pseudophyllidea* (假叶目) is shuttle-like, its holdfast organs are two grooves named bothrium (吸槽).

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

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

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 (微毛)" that are underlaid by the tegumental distal cytoplasm. The microtriches are similar in some respects to the microvilli (微绒毛) found on gut mucosal cells and other vertebrate and invertebrate transport epithelia (上皮细胞), and they completely cover the worm's surface, including the sucker.

Calcareous corpuscles (石灰小体) 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 (石灰小体)** has been the

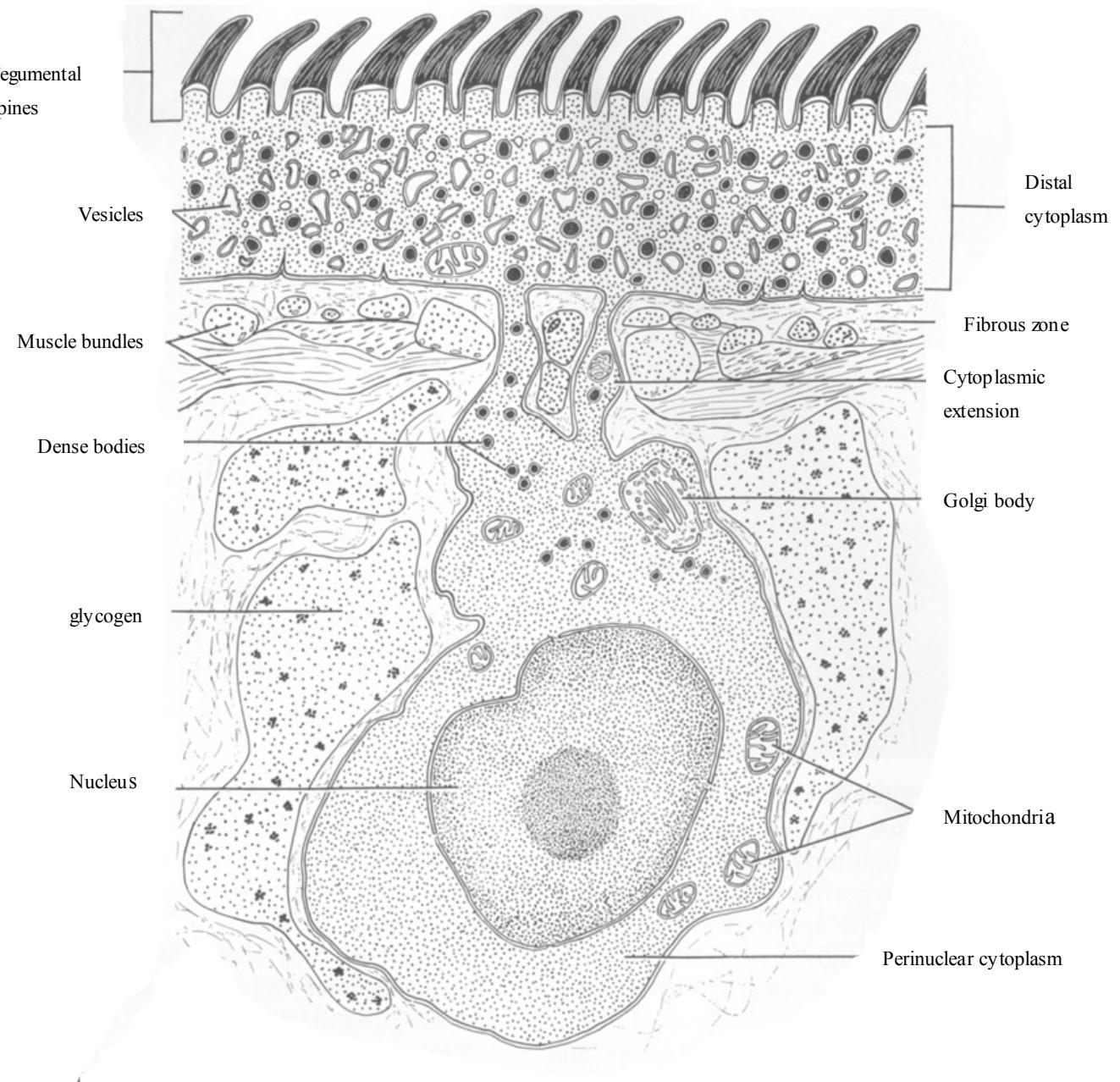


Fig IV- I -1 Diagram of the tegument of *Echinococcus granulosus*[from Moeseth, 1967]

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 (雌雄同体的). 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** (睾丸), **vas efferens** (输精管), **seminal vesicle** (储精囊), **deferens cirrus pouch** (阴茎囊), and **cirrus** (阴茎) etc. The female reproductive system consists of an **ovary** (卵巢) and associated structures, including **vitelline follicles** (卵黄腺), **vitelline duct** (卵黄管), **uterus** (子宫), **seminal receptacle** (受精囊) and **vagina** (阴道) etc.

LIFE CYCLE

Cestodes (绦虫) complete their life cycle in two or their different hosts(exception: *Hymenolopis nana* 微小膜壳绦虫 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**"(中绦期) when larva tapeworm live 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:(1) embryogenesis (胚胎发育) within the egg to result in a larva, the **oncosphere**(六钩蚴);(2)hatching of the oncosphere after or before being eaten by the next host, where it penetrates to a parenteral(extraintestinal) site;(3)metamorphosis of the larva in the parenteral site into a juvenile(**metacestode**) usually with a scolex;(4)development of the adult from the metacestode in the intestine of the same or another host.

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

The development stages of Cyclophyllidea: **egg**, **oncosphere**(in intermediate host), **bladder worm** (囊虫) /**coenurus** (多头蚴) /**hydatid cyst** (棘球蚴) containing **proto-scolex** (原头蚴) and **alveolar hydatid cyst** (泡球蚴)/**multilocular hydatid cyst**(多房棘球蚴) 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* 阔节裂头绦虫).
- 2) Causing mechanical obstruction or migration to the unusual sites(e.g., *Taenia species* 带绦虫).
- 3) Evoking local inflammatory reactions(e.g., *Taenia solium* 猪带绦虫).

Larvae Tapeworm larvae can invade almost any internal organ in a human being, and may cause serious pathological damages. Bladder worms or plerocercoids (裂头蚴) migrate in subcutaneous tissue and develop cysts. If invading eye or brain, the infection will have serious consequences. Hydatid cyst (棘球蚴) 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.

MAIN HUMAN TAPEWORMS

Pseudophyllidea: *Spirometra mansoni*(曼氏迭宫绦虫),

Diphyllobothrium latum(阔节裂头绦虫)

Cyclophyllidea: *Taenia solium*(链状带绦虫)

Taenia saginata(肥胖带绦虫)

Echinococcus granulosus(细粒棘球绦虫)

Echinococcus multilocularis(多房棘球绦虫)

Hymenolepis nana(微小膜壳绦虫)

Hymenolepis diminuta(缩小膜壳绦虫)

II **TAENIA SOLIUM** (猪带绦虫/链状带绦虫)

T. solium called the pork tapeworm can cause the infection of *T.solium taeniasis* (猪带绦虫病), and its cysticercus (囊尾蚴) cause human cysticercosis (囊虫病). The life cycle of the parasite was first described by Kuchemeister(1855) and Leukart(1856). 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. Differences between the adult worms of *T.solium* and *T.saginata* are described in the below Table.

1) Scolex Typically, it is provided with hooklets (小钩), 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 (顶突). The latter consists of two small and large hooks(25-50 hooks).

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

3) Proglottid The strobila (链体) 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 are provided with a two layered shell. The outer shell is thin, transparent and does not always remain with the eggs. The inner embryophore (胚膜) is a thick, brown, roughly structured wall which surrounds the embryo.

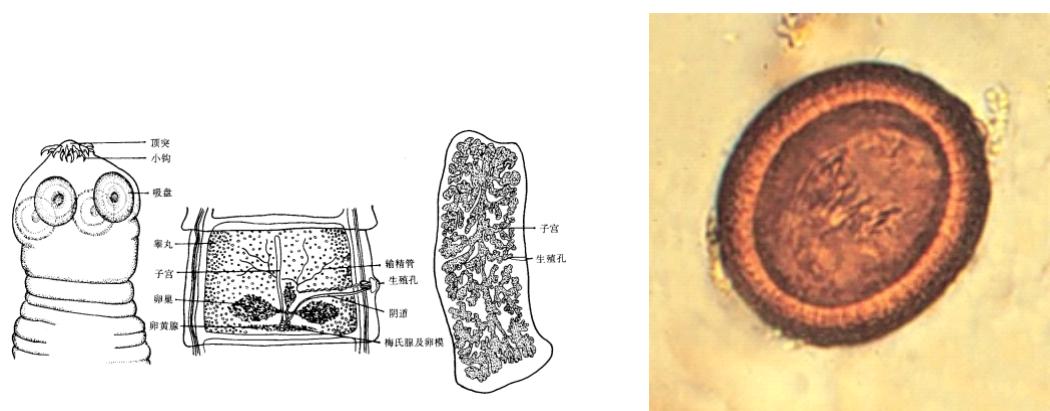


Fig IV-II-1 Scolex, mature and gravid proglottids of *T.solium*

Fig IV-II-2 The egg of *T.solium*

5) Cysticercus cellulosae (猪囊尾蚴) Cysticercus cellulosae is the larval stage of *T.solium*. Cysticerci are small, oval and milky white bladder-like structure. They are filled with a fluid rich in albumin and salts. It shows a small white spot representing the future head invaginated (内翻) 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 live in the intestine, and fix the wall by its scolex. The gravid proglottids become detached from the strobila (链体), 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.

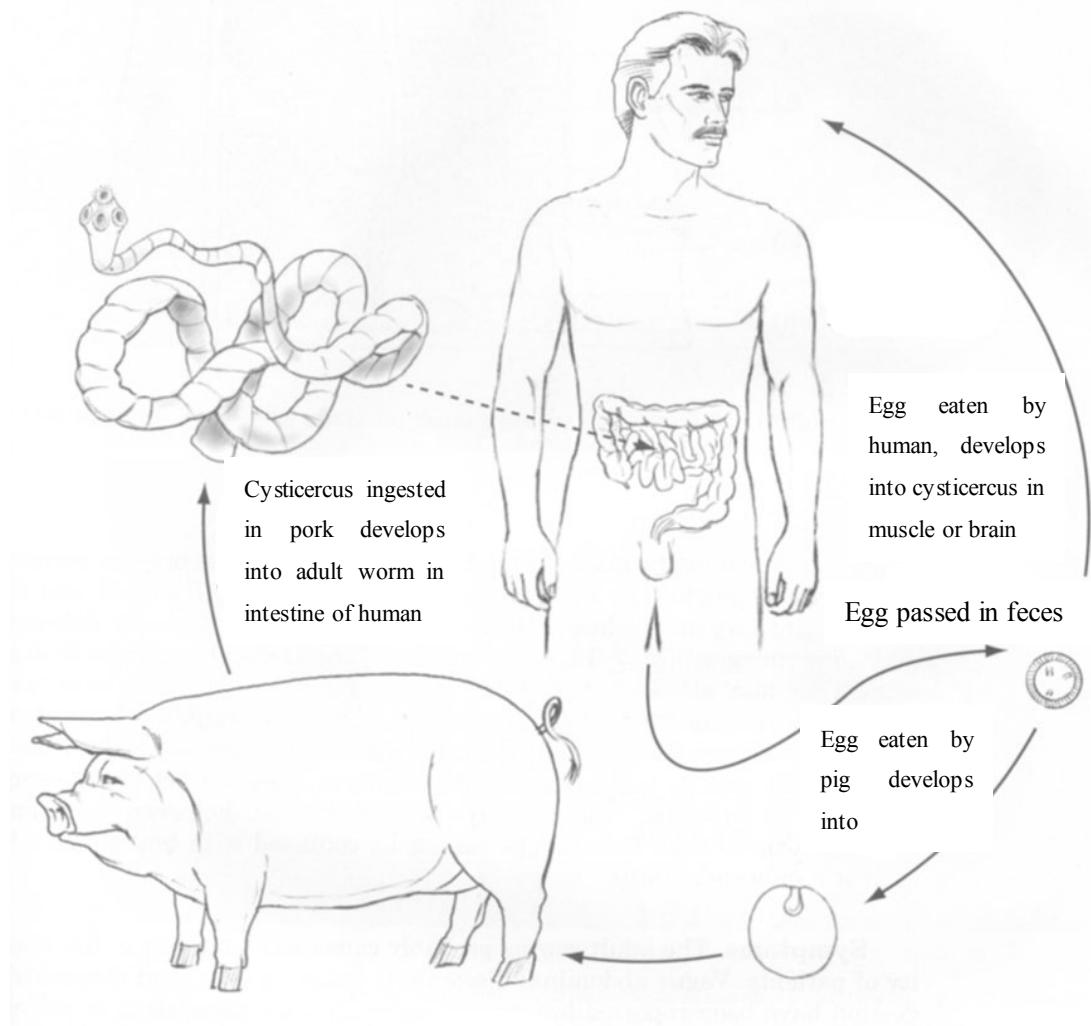


Fig IV-II-3 Life cycle of *Taenia solium*

The oncosphere (六钩蚴) 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 develop bladder worm like in pig. But it can't continue to develop adult worm.

PATHOGENESIS 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 ***Tsolum 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 disease 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 ophthalmic(眼的) cysticercosis. The *cysticerci* are often present in the subcutaneous tissue, vitreous humour(玻璃状液), anterior chamber(眼前房) 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(脑脊膜), cerebrum(大脑), ventricles and subarachnoid space(蛛网膜下腔), 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 (脑囊虫病) 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 range from one month to one year, but can last 30 years in a few cases. The symptomatology of cerebral cysticercosis is characterized by three basic syndromes: convulsions (惊厥), intracranial hypertension, and psychiatric disorder, occurring separately or in combination. The prognosis of cerebral cysticercosis is highly variable and unpredictable.

Ocular cysticercosis (眼囊虫病) may cause irreparable damage to the retina, iris, uvea, or choroids. This disease constitute about one-fifth of human neurocysticercosis cases. Host reactions to cysticerci vary from slight to severe inflammation with complication such as chorioretinitis (脉络膜视网膜炎), and iridocyclitis (虹膜睫状体炎).

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(颅内) 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. In our country, the cases were found from 27 provinces, major endemic areas are located in Yuan, Healongjiang, Jiling, Shandong, Henan, and Hebei etc. In serious endemic villages with high prevalence of *T. solium* infection there are high prevalence of cysticercosis in pigs and humans. 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 males and in others more in females, depending on the eating habits.

The prevalence of *T. solium* infection varies greatly according to the regional level of sanitation, the pig husbandry pattern, and the eating habits.

Man is the usual definitive host, and pigs is a major intermediate host.

The way to acquire the infection of *T. solium* is eating raw pork containing bladder worm(s). The way to acquire the cysticercosis is due to ingestion or swallow the eggs, which include three ways: (1) persons who are infected worms may contaminated their households or food with eggs that are accidentally eaten by themselves, called **auto-infection (自体外感染)**; (2) possible, a gravid proglottid may migrate from the lower intestine to the stomach or duodenum, or it may be carried there by reverse peristalsis, called **internal-auto infection (自体内感染)**; (3) ingestion of the eggs in contaminated food or water, called **hetero-infection (异体感染)**. It was reported that 16-25% cases of *T. solium* infection was the patients of cysticercosis; meanwhile, 55.6% cases of cysticercosis was the patients of taeniasis.

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 husbandry methods and avoiding human fecal contamination;

d) Treatment of infected persons.

The cysticercosis can be prevented by

- a) Early detection and chemotherapy by taeniacides (杀绦剂), such as praziquantal;
- 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.

III ***TAENIA SAGINATA* (牛带绦虫/肥胖带绦虫)**

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

The morphological differences between *T.saginata* and *T.solium* are presented in table IV - II -1.

Table IV-III-1 Morphological differences between *T.solium* and *T.Saginata*

	<i>Tsolium</i>	<i>Tsaginata</i>
Entire body		
Length(m)	2-4	4-8
Maximal breadth(mm)	7-10	12-14
Proglottids(number)	700-1000	1000-2000
Scolex		
Diameter(mm)	0.6-1.0	1.5-2.0
Sucker(number)	4	4
Rostellum	Present	Absent
Hooks(number)	25-50	Absent
Mature proglottids		
Testes(number)	150-200	800-1200
Ovary(number of lobes)	3	2

Vaginal sphincter	Absent	Present
Gravid proglottids		
Uterus(number of branches each side)	7-13	15-30

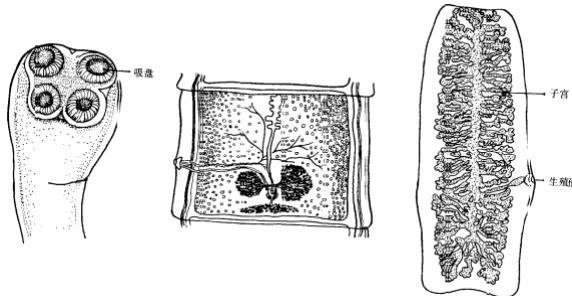


Fig IV-III-1 Scolex, mature and gravid proglottids of *T.saginata*

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 (六钩蚴) leave its embryophore in the cow's intestine and migrates to the muscles, where within 60-70 days the next larval stage-the cysticercus-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 (空肠) 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 (中性粒细胞减少症).

Many cases of the infection are asymptomatic. Verminous intoxication, 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 increased 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. In our country, endemic areas have been reported from Xinjiang, Neimeng, Xizang, Yunan, Ningxia, Sichuan etc.

Human infection is acquired from eating raw beef containing the viable bladder worm(cysticercus larvae).

PREVENTION AND CONTROL

The main measures is as follows:

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

IV *ECHINOCOCCUS GRANULOSUS* (细粒棘球绦虫)

The adult worm of *Echinococcus granulosus* live in the intestine of carnivores (食肉动物)

物), and the larval stages parasitize in various mammalian intermediate hosts. The larval or metacestode forms are referred to as hydatid cysts (包虫) and the diseases caused by them as **hydatidosis** or **hydatid disease** (棘球蚴病 / 包虫病). Adult worm was described by Hartmann(1695) in the intestine of the dog. The larval form(hydatid cyst) subsequently was described by Goeze(1982).

MORPHOLOGY

Adult worm It is a small tape worm and measures 2-7 mm in length; having a pyriform (梨状的) 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. 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 germinative layer, which can bud many protoscoles (原头蚴), brood capsules (发生囊) 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 (囊液), which fluid is called as "hydatid sand 囊砂 / 囊球蚴砂".

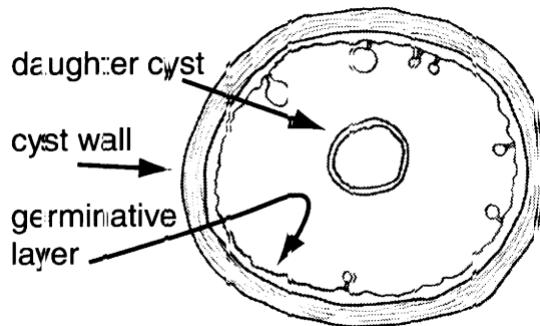


Fig IV-IV-1 Hydatid cyst of *E.granulosus*

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.

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 activated oncospheres. 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 develops an adult worm in the intestine.

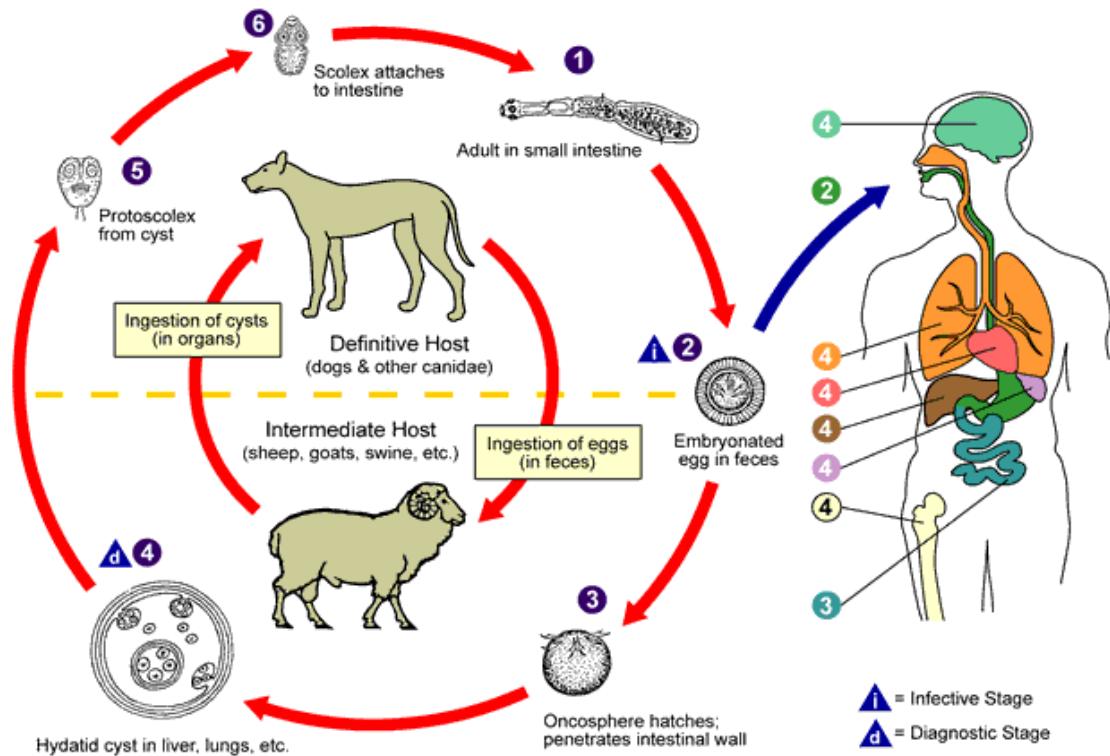


Fig IV-IV- 2 Life cycle of (from Parasite image library of CDC, USA)

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 caused by 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 throughout 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 depend on the size, location and number of hydatid cysts. Occasionally, due to high intracystic (囊内) 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 (过敏性休克) which may be fatal (致死性).
- 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 an 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. In our country, hydatid disease is prevalent in Northwest parts of China, such as Xinjing, Qinhai, Gansu, Ningxia, Xizang and Neimeng etc. It is still serious parasitic disease.

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 contaminated with 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 man 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 (废料) of domestic animals (sheep, etc) in the endemic areas.
- d) Health education and strict personal hygiene.
- e) Avoidance of unnecessary contact with infected dogs.

Section V NEMATODE(线虫)

I. INTRODUCTION

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* 秀丽杆线虫), a few are parasitic. Parasitic nematodes that infect humans have about 10 species, including *Ascaris lumbricoides* (蛔虫) , hookworm (钩虫) , filarial (丝虫) and *Trichinella spiralis* (旋毛虫).

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(假体腔), in which the reproductive system and other structures are found. Sexual dimorphism(dioecious, 雌雄异体) is evident: at the curved, posterior end of the male are a copulatory (交配的) organ and other specialized organs, male are also usually smaller than females.

Parasitic nematodes vary widely in size according to species. Nematodes are colorless and vary from translucent(半透明的) to opaque(不透明的) 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 (卵黄膜或受精膜) 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(壳质层). The inner membrane, the lipid layer/ascaroside(脂层或蛔肽层), is formed by the zygote.

- A) Embryo member (受精膜) : consist of lipid protein;
- B) Chitonous layer (壳质层) : consist of chitonous and protein, and process the function of resisting the mechanic pression;
- C) Lipid layer/ascaroside (脂层或称蛔肽层) : consist of lipid protein and ascaroside, and process the function of regulating.

LIFE CYCLE

The basic process of 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 infective-stage larva, e.g. filarial.

Nematodes undergo four molts, the sequence of events is controlled by exsheathing fluid(脱皮液) 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(脱皮); 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.(L1-L4), named Rhabditiform larva(杆状蚴)、filariform larva(丝状蚴) or microfilaria(微丝蚴)。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* (斑氏丝虫) are known as microfilariae. This larva, generally found in circulating blood.

The adult worm of nematodes parasitize 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 (土源性线虫), 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 (生物源性线虫), such as filaria (丝虫) .

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 cause 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 *Ascaris lumbricoides*(蛔虫, 似蚓蛔线虫), *Enterobius vermicularis*(蠕形住形肠线虫, 蛲虫), *Trichuris trichura*(毛首鞭形线虫, 鞭虫), *Ancylostoma duodenale*(十二指肠钩口线虫), *Necator americanus*(美洲板口线虫), *Wuchereria bancrofti*(班氏吴策线虫, 班氏丝虫), *Brugia malayi*(马来布鲁线虫, 马来丝虫), *Trichinella spiralis*(旋毛形线虫, 旋毛虫)。

II *Ascaris lumbricoides* (蛔虫, 似蚓蛔线虫)

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

MORPHOLOGY

In *Ascaris 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. The posterior end of the female is straight while that of the male curves ventrally. The females is 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 × 35~50 μ m, there are three layers in the shell and one embryo cell in the egg. Some time the protein membrane(蛋白膜) 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 × 39~44 μ m, there is no ascaroside in the shell and

embryo cell in unfertilized egg.



Fig V-II-1 The fertilized egg and unfertilized egg of *Ascaris lumbricoides*

LIFE CYCLE

The life cycle of *Ascaris* 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 uncleaved, 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 $21 \sim 30^{\circ}\text{C}$. Development ceases at temperatures below 15.5°C , and eggs cannot survive at temperatures more than slightly above 38°C .

After 2-4 weeks 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.

After being ingested by a human, eggs containing infective larvae hatch in the duodenum. The larvae actively 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 200-300 μ m 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.

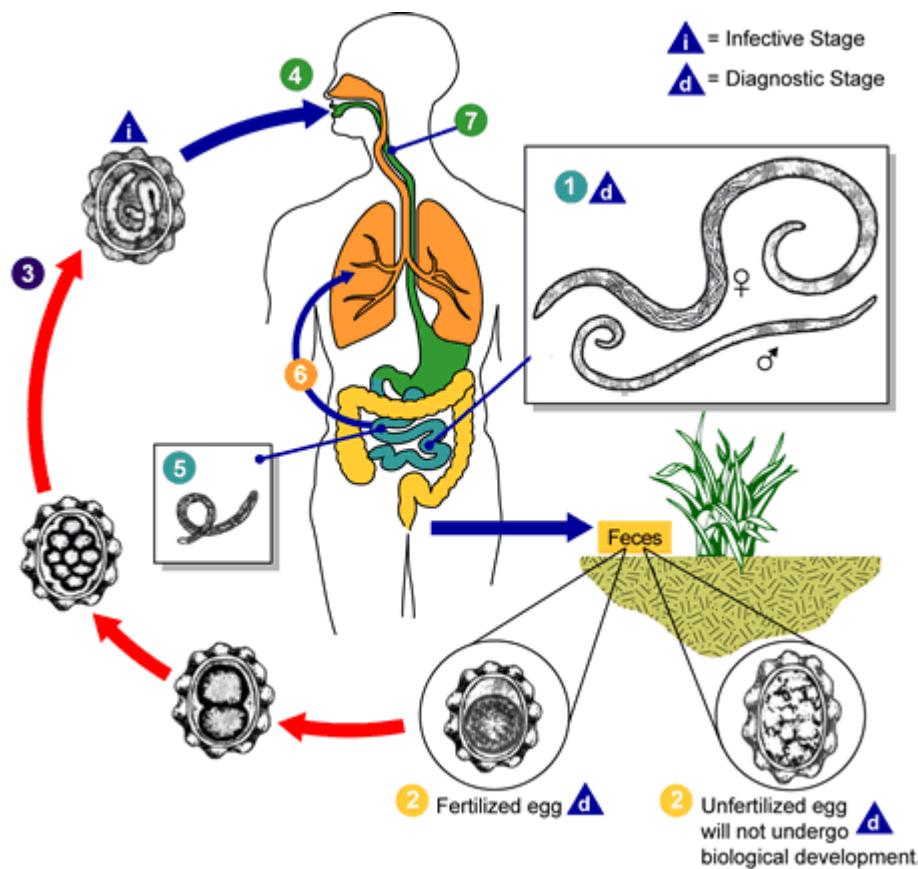


Fig V-II-2 Life cycle of *Ascaris lumbricoides* (from Parasite image library of CDC, USA)

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 (哮喘), transient eosinophilic pulmonary infiltrates (一过性嗜酸细胞浸润, 肺蛔虫症, Loeffler's syndrome). Angioneurotic edema (班尼斯特病或血管神经水肿), and urticaria (风疹) .

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(A,B,C), and cause the poor nutritional status, especially in children with lower nutritional intake.

Clinical symptoms include anepithymia (食欲不振), nausea (恶心), vomiting (呕吐), vague abdominal pains (脐周疼痛)。

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(风疹), itch (皮肤瘙痒) and Angioneurotic edema (血管神经水肿) .

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 migration of adult Ascaris may be promoted by some drugs, including some antihelmintics 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 (劍突). 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 city, children higher adult.

According to the data of the nationwide survey of human parasites conducted in 1988-1992, *A. lumbricoides* has a wide-spread distribution in China, extending across from south to north, the different temperature zones as tropical, subtropical, warm-temperate ,meso-temperate, and frigid zones as well as the special temperature zone of Qinghai-Xizang Plateau. The infection rate was 46.9%. 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 Ascaris produces large number of eggs and the eggs possess the strong ability to resist environmental conditions, humans are easy to expose to the eggs. This is main reason why the infection of Ascaris is one of the most common parasitic diseases.

It are 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 Ascariasis is considered to be very closely related 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 seen in the intestine but who do not require hospitalization, a single dose of pyrantel pamoate(噻嘧啶) and Mebendazole (甲苯咪唑) are highly effective. The case with complication should be sent to hospital.

The preventive and control measures include:

- a) Treatment of infected children and other members of the family. For optimal effectiveness, such a program should be combined with treatment of the population with broad-spectrum anthelmintics 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.

III *Trichuris trichiura* (毛首鞭形线虫/鞭虫)

Trichuris trichiura, called whipworm also, is one of most common human parasites. Human infection with *T.trichiura* causes 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. 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(纺锤状) with two polar plugs. These are

yellowish brown and double shelled. The eggs measure $50\sim 54 \times 22\sim 23 \mu m$. The eggs contain an unsegmented ovum each, when passed in the faeces. These freshly passed eggs are not infective to humans.



Fig V-III- 1 Ovum of *Trichuris trichiura* (from Parasite image library of CDC, USA)

LIFE CYCLE

The life cycle of *T.trichiura* is simple, complete in a single host, the man. 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.

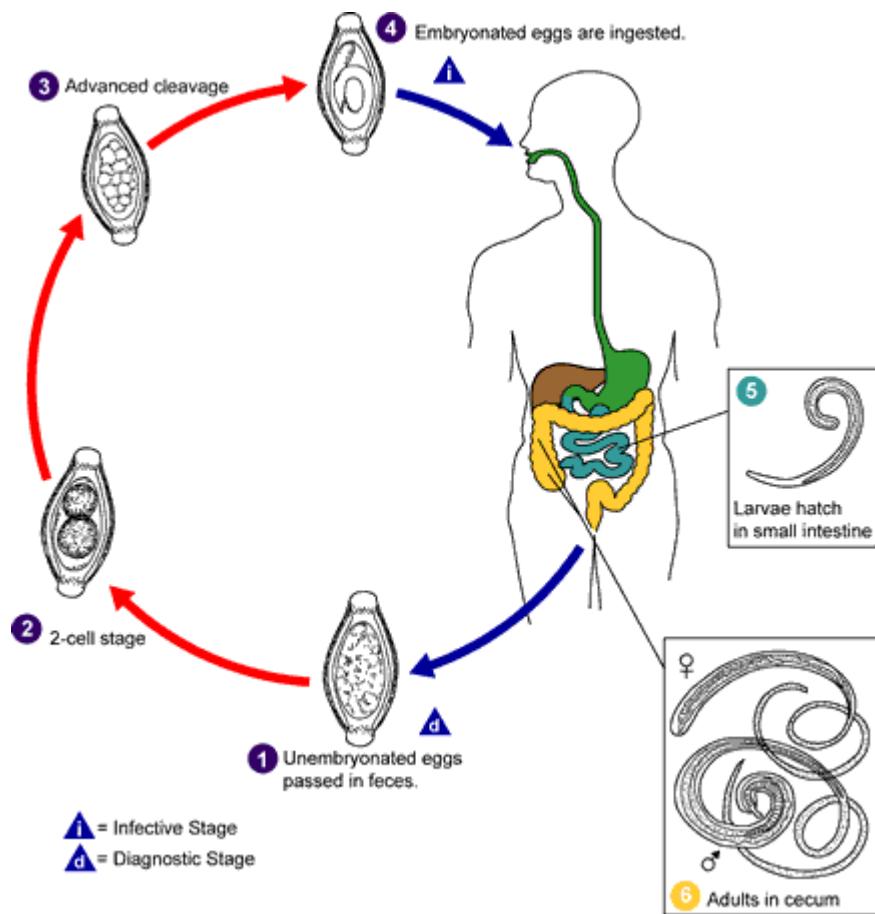


Fig V-III-2 Life cycle of *Trichuris trichiura* (from Parasite image library of CDC, USA)

PATHOGENESIS AND CLINICAL MANIFESTATION

The major pathology resemble that of inflammatory bowel disease due to mechanical disruption and toxicity of whipworms. The pathological changes include hyperemia(充血)、edema(水肿) or hemorrhage/bleeding(出血). In few cases, there are cellular proliferation (细胞增生) and 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(直肠脱垂), anemia(贫血). It was reported that 73% of persons infected with whipworm were identified to be the cases of chronic colonitis by fibrescopy (纤维镜) .

DIAGNOSIS

The clinical manifestation are 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. In our country it is estimated that the prevalence of whipworms infection was 18.8%, and 212 million humans infected whipworms.

Warm climate(30 °C), 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 those for Ascaris control, e.g. health education and sanitation.

IV *ENTEROBIUS VERMICULARIS* (蠕形住肠线虫/蛲虫)

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(1865) 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. True buccal capsule is absent. Female pinworms, measuring 8-13 mm by 0.3-0.5mm, are characterized by the presence of winglike expansions(alae) of body wall at the anterior end, distension of the body due to the large number of eggs in the uterus, and a pointed tail. Males are 2-5mm long and possess a curved tail.

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



Fig. V-IV-1 The egg of *E.vermicularis*

LIFE CYCLE

Life cycle of *E.vermicularis* is simple and is completed in a single host. Man is the natural host.

No intermediate.

Sexually mature worms usually inhabit the human intestinal tract, but they can spread to adjacent regions of the small and large intestines(blindgut/cecum/盲肠、 appendix/阑尾、 colon/结肠、 rectum/直肠 or below portion of ileocaecal/回肠下段). 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.

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 scratches the perianal zone to relieve itching caused by nocturnal 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.

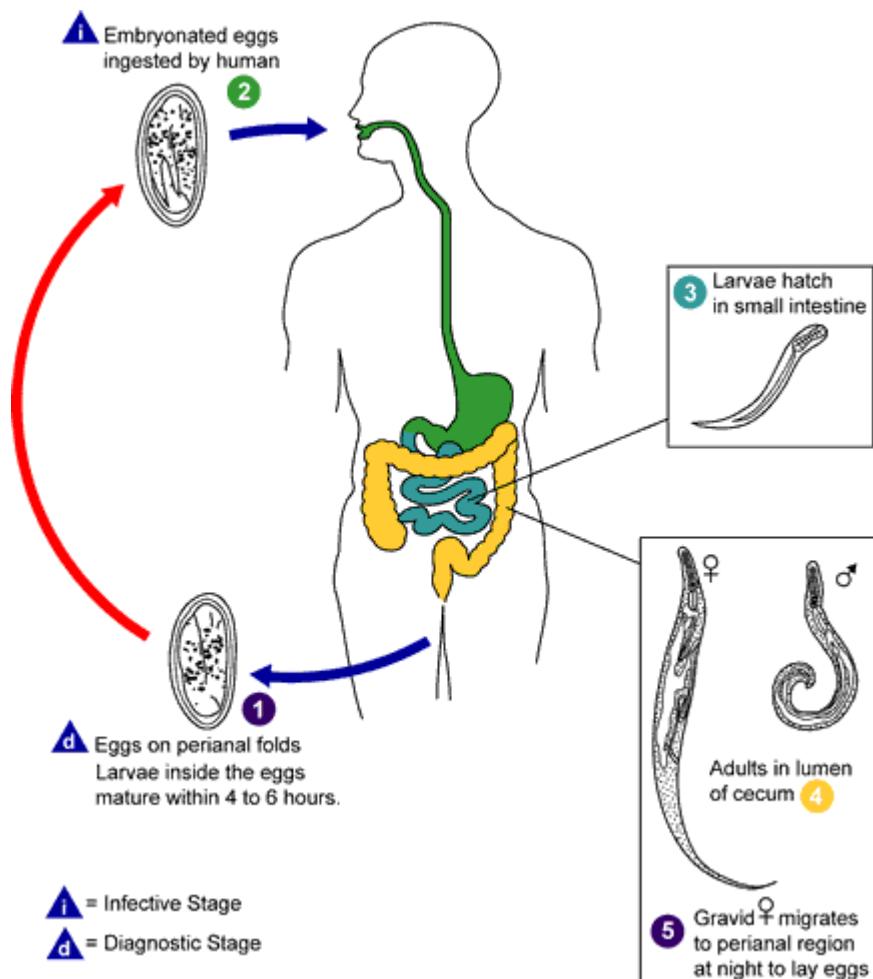


Fig V-IV-2 Life cycle of (from Parasite image library of CDC, USA)

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(阴道炎), endometritis(子宫内膜炎) and granulomata in the uterus and fallopian tubes. They may also migrate to the appendix, the peritoneal cavity, or even the urinary bladder.

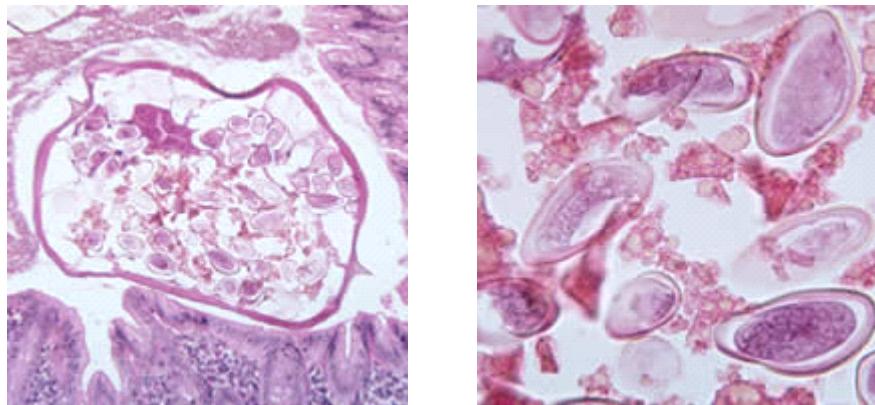


Fig V-IV-3 Cross-section of human appendix containing *Enterobius vermicularis*

(from Parasite image library of CDC, USA)

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.

In China, enterobiasis cased were found in all provinces and be recorded in any age group. The infection rate was higher in children and in urban than in adult and in rural areas, respectively. According to a national survey on infection status of intestinal helminthiasis the infection rate were

30.4%, 29.5% and 31.4% in children aged 7-12 years, males and females, as an average, respectively.

Humans is only host of pinworm. Infections occur in one of four ways: (1) retroinfection (逆行感染), when hatched larvae migrate back into the large intestine; (2) self-infection (自身感染), when the patient is reinfected by hand-to-mouth transmission; (3) cross-infection (交叉感染), 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 (4) inhalation of airborn eggs (吸入感染). 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.

V *Ancylostoma duodenale* and *Necator americanus*

(十二指肠钩口线虫和美洲板口线虫)

The hookworms parasitizing humans include *Ancylostoma duodenale* (十二指肠钩口线虫) and *Necator americanus* (美洲板口线虫). 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. 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, 交合伞), with riblike rays(辐肋). The mouth or buccal capsule(口囊) of *A.duodenale* has two pairs of curved teeth(钩齿) on the ventral wall of its buccal capsule, *N. americanus* has a conspicuous pair of semilunar(半月形) cutting plates (板齿) on the dorsal wall(背侧).

Egg The eggs are oval($56 \sim 76 \times 36 \sim 40 \mu\text{m}$) thin -shelled and colourless. These are surrounded by a thin transparent membrane. The eggs usually contain two or four blastomere (胚细胞) 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.



Fig V-V-1 The egg of hookworm



Fig V-V-2 filiform larva of hookworm (from Parasite image library of CDC, USA)

Infective form Third stage larva(filaiform larva) is the infective form. It is slender and measures $0.5 \sim 0.7 \times 0.025 \text{ mm}$. 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 (and two molts) they become filariform (third-stage) 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 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.

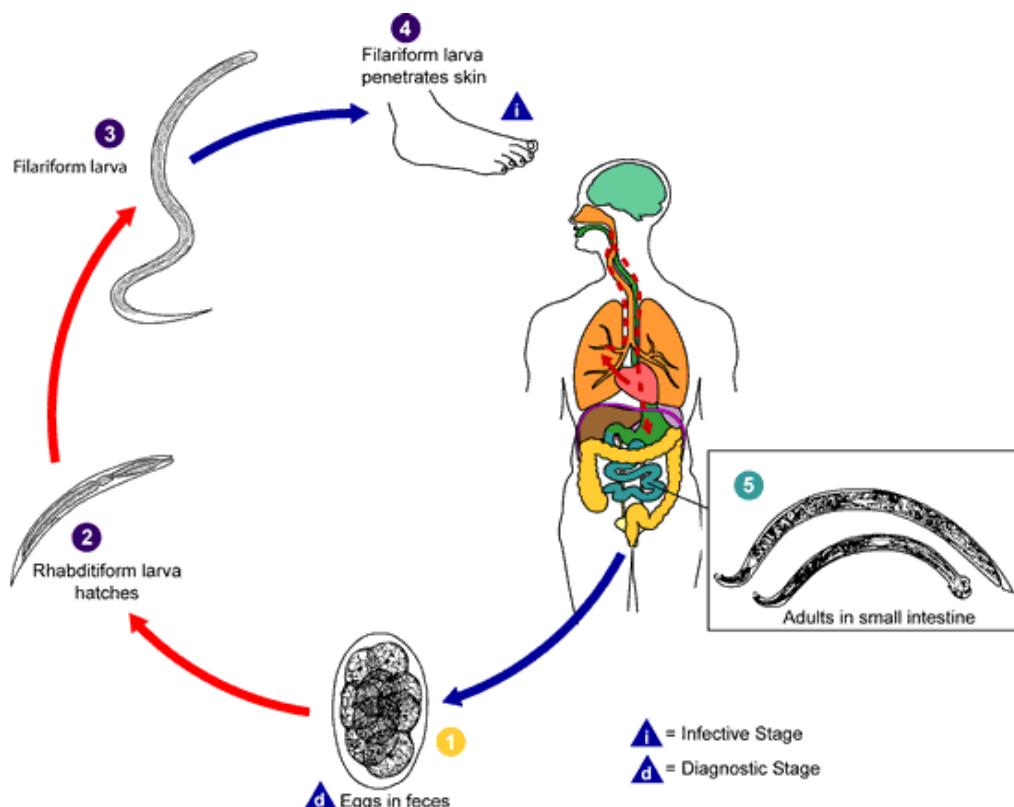


Fig V-V-3 Life cycle of hookworm (from Parasite image library of CDC, USA)

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 is 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) Seepage (渗出) of the blood around the site of attachment of the worm.
- c) Oozing (渗出) of the blood from the burrowed site previously attached by the worm, and
- d) Anticoagulants(抗凝素) 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* (低色素小细胞性贫血). The anaemia can frequently become serious and even fatal in the persons with low iron intake and low level of iron absorption. Loss of protein leads to hypoproteinemia (低蛋白血症)and oedema. (水肿) .

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

(呼吸困难), pharyngitis(咽炎) and occasionally, haemoptysis(咯血) are the important symptoms.

DIAGNOSIS

Eosinophilic leucocytosis (嗜酸性细胞增多症) and hypochromic microcytic anaemia (低色素小细胞性贫血) 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 severe 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 disease is widely epidemic parasitic disease in the world. Hookworm distribute these areas between northern latitude(北纬) 45°C to southern latitude(南纬) 30 °C. *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 Beijing, Jilin Heilongjiang, Qinghai etc, the average infection rate(prevalence) is 17.16%. In Hainan, the infection rate was up to 60.89%, which was the most 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 are absent. The infection route is the infective filariform larvae penetrate the skin (cutaneous route). 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 are 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 (排便)indiscriminately in the open ground or use faeces as fertilizer(肥料) 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(52.0%) in some mine district.

PREVENTION AND CONTROL

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

The most important control measures consist 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 latrines and use of foot wears;

VI *Filaria*(丝虫)

Filaria is one species of nematoda. Adult worm resides in lymph nodes and adjacent lymphatics or in the subcutaneous tissue. Female worm produce microfilaria (微丝蚴) , which belong to viviparous (胎生) .The microfilariae migrate into lymph and blood stream. When insects (昆虫) bits infection person with microfilaria, the microfilaria invade 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 filarial parasites commonly infect humans, two species account for most of the pathology associated with these infections in China. They are filariae *Wuchereria bancrofti*(班氏吴策线虫又称班氏丝虫) and *Brugia malayi*(马来布鲁线虫又称马来丝虫). *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 1.0mm long. 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.

Microfilariae Microfilariae with sheaths(鞘) are bluntly rounded (钝圆) anterior end 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 used to differentiate the Microfilariae of *W. bancrofti* from *B. malayi*. These are :a) nerve ring(神经环), b) body cell (体核), c) tail nuclei(尾核). The morphological differences of the microfiliariae of *W.Bancrofti* from *B.malayi* are as follows:

	W. Bancrofti	B. malayi
Length(μm)	224~296×5.3~7.0	117~230×5.0~6.0
Appearance (体态)	Graceful,sweep curves	Stiff, irregular kinky curves
Gephalic space (头间隙)	As long or half as broad	Twice as long as broad
Body nuclei	Well defined, discrete, round, uniform sized	Blurred, intermingled
Tail	Tapers to a delicate point, terminal nuclei absent	Slight bulb at tip, 2 terminal nuclei

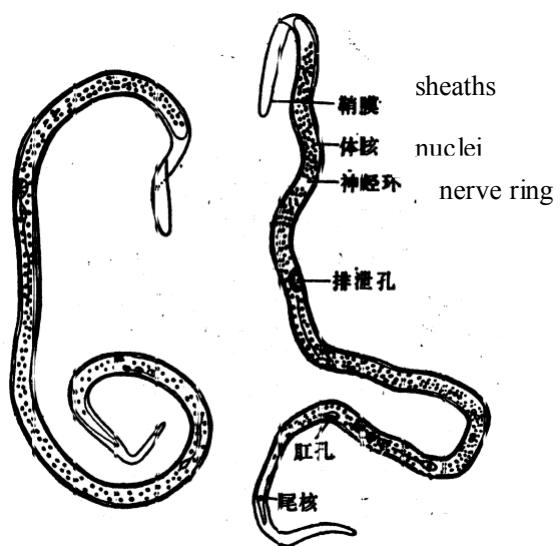


Fig V-VI-1 Microfilariae of *W.Bancrofti* and *B.malayi*

LIFE CYCLE

The development include two stages; larva(microfilaria) in mosquito (intermediate host), adult in man (definitive host).

Development in the human host It is commonly accepted that the infectived-stage larvae, after penetrating the skin, pass through peripheral lymphatics(外周淋巴管), in which they migrate (移行) and grow, then settle down in certain lymphatic vessels retrograde to lymph nodes (淋巴节), grow to maturity and mate, followed by parturition (生产) 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(final 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 (夜间) 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.

Development in mosquito When the microfilaria are 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 (血腔). From there they turn anteriorly to penetrate the thoracic musculature(胸肌), where they rest and begin to grow. The larva moult(蜕皮) to the first stage larva, second stage larva(sausage -shaped larva), and finally third stage larva(infective larva). The third stage larva migrate to the salivary glands of mosquito. When the mosquito bites a man during blood meal, the larva are released from the tip of proboscis of mosquito and the life cycle is continued. Development in mosquito is usually completed within 10-14 days(*W. bancrofti*) or 6-6.5 days(*B. malayi*).

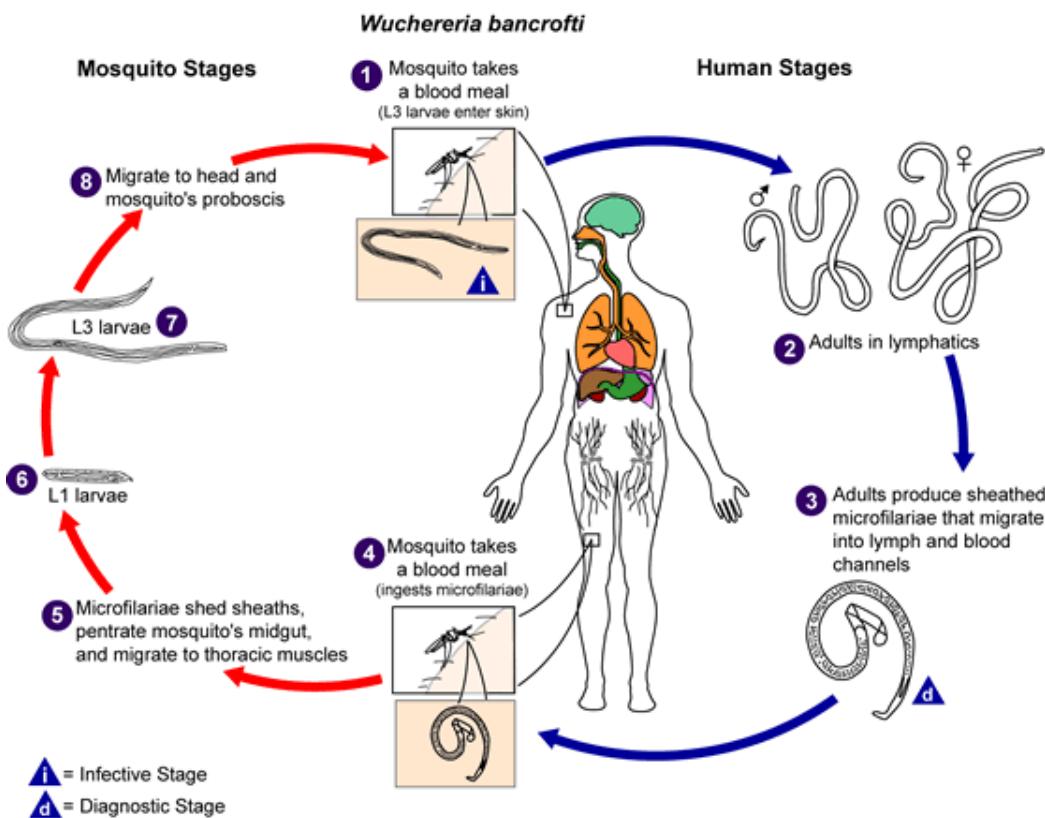


Fig V-VI-2 Life cycle of *Wuchereria bancrofti* (from Parasite image library of CDC, USA)

PATHOGENESIS AND CLINICAL MANIFESTATION

Pathogenesis The microfilariae do 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 (淋巴管) and to temporary lymphatic obstruction (阻塞). 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(破裂) 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 (变态反应). In early stage, there is edema (水肿) and thickening of lymphatic vessels. And then, the wall and tissue around vessel were infiltrated (浸润) by eosinophils (嗜酸性粒细胞), plasma cells, lymphocytes, and macrophages which tended to form into nodules.

Frequent early manifestations of filariasis are fever, lymphangitis (淋巴管炎), lymphadenitis (淋巴结炎) and dermatitis (皮炎). The characteristic symptom of lymphangitis is erythema (红斑) along the course of inflamed lymphatic called “liu huo (流火)” in Chinese.

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 pressure of lower lymphatic vessel obstructed becomes higher, then lymphatic rupture and lymph spills into tissues. The infected people have different clinical manifestations based on the location of obstruction.

1) Elephantiasis (象皮肿) Elephantiasis is a common symptom of chronic filariasis. Lymphatic rupture and lymph spills into tissues, and then progressive enlargement, coarsening (变粗), corrugation (变皱) and fissuring (裂开) 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.

2) Hydrocele testis (阴囊肿) Obstruction of spermatic cord(精索) and testis lymphatics may be caused by hydrocele testis. The manifestation is commonly found from the patient with *W. bancrofti* infection.

3) Chyluria (乳糜尿) Obstruction of abdominal or thoracic lymphatics may lead to chyluria (乳糜尿), chylous ascite (乳糜性腹水) or a chylous pleural effusion (乳糜性胸腔积液). 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/defined 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 manifestations are non-specific, the laboratory diagnosis plays an 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(乳糜尿) and hydrocele fluid (阴囊积液) and by demonstration of adult worm。 Microfilariae can be demonstrated in the blood by the following methods:

1) Thick blood smear Thick blood smear The thick blood smear was made using 60 μ 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(海群生) 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 (近端的) to the affected lymphatic vessels.

Immunodiagnosis 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 epidemiological survey.

- 1) Interdermal test (IDT) 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. In China 16 filariasis endemic provinces were reported, including Shangong, Henan, Jiangsu, Zhejiang, Shanghai, Fujian, Taiwan, Guangdong, Hainan, Guangxi, Anhui, Jiangxi, Hubei, Hunan, Sichuan, and Guizhou. Of which, Shangdong, Taiwan and Guangdong were endemic areas of bancroftian filariasis, while malayan filariasis occurred in other endemic provinces. The 30 million estimated cases comprised microfilaremia(微丝蚴血症) and /or symptomatic cases. After more than 40 years control, of 15 endemic provinces have control the

prevalence of filariasis including Guangdong province.

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 μl 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* (淡色库蚊) ,*Culex fatigans* (致乏库蚊) and *Anopheles sinensis* (中华按蚊) for *W. Bancrofti* and *Anopheles anthropophagus* (嗜人按蚊) , *Anopheles sinensis* (中华按蚊) and *Aedes togoi* (东乡伊蚊) for *B. malayi*.
- 3) Susceptibility of population Humans is susceptible to filaria infection. But in endemic areas the peak of prevalence occur 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(DEC , 海群生/乙胺嗪). In our country, drug salt with DEC (DEC medicated salt) was the common measure for mass treatment. 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 biological 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(康复).

VII *Trichinella spiralis* (旋毛虫)

Trichinella spiralis (旋毛虫) is a nematode parasite of humans that is cosmopolitan (国际性) 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. *Trichinella spiralis* causes trichinellosis, a zoonotic infection in human. Humans are infected when parasite-infected meat (port in most instances) is ingested.

Tidemann(1821) in Germany and Peacock and Owen(1935) 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. The male measures $1.4\sim1.6$ mm in length and $0.04\sim0.05$ mm in diameter. The female size is $3\sim4\times0.06$ mm. Its pharynx (咽) is one third or half of worm body long, and posterior part of pharynx consists of a column of cells called of stichocytes (杆状体). The reproductive system of both sex worm is single tract, and the single uterus is filled with developing eggs in its posterior portion, whereas the anterior portion contains fully developed, hatched juveniles or larva.

Larvae cyst (幼虫囊包) The cyst are found in skeletal muscle commonly, its size is about $0.25\sim0.5\times0.21\sim0.42$ mm. Usually, there is more than one larvae in a cyst.

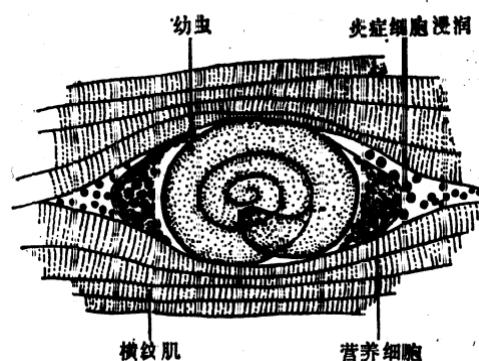


Fig V-VII-1 **Larvae cyst**(幼虫囊包) of *Trichinella spiralis*

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 (杂食) 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 *Trichinella*, the cysts are digested out of the muscle in the stomach; the larvae(first stage) are resistant to gastric juice(胃液). 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 (受精 / 交配), 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 (扩散) via lymphatics and the bloodstream, larvae enter most organs, but persist only in individual skeletal muscle fibers. Increasing almost ten-fold in size(to 1.0mm) 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.

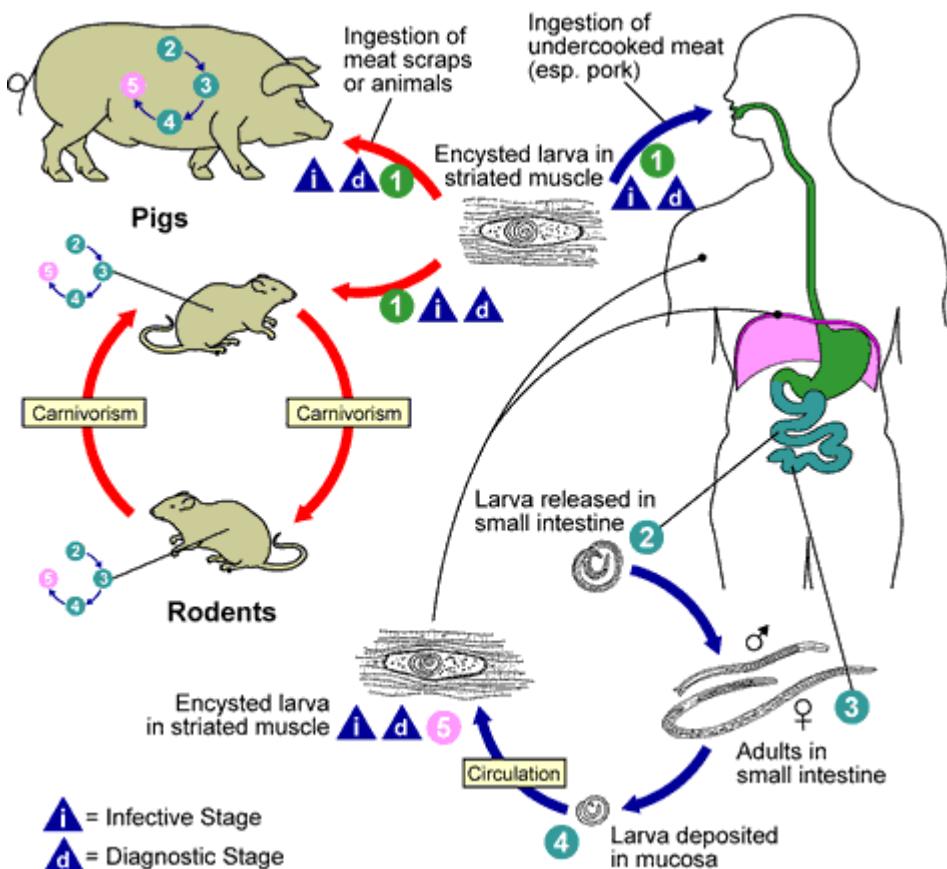


Fig. V-VII-2 Life cycle of *Trichinella spiralis* (from Parasite image library of CDC, USA)

PATHOGENESIS AND CLINICAL MANIFESTATION

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 occur 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(溃疡), mucosal hyperemia (粘膜充血), localized edema (局限性水肿), punctate hemorrhages (出血), 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 newborn 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 (肿) 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(肺炎), and pleural transudate (胸腔积液) may occur. Serious complications, including myocarditis (心肌炎), and meningoencephalitis (脑膜炎), occur most often in the third to ninth week of the disease.

Encystation of the larvae and tissue repair The formation of cyst result in the stimulation of larvae and tissue reparation. With encystation, the inflammation disappear 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(囊内) *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 (IDT) 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. In China, 15 provinces have reported the infections individuals or patients. In Yunan trichinosis is the most serious zoonosis.

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 have stronger resistance to low temperature, freezing at -15 °C for 20 days can destroy the parasites in the pork. Cyst can be killed at 70°C, so eating non-properly processed meat products is the way to require infection.

PREVENTION AND CONTROL

Deep freezing at -15°C for 20 days or -30°C for 6 days and thorough cooking at 70°C or above kills the larvae in the pork. Smoking, curing or drying of meat are not dependable methods 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(丙硫咪唑) is the effective drug for trichinellosis. Mebendazole (甲苯咪唑) is also recommended, it is believed to kill both adult worms and larvae.

Section VI LARVA MIGRANS(幼虫移行症) AND ACCIDENTAL PARASITES OF HELMINTHES(偶然寄生蠕虫)

I LARVA MIGRANS

Larva migrans is a syndrome of the infection with a larval helminth, which invade/penetrate into un-suitable definitive hosts such as human but can develop into adult worm. The migration of

the larva in human body can cause partial and general pathological changes.

- 1) Characteristic presentation Symptom of hypersensitivity: Eosinophilia, fever, hyper, hyperglobulinemia et al
- 2) Pathological changes: hepat- granuloma, lung granuloma, cerebral granuloma, ocular granuloma, and bowel/intestinal granuloma

Clinical sorts include Cutaneous larva migrans(CLM, 皮肤幼虫移行症) and visceral larva migrans(VLM, 内脏幼虫移行症)。

CUTANEOUS LARVA MIGRANS(皮肤幼虫移行症)

Cutaneous larva migrans(CLM) is a ubiquitous selflimited (自限性) skin eruption (皮疹), most frequently caused by third-stage larvae of nematode or cercariae of trematode or sparganum (裂头蚴) of cestode. In the human host, larvae/ cercariae/ sparganum are not able to complete their natural cycle and thus remain confined to the upper dermis of shin. During the migrating through the skin, a local inflammatory response is provoked by release of larval secretion consisting largely of proteolytic enzymes (蛋白水解酶). The route of migration is marked by an intensely pruritic(搔痒症), linear lesion known as “creeping eruption”(匐行疹)。

Pathogens and the way of acquiring infection Summary in following table.

Parasite	The way of acquiring infection	Definitive host
● Trematode		
<i>Schistosoma sp</i>	cercaria, skin	bird and livestock
裂体吸虫		
<i>Trichobilharzia sp</i>	cercaria, skin	bird
毛毕吸虫		
<i>Orientobilharzia sp</i>	cercaria, skin	livestock
东毕吸虫		
<i>Pagumogonimus skryabinii</i>	metacercaria, mouth	cat and raccoon dog(狸)
斯氏狸殖吸虫		
● Nematode		
A. <i>Caninum</i>	infective larva, skin	dog and cat

犬钩口线虫

<i>B.Ancylostoma braziliense</i>	infective larva,skin	dog and cat
巴西钩口线虫		
<i>Gnathostoma spinigerum</i>	Third stage infective larva,	murine, cockroach(蟑螂)
棘颚口线虫	mouth	pig,fish,frog,snake
<i>Strongyloides spp</i>	infective lava,skin	dog,cat,sheep,pig,monkey
类圆线虫		

- Cestode

<i>Spirometra mansoni</i>	plerocercoid/sparganum,	dog and cat
曼氏迭宫绦虫	Skin or mouth	

Pathogenesis and clinical manifestations

1) Infection by nematoda larva Such as *Gnathostoma spinigerum* (棘颚线虫) :

Filariform larva or infective larva penetrate the skin of host and migrate in germinal layer of skin(生发层), and make a snake-form canal(creeping canal). The pathological change cause inflammatory reaction with infiltration with eosinophils along the path of migrating of larva, the clinical symptoms include erythema of shin firstly, and then linefrom rash or herpes with light edema(水肿). IgE level in blood become high. Complications are main bacterial infection caused by scratching(抓痒). After weeks, inflammation get fadeaway and the scab form.

2) Infection by *Pagumogonimus skrjabini* (斯氏狸殖吸虫), *Gnathostoma spinigerum* and *Spirometra mansoni* (曼氏迭宫绦虫): These larva migrate into hypodermis(皮下) or muscle and form moving subcutaneous lumps (皮下包块). The lumps may appear different locus interval. This infective individuals usually present the symptoms of VLM, such as general hypersensitivity(fever, nettle rash, eosinophilia, weak/inertia, muscle pain and anepithymia etc).

3) Infection by animal cercariae such as *Trichobilharzia sp* (毛毕吸虫) and *Orientobilharzia sp* (东毕吸虫): These cercariae caused cercarial dermatitis, or swimmer's itch, or "rice-field dermatitis, 稻田性皮炎". After penetrating the skin of human, they are destroyed by the victim's immune response. Allergenic substances released from dead and dying cercariae produce a localized inflammatory reaction. The pathological changes usually appear the skins of hands or feet, which parts of body contact infested water frequently. The pathogenesis belong to

immediate hypersensitivity, and delay hypersensitivity. The clinical symptoms include initial tingling sensation, erythema, maculopapular rash, vesicles and edema.

Diagnosis The diagnosis for the dermatitis of CLM is relatively straightforward because of its very characteristic clinical picture. But the differential diagnosis should be noticed.

In CLM, most cases are caused by third-stage larvae of dog and cat hookworms. So the dermatitis of CLM is misdiagnosed as the dermatitis of human hookworm. If hookworm eggs are found from stool/feces after fading of dermatitis, the dermatitis should be diagnosed as the dermatitis of human hookworm, not the dermatitis of CLM.

The cercarial dermatitis is not easy to differ from the dermatitis caused by human schistosome.

The CLM cause by *Pagumogonimus skrjabini*, *Gnathostoma spinigerum* and *Spirometra mansoni* can be diagnosed by using skin biopsy.

VISCERAL LARVA MIGRANS(VLM, 内脏幼虫移行症)

Visceral larva migrans(CLM) was considered to be a type of parasitism in man caused by young worm of helminthes parasites of other animals. It was thought that man was an abnormal host, and therefore was unsuitable for normal migration and development of the parasite. In the human host, larva migrate into various tissues and organs. During the migrating through the tissues or organs, a local or general inflammatory response is provoked by release of larval secretion consisting largely of proteolytic enzymes, and local lesion or cyst with young worm is formed later.

Pathogens and the way of acquiring infection Summary is as follows:

Parasite	The way of acquiring infection	locus of lesion
● Trematode		
Pagumogonimus skrjabini	<i>metacercaria, mouth</i>	viscus
	斯氏狸殖吸虫	
● Nematode		
Toxocara canis	Infective egg, mouth	viscus
	犬弓首线虫	
Gnathostoma spinigerum	third-stage larva, mouth	viscus

棘颚口线虫

Ascaris suum	egg with larva, mouth	viscus
猪蛔虫		
Angiostrongylus cantonensis	infective larva, mouth	meningoencephalitis with
广州管圆线虫		eosinophilia

● Cestode

<i>Spirometra mansoni</i>	procercoid, mouth	viscus
曼氏迭宫绦虫	plerocercoid/sparganum, skin or mouth	

Pathogenesis and clinical manifestations

1) Infection by *Toxocara canis*(犬弓蛔虫) larva: *Toxocara canis* is most common pathogen caused VLM. The larva migrate into liver, lung, brain, eye etc, and form lesion(eosinophilic granuloma). The clinical symptoms include hepatomegaly(80%), eosinophilia, Loeffler syndrome(cough, fever, breath difficulty etc). If the larva migrate into brain, the symptoms such as epilepsia(癫痫) may appear.

2) Infection by *Gnathostoma spinigerum* (棘颚口线虫) : *Gnathostoma spinigerum* can cause VLM except CLM. The symptoms of CLM appear after one month of infection, while the symptoms of VLM appear. The larva migrate through the wall of intestinal into the abdominal cavity, and then invade into liver or muscle or connective tissue. The clinical manifestation include nausea (恶心), vomiting and abdominal pain etc. These symptoms should be differed from other diseases such as acute abdomen. The symptoms caused by *Pagumogonimus skrjabini* is similar to *Gnathostoma spinigerum*. But the CLM appear more than VLM.

3) Infection by *Angiostrongylus cantonensis* (广州管圆线虫) : The worm parasitize in pulmonary artery of murine (鼠), the egg develops into first stage larva in capillary of lung and migrate into digestive tract, and then out of body with feces. The larva can survive by free-living for 3 weeks. Murine acquire infection by eating intermediate or transport hosts or foods containing third-stage larva. Human is an unsuitable host of *Angiostrongylus cantonensis*, when the third-stage larva enter body by eating raw snail-meats or transport hosts' meats, it invades into central nervous system frequently.

4) Infection by *Spirometra mansoni* (曼氏迭宫绦虫) : The sparganum of *Spirometra mansoni* frequently migrate into eye, brain and viscus except the upper dermis of the skin. The movements and secretions of living sparganum /plerocercoids (裂头蚴) can induce localized inflammatory reactions; dead and degenerating larvae sometimes cause edema of the surrounding tissue. Chills and fever may accompany infections. So the symptoms caused by *Spirometra mansoni* correlated to the parasitic locus of the sparganum. Most common symptoms is ocular sparganosis (裂头蚴病) (45.6%) or eye infection, which produce conjunctivitis (结膜炎) and swelling. In general, the severity of infection is determined by the location of the larvae and how quickly and completely the patient can be rid of them.

Diagnosis

1) *Toxocariasis*(弓首线虫病) a) Clinical diagnosis: clinical symptoms such as hepatomegaly, Loeffler syndrome(cough, fever, breath difficulty etc); b) Laboratory examination: esinophilia, hyperglobulinemia(IgG and IgM as well as IgE level) and special antibodies by immunodiagnosis e.g. ELISA; c) Differential diagnosis: intestinal helminthes, pneumonitis(肺炎), retinoblastoma(视网膜母细胞瘤) and ophthalmitis(眼炎) etc.

2) *Gnathostomiasis*(颚口线虫病), *sparganosis*(裂头蚴病), *Pagumogonimiasis*(斯氏狸殖吸虫病): With similar clinical symptoms, immunodiagnosis or biopsy are main methods.

3) *Angiostrogyliasis*(管圆线虫病) : a) Clinical symptoms such as eosinophilia and symptoms of meninges; b) finding larva from cerebrospinal fluid; c) immunodiagnosis e.g. ELISA.

Control

The way of acquiring infection include a) for feeding dog or cat, it is easy to swallow infective egg e.g.egg of *Toxocara canis*; b) eating raw meats containing infective larvae or plerocercoid/sparganum; c) infective larvae penetrate through skin for contacting infested water or contaminative fields while traveling or working.

Health education can play an important role in CLM control.

The drugs for CLM caused by nematoda larvae include thiabendazole(噻苯咪唑) and inunction (涂擦). The operation and chemotherapy with praziquantel is available for CLM caused by *Pagumogonimus skrjabini*, *Gnathostoma spinigerum* and *Spirometra mansoni*. For VLM caused by *Toxocara canis*, hetrazan(海群生/乙胺嗪, DEC) or thiabendazole is recommended.

II ACCIDENTAL PARASITES OF HELMINTHES(偶然寄生蠕虫) TREMATODE

These parasite commonly include *Pagumogoniumus skrjabini*, *Heterophyes heterophyes*(异形异形吸虫), *Metagonimus yokogawai*(横川后殖吸虫), and *Echinostomatidae*(棘口吸虫) etc.

Pagumogoniumus skrjabini The worm was found by Chen XT in 1959. Adult worms are 3.5-6.0mm × 11.0-18.5 mm. The greatest width is upper of ventral sucker. The ratio of length and width is 1:2.4~1:3.2. Shapes at both top of body is sharper. The ovary is also lobated and found to left of post acetabular. The life cycle is similar to *Paragonimus westermani*, the definitive host include raccoon dog etc. Human is unsuitable host . Human acquire the infection by eating raw crabs(second intermediate host) or frog, bird, duck and rats(transport host). The disease cause by *Pagumogoniumus skrjabini* was found in China only.

Heterophyes heterophyes The adult worm is pear shape(梨形) with $1 \sim 1.7 \times 0.3 \sim 0.4$ mm. Oral sucker is smaller than ventral sucker. The reproductive sucker(生殖吸盘) is upper ventral sucker. Uterus is longer with tortuous and hovering to reproductive sucker. The life cycle include egg, miracidium, sporocyst, redia, metacercaria and adult worm. Adult worm parasitize in the intestinal tract of birds and mammals. The first intermediate host is fresh water snails , and second intermediate host is fresh water fish or frog. The egg is similar to the egg of *C.sinensis*. Human can acquire the infection accidentally for eating raw fish or frog meats. The clinical symptoms include digestive manifestation. If the worm invade other organs, the serious local symptoms will be cause by the worms or eggs.

Echinostomatidae (棘口吸虫) There are about 600 species in Echinostomatidae. Most of these worms parasitize in birds, some in mammals, a few in snake. Several members of the genus *Enchinostoma* and relation genera occasionally infect humans. In our country, there 10 species of Echinostomatidae that parasitize in human such as *Echinocasmus japonicus*(日本棘隙吸虫). Adult echinostomes, while varying greatly in size, are easily identified by the collar of spines. In general appearance, the adult worm is elongated, with a relatively large ventral sucker situated immediately behind the anterior end. The testes lie in tandem in the posterior portion of the body. The life cycle is typical of most echinostomes. Operculated eggs are passed from the definitive host with feces and much reach fresh water for the cycle to continue. The miracidium enclosed in egg

develops and hatches, and then penetrates a fresh water snail(first intermediate host). A single sporocyst generation and two redial generations develop in the molluscan host. Free-swimming cercariae escape from daughter rediae, enter the water, and penetrate and encyst either in a variety of aquatic animals including mollusks(软体动物), tadpole(蝌蚪), or fish (麦穗鱼) . When the definitive host ingests encysted metacercariae, which excyst and develop to sexual maturity in the small intestine of vertebrate(脊椎动物). Human infections of echinostomes are most frequently reported from Oriental countries such as the Philippines, China, and Indonesia. Infection occurs when the infected second intermediate host is eaten either raw or improperly cooked. Echinostomiasis in human is usually a minor affliction, often causing nothing more serious than diarrhea. In heavy infections, the spinose collar may cause ulceration of the intestinal mucosa. Children sometimes experience abdominal pain, diarrhea, anemia, and/or edema. The principal diagnostic technique, identification of eggs in feces, is facilitated by a number of distinctive features of echinostome eggs, their dark brownish color and the very immature larvae, even uncleaved zygotes, that are unlike those of other intestinal trematodes. The disease can be treated using praziquantel.

CESTODE

Spirometra mansoni is one of Pseudophyllidea (假叶目). The adult worm parasitize in cats, or infect human accidentally. If human infect plerocercoid/sparganum (裂头蚴) by contacting or eating intermediate host , the plercoercoid larvae(中绦期幼虫)are capable of infecting tissues of humans , causing human sparganosis (裂头蚴病) .

NEMATODE

Angiostrongylus cantonensis(广州管圆线虫) was described by Prof Chen Xintao in 1933. The worm parasitize in pulmonary artery of murine, the egg develop into first stage larva in capillary of lung and migrate into digestive tract, and then out of body with feces. The larva can survive by free-living for 3 weeks. Murine acquire infection by eating intermediate or transport hostsor foods containing third-stage larva. Human is a unsuitable host of Angiostrongylus cantonensis , when the third-stage larva enter body by eating raw snail-meats or transport hosts' meats, it invade into central nervous system frequently. Common symptoms of Angiostrongyliasis

are meningoencephalitis with eosinophilia, including acute headache, nausea, vomiting and fever. Serious cases may present paralysis(瘫痪), drowsiness(嗜睡), coma(昏迷) or death. The disease is found in tropic or subtropic areas including China, Thailand, Japan, and Vietnam etc. In Taiwan of China, it reported more 300 cases of Angiostrongyliasis . 2 cases was diagnosed in Guangdong. Up to now, there are any specific drugs for the disease.

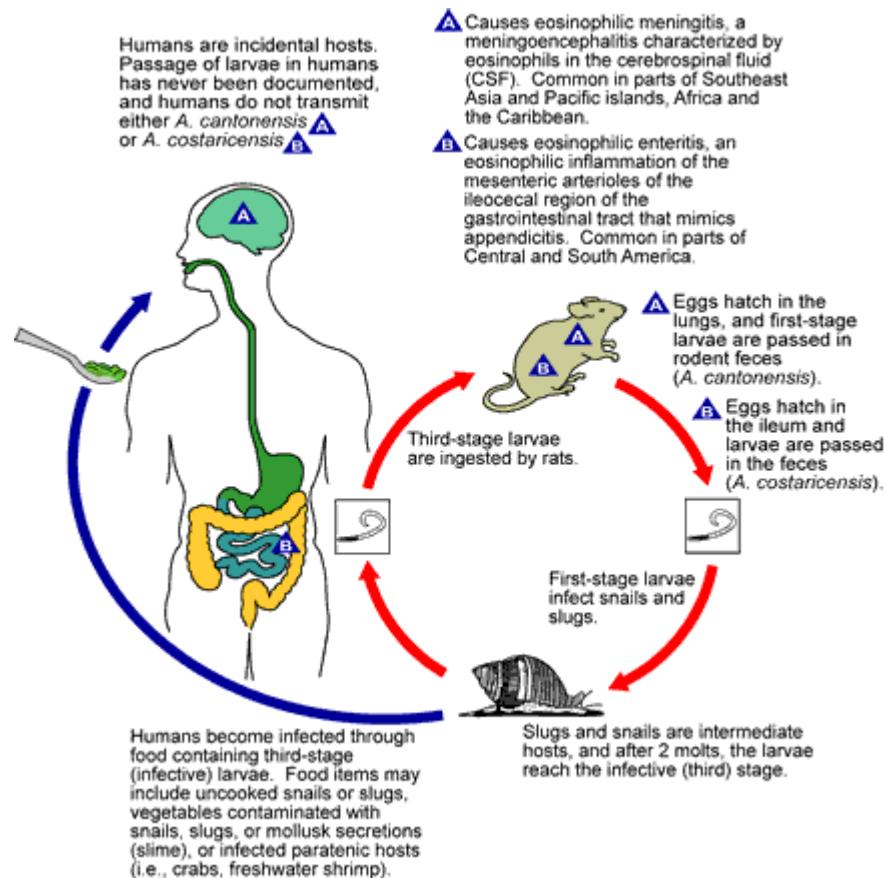


Fig VI-II-1 Life cycle of *Angiostrongylus cantonensis*

Section VII MEDICAL ARTHROPOD(医学节肢动物)

I INTRODUCTION

Arthropods are as intimately associated with humans' welfare as any other animals. The economic importance of this group to agriculture, in terms of both beneficial and destructive effects, can hardly be overemphasized. In addition, many species have a direct relationship to human health and well-being. The majority of arthropods function indirectly in human diseases, which they transmit but do not produce; some species are true parasites, whereas others may inflict direct

injury by their bites, stings, or other activities. Some species are both parasites and vectors of disease. These arthropods related with human health are named “Medical arthropod(医学节肢动物)”.

Medical arthropodology (医学节肢动物学) is a science that study the morphology, taxonomy, cycle life, zoology, geographic distribution of medical arthropodology, and the relationship of medical arthropods with the transmission of the disease , as well as the measures for medical arthropods control.

PHYLOGENY, MORPHOLOGY, MOLTING, AND DEVELOPMENT

The organisms in the phylum Arthropoda belong to diverse group, but they have some features in common.

- 1) Bilateral symmetry (两侧对称) .
- 2) Chitinous exoskeleton(外骨骼) with jointed legs.
- 3) Growth by molting, which is controlled by hormones (激素) .
- 4) A complete digestive tract extending from an anterior mouth to a posterior anus.
- 5) A nervous system consisting of an anterior set of ganglia (神经节) and commissures, which extend around the esophagus and pass posteriorly as two fused chains of ventral ganglia.
- 6) True segmentation or metamerism (分节) . Primitively, each segment had a pair of legs, neural ganglia, probably an excretory unit, and a set of muscles. In primitive (原始的) arthropods there is little difference among the segments along the length of the animal(homonomous metamerism), but in more advanced forms, there is movement toward specialized changes in segments(heteronomous metamerism) or the merging of segments into distinct body parts(tagmatosis).
- 7) The body cavity is a hemocoel (血体腔) and the circulatory system is open. In an open circulatory system, blood moves into the heart through openings or ostia (口) and is pumped out to various parts of the body, where it leaves the vessels and bathes the tissues directly.

The development of arthropod include embryonic development (胚胎发育) and postembryonic development (胚后发育)。Embryonic development is completed in egg. From eclosion (孵化) of larva or nymph(若虫) to adult , there is widely differences in the morphology, physiological function and living habits etc. The process of the change is called as

metamorphosis(变态). Metamorphosis include two types: complete metamorphosis(including egg, larva, pupa(蛹) and adult) and incomplete metamorphosis(including egg, larva/nymph, adult). Larva develop into the next stage by molting (蜕皮) . After 4 times of molting, larva develop into pupa, which process is called as pupation(化蛹)。 The process from pupa to adult stage is emergence(羽化)。

CLASSIFICATION

Medical arthropod belong to Class Crustacea (甲壳纲), Diplopoda (倍足纲), Chilopoda (唇足纲), Arachnida (蛛形纲), and Insecta (昆虫纲) . Among them, most medical arthropod is from Class insecta and archnida.

Insecta: mosquito (蚊), fly (蝇), sandfly (白蛉), flea (蚤), louse (虱), cockroach (蟑螂), etc.

Archnida: tick (蜱), mite (螨), spider (蜘蛛), etc

Crustacea: crab (蟹), shrimp (虾), etc.

Chilopoda: centipede (蜈蚣) .

Diplopoda: millipede (马陆) .

HARM FOR HUMAN HEALTH

Medical arthropod can cause harm to human by direct or indirect ways, such as bites, stings, defensive secretions or as vectors of disease.

Direct harms

1) Harassment and sucking blood 骚扰 and 吸血) Bloodsucking arthropods such as mosquito, louse, tick, mite, etc bite human by penetrating the skin with their mouthpart and cause harassment to humans.

2) Allergy and toxicosis When bloodsucking arthropod bites human, various secretions including salivary fluid are injected into the body, and may be cause hypersensitivity(allergy) or toxicosis. After contacting with the proteins of arthropods such as the secretions of cockroach can, some individual occur serious allergic reaction e.g., asthma of child.

3) Invading tissue Some larva of flies can parasitize in the skin or the cavity , and cause myiasis(蝇蛆病). Itch mite can invade the subcutaneous and cause scabies(疥螨).

Indirect harms

Aethropods are of great importance as vectors of disease-producing agents to humans and other animals. Disease transmission can be accomplished in two general ways. It may be mechanical, which means that the arthropod carries an infectious organism from one person or object to the next without serving as a host for the development or multiplication of this organism. Transmission may also be biological, in which case the infectious organism develops or multiplies within the arthropod host and is only then transmitted to the vertebrate host.

1) Mechanical transmission(机械性传播)

Among those diseases that may be transmitted in a mechanical manner are the bacterial enteritis (肠炎). Enteric organisms may be carried by flies that feed on fecal material to foods destined for human consumption. Pathogenic bacteria may be found on the mouth parts, legs, or intestinal contents of flies feeding on excreta; some protozoan cysts may be carried in a like manner. Flies have long been thought to play a role in the mechanical transmission of those viral diseases in which the organisms are passed in the feces.

2) Biological transmission(生物性传播)

Some infectious organisms require an arthropod host for completion of their life cycle and also utilize this host as a vector. Most arthropod-borne diseases are carried in this fashion, reaching the vertebrate host through the agency of the bite of the vector. Examples of such diseases are malaria and filariasis. An arthropod may serve as intermediate host for an organism that is acquired by the vertebrate host when that host ingests the infected arthropod. There are four types of biological transmission.

- a) for development: the pathogen develop to infective stage in the arthropod, but don't proliferation, e.g., larva of filarial develop in mosquito;
- b) for proliferation: the pathogen proliferate in the arthropod, but it's form don't change, e.g., *yersinia pestis*(鼠疫杆菌) proliferate in flea ;
- c) for development and proliferation: the pathogen either develop or proliferate in the arthropod, e.g., *plasmodium* in mosquito;
- d) transmission by egg: the pathogen not only can develop or/and proliferate in the arthropod, but also invade the ovum of female arthropod. The pathogen can be transferred to filial generation(子代) of the arthropod by egg. The filial generation also become infective vector. For example, *Rickettsia tsutsugamushi*(恙虫病立克次体) in *Leptotrombidium deliensis*(地里纤恙螨)。

ARTHROPOD AS A VECTOR

Arbo-diseases is the disease transmitted by arthropods. When a arbo-disease occur, how to judge the vector of the disease? The evidences for the judgment are as follows.

Biological evidences As vector of a arbo-diseases, the arthropod should have below biological features:

- 1) It is closed relationship with human, e.g., having the habit of biting or sucking humans; its activity is correlated with humans foods, e.g., lapping foods(舐吸) or contaminating foods.
- 2) The arthropod is a common species of arthropods at local area, or the population of the arthropod is dense .
- 3) The life span of the arthropod is long enough and can provide the time for the pathogen to complete the development or proliferation.

Epidemiological evidences The geographic distribution and seasonal distribution of the arthropod are same as the arbo-disease.

Laboratory evidences The arthropod can be infected with the pathogen by experimental methods and the pathogen can develop into infective stage in the arthropod in the laboratory.

Natural infection evidences In the epidemic season, the pathogen can be examined from the arthropod at the field. This is the most important evidence to judge the vector.

SOME IMPORTANT DISEASES TRANSMITTED BY ARTHROPODS

Following table lists some important diseases transmitted by arthropods in our country.

Arthropod	Disease
Hard tick/Ixodidae(硬蜱)	Forest encephalitis(森林脑炎) Xingjing haemorrhagic fever(新疆出血热), Lyme disease(莱姆病), Q fever(Q热)
Soft tick/Argasidae(软蜱)	Tick-borne recurrent fever(蜱媒回归热), Q fever(Q热)
Chigger/Trombiculid mites(恙螨)	Scrub typhus(恙虫病)
Itch mite/Sarcoptidae mite (疥螨)	Scabies(疥疮)
Demodicidae mite(蠕形螨)	folliculitis (毛囊炎) etc

Dust mite/Pyroglyphidae(尘螨)	Asthma(哮喘), Allergic rhinitis(过敏性鼻炎), Allergic dermatitis(过敏性皮炎)
Mosquito(蚊)	Malaria(疟疾), Filariasis(丝虫病), Japanese B encephalitis(流行性乙型脑炎), Dengue fever(登革热), Yellow fever(黄热病)
Fly/Musca(蝇)	Dysentery(痢疾), Typhoid fever(伤寒), Cholera(霍乱), Poliomyelitis(脊髓灰质炎), Amebic dysentery(阿米巴痢疾), Myiasis(蝇蛆病)
Sandfly(白蛉)	Kala-azar disease/ visceral leishmaniasis(黑热病)
Flea(蚤)	Plague(鼠疫), Murine typhus(鼠型斑疹伤寒), Hymenolepiasis diminuta(微小膜壳绦虫病)
Lice(虱)	Epidemic typhus(流行性斑疹伤寒), Lice-borne relapsing fever(虱媒回归热)

CONTROL

Since the recognition that insects transmit infectious agents and the elucidation of the life cycles of parasites in vectors, the vectors(insects, arachnids and snails) have been targets through which disease control can be achieved. Initial attempts at arthropods control depended on environmental management to reduce arthropod populations before insecticides became available and application techniques were developed. Up to now, the integrated measure(综合性措施) is considered as best measure for arthropods control. These measures are as follows:

Environmental management It 's objective is to reduce or control the resting/growing field or breeding sites(滋生地), and reduce the arthropod population by environmental modify and sanitation.

Physical measures It's objective is to control or drive away medical arthropod. For example, bed-net is usually useful tool to avoid the bite of mosquito.

Chemical measures Since 1940s, four types of insecticides have been used widely. The use of DDT achieved eradication in controlled malaria transmission at some subtropical region. Gradually, however, DDT resistance developed and alternative insecticides were required; Organophosphates , Carbamates and Pyrethroids have been introduced as the spectrum of resistance

has widened. In recent year the development of the insecticide **growth regulator**, a new insecticide, has been used in the experimental areas.

- 1) Organochlorines (有机氯杀虫剂) e.g., DDT (dichlorodiphenyltrichlorethane).
- 2) Organophosphates(有机磷杀虫剂) e.g., malathion (马拉硫磷), fenitrothion (杀螟松) etc
- 3) Carbamates (氨基甲酸酯杀虫剂) e.g., bendiocarb (虫威), and propoxur (残杀威) etc.
- 4) Pyrethroids(拟除虫菊酯杀虫剂) e.g., permethrin(朴杀司林). Etc.

Biological measures *The bacterium Bacillus thuringiensis* (苏云金杆菌), and *B.sphaericus* (球形芽孢杆菌), as well as *Romanomermis culicavorax* (罗索线虫) can infect the larva of mosquito and kill them. In the rice field, breeding fish is also a useful method to control the larva of mosquito.

Genetic measures By applying molecular biological methods such as mutation or gene transfer, to produce infertility males of medical arthropod and let them mate with wild female of medical arthropod, which female will not reproduce filial generation.

II CLASS ARACHNID(蛛形纲)

The morphological features of the arachnids are as follows:

- 1) There is no head, as such, because the segments are fused to form a cephalothorax (头部) and abdomen, which make up the body regions; in the mites, there is further fusion of body regions.
- 2) Antennae(触须) are lacking and they have only simple eyes.
- 3) They have four pairs of legs and are lacking wings.
- 4) Developmental patterns are such that the larva form have much the same body form as the adults.

The only group that sucks blood from vertebrates and serves as vectors of disease agents is the Acari(ticks and mites). The Acari(蜱螨亚纲), whose members are commonly called mites or acarines, includes both mites and ticks. The body consists of gnathosoma (颚体), also called

capitulum (假头), and idiosoma (躯体).

The developmental pattern is basically the same in mites and ticks:

Egg->larva->nymph->adult

The stages are usually similar to one another in general form, but the larva has three pairs of legs and the nymph(若虫) and adult have four pairs. The life cycle vary in that there may be more than nymphal stage and there may be quiescent(静止的) stages such as the nymphochrysalis (若蛹) in the chigger(恙螨)(Trombiculidae). In some case, females die after laying eggs, but in a few instances such as the soft ticks, female may continue to lay batches of eggs for months.

TICK (蜱)

Ticks belong to *Order Parasitiformes*(寄螨目) and are divided into two families, the Ixodidae or hard ticks, and the Argasidae, or soft ticks. The division between the two families is based on the following characteristics:

Ixodidae(硬蜱) An inflexible, dorsal scutum(盾板) covers the idiosoma(躯体) of the male and the anterior part of the idiosoma (躯体) of the female; mouthparts (口器) are terminal and visible from above; stigmata are located posterior to coxae IV; the body is usually smooth.

Argasidae(软蜱) The scutum is lacking; mouthparts are ventral(腹部) and not visible from above; stigmata are usually located between coxae III and IV; the body is often wrinkled.

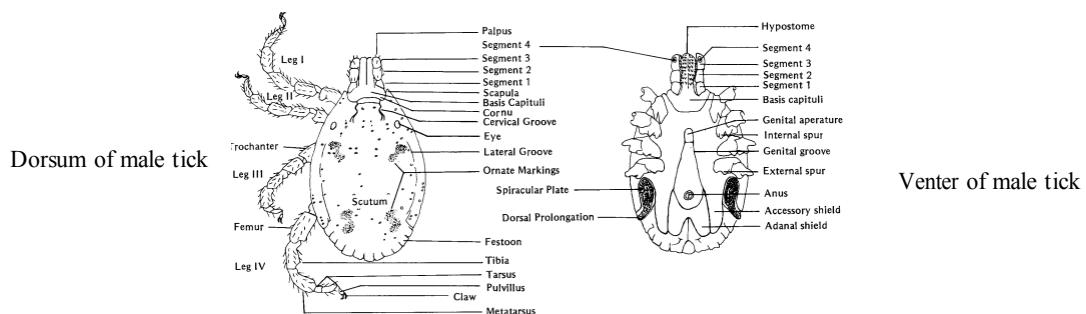


Fig VII-II-1 Dorsal and venter view of hard tick

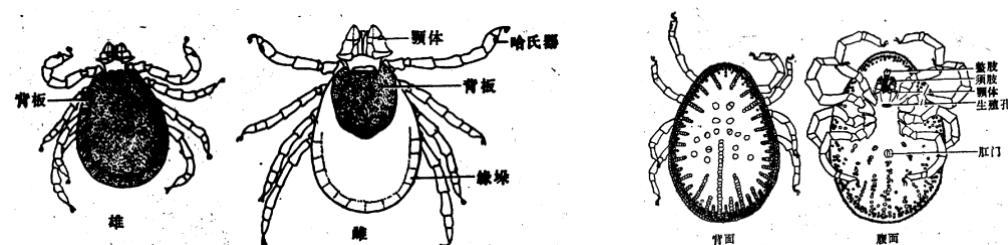


Fig VII- II -2 hart tick(male and female)

Fig VII- II -3 Soft tick (Dorsum and venter)

Life cycle egg-> larva-> nymph->adult

During development the tick feeds and molts, feeds and molts. The adults copulate while on the host, the female then drop off, lay eggs, and die. Hard ticks only lay eggs one time within whole life. Soft ticks can lay eggs a few times. The male can mate with female much time. Under suitable conditions, the larva hatches in 2-4 weeks and seek a host to feed on. After 1-4 week, The six-legged larva develop to nymph by molting. The nymph seeks a host to feed on again, drops off, molts and remains in the ground. After 1-4 times of molting, the nymph develop to the

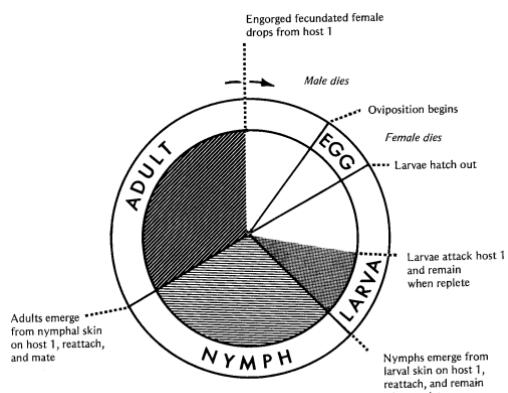


Fig VII- II -4 One host type of tick life cycle

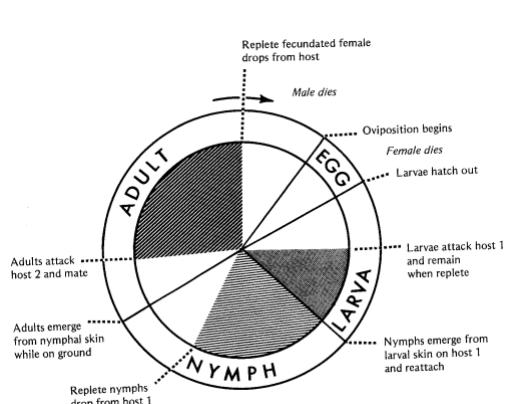


Fig VII- II -5 Two host type of tick life cycle

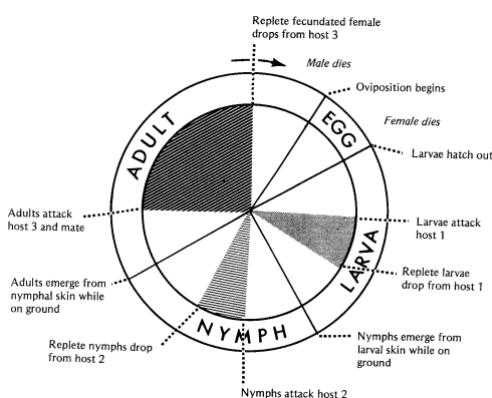


Fig VII- II -6 Three host type of tick life cycle

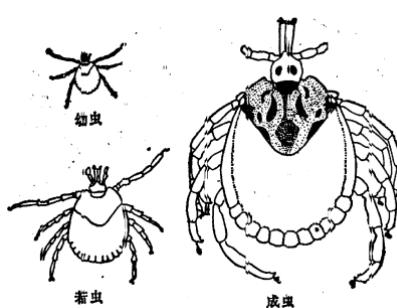


Fig VII- II -7 The life cycle of hard tick

adult stage. The life cycle of hard ticks is complete in two months to 3 years, most of soft ticks is in 6 months to two years. The life span of hard ticks is about one month to ten months, of soft ticks is about five to ten years. All of the stage can survive a long time without feeding allowing the life

cycle to be further stretched out if host are not available. There are three pattern hard ticks, one-host, two-host and three-host ticks(see below figures).

Ecology The larva, nymph and adult all need to suck host blood. The hosts rang include birds, amphibian (两栖动物), reptile (爬行动物), mammalian and humans.

1) Hard ticks They suck in day, and feed on host a few days usually. The resting sites of hard ticks are found at forest /woods, grassland, and pasturage(牧场) commonly.

2) Soft ticks They suck the blood at night and only feed on host from minutes to one hour. The resting sites are located at host's nests and hovel.

Important species of ticks They are *Ixodes persulcatus*(全沟硬蜱), *Dermacentor muttalli* (草原革蜱), *Hyalomma asiaticum kozlovi* (亚东玻璃蜱) and *Ornithodoros papillipes* (乳突钝缘蜱).

Harm to humans Ticks can cause harms to humans by direct injures and transmission of diseases.

1) Direct injures **a)** Irritation: The insertion of the capitulum into skin produces an inflammatory reaction of the perivascular (血管周围) tissue of the corium (真皮) with local hyperemia (充血), edema, hemorrhage and thickening of the stratum corneum (角化层). Occasionally, the ticks can beneath the skin. **b)** Tick paralysis (蜱性麻痹): This disease is caused by the biting of certain female ticks. It's clinical characteristics show that the person can not walk or stand, has difficulty in speaking, swallowing and breathing due to paralysis of the motor nerves.

2) Transmission of diseases a) Tick-borne encephalitis/forest encephalitis(森林脑炎) is mainly transmitted by *Ixodes persulcatus*, and found in forest areas of Northeast and Xinjiang of China; b) Xinjiang hemorrhagic fever(新疆出血热) is mainly transmitted by *Hyalomma asiaticum kozlovi* (亚东玻璃蜱) and found in the pasturage of Xinjiang; c) Tick-brone relapsing fever(蜱传回归热) is transmitted by *Ornithodoros papillipes* (乳突钝缘蜱) and found in Xinjiang areas. Its pathogen is *Borrelia persica* (伊郎包柔螺旋体) or *B.latysheviyi* (拉氏包柔螺旋体); d) Lyme disease is transmitted by *Ixodes persulcatus*, and found in 20 provinces of China. The pathogen is *B.burgdorferi* (伯氏包柔螺旋体); e) Q fever and tick-borne typhus.

The tick-borne diseases are zoonosis, ticks can act as vectors and reservoirs host. The pathogens may be transmitted by tick's feces, saliva or secretions. The pathogen can also be transmitted into filial generation by the eggs, which called transovarial transmission (经卵传递).

Control

The control measures include Environmental management, chemical measure(application of insecticides such as DDT etc), and personal protection. For humans, it is best to avoid allowing ticks to embedded by using a repellent such as deet, trying clothing tightly at the ankles and wrists, and searching for ticks in the clothing and on the body after a day out of doors while you stay at forest or pasturage.

TROMBICULID MITE (恙螨)

Trombiculid mite, which common name is chigger, red bug, or harvest mite, belong to Family Trombiculidae. Among the common genera in this family is *Leptotrombidium deliensis* (地里纤恙螨) in China.

Morphology Keys for identifying chiggers are based on the larvae. They are tiny mites—0.2 to 0.5 mm long—with three pairs of legs. They are typically reddish or orange, are well supplied with setation on the body, and the palps (触须) have five segments.

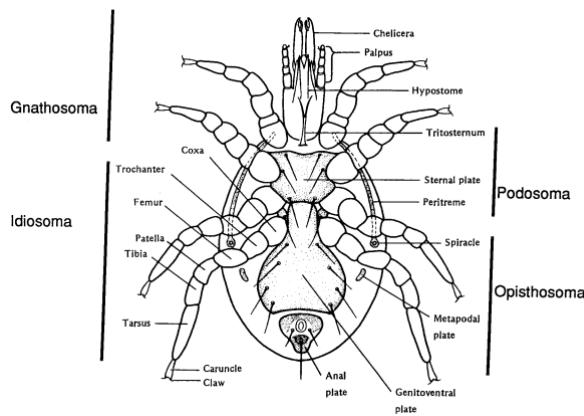


Fig VII- II -8 Diagram of the external anatomy of a mite

Life cycle

The stage in the life cycle of the mites are as follows:

Egg->deutovum (前幼虫 -> larva-> nymphochrysalis (若蛹) ->nymph ->imagochrysalis (成蛹) ->adult

The larva is the only parasitic stage; It usually feeds on a wide range of hosts. Adult and nymph stage are free living. Female lay eggs in the soil. Life cycle of chiggers are dependent upon the weather. In cooler climates they may have three generations each year, but in tropical

and semitropical climates, development takes place year-round. Three months is about an average time for completion of life cycle.

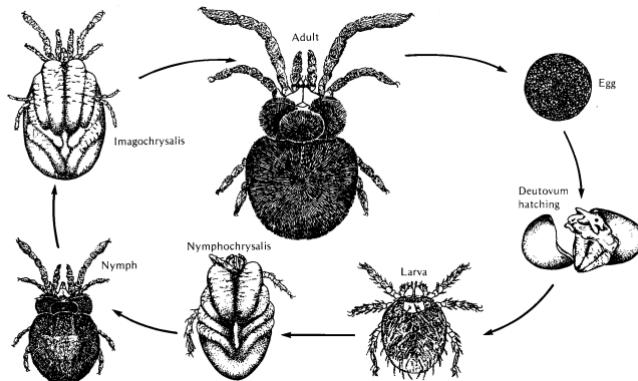


Fig VII- II -9 The life cycle of *Leptotrombidium deliensis*

Ecology The parasitic stage have a low host specificity. They feed on small mammals such as rat, and birds etc. Sometime it feed on humans. Chiggers remain at the surface of the skin of host to feed. In China, the common species of the mite is *Leptotrombidium deliensis* (地里纤恙螨), which distribute the south areas especially in Guangdong and Fujian provinces. *Rattus* is main host of *Leptotrombidium deliensis*.

Harm to humans The mite can causes chigger dermatitis or trombiculosis(恙螨皮炎). The principle agent that it transmit causes scrub typhus or tsutsugamushi disease(恙虫病)。The pathogen of scrub typhus is *R. tsutsugamushi*(恙虫立克次体) or *Orientia tsutsugamushi* (东方体), which pathogen can be transmitted into filial generation of the mite by egg. In China, scrub typhus is endemic in Taiwan, Guangdong, Fujian, Zhejiang, Yunnan, Guangxi, Guanzhou provinces ect. In recent years, it was reported that the cases were found in Henan and Shanxi provinces.

Diagnosis Raised, itching papules and a history of having recently been in a grassy or forest edge area usually adequate to determine that a persons has been attacked by chiggers. The papules are usually located where the clothing is tight: at the belt, at the top of the socks, and so on.

Control The control measures include Environmental management, chemical measure(application of insecticides such as DDT etc), and personal protection.

SCAB MITE(疥螨)

The Astigmata includes both parasitic and free-living mites. Scab mite, *Sarcoptes scabiei*

parasitize on humans and mammalian.. There is a single species in the genus with a number of varieties named for the hosts on which they occur. *S.scabiei* var. *humani*(人疥螨) is on humans, which can cause sarcoptic mange or scabies (疥疮) and *S.scabiei* var. *suis* is on swine and so on. There is some cross-transmission possible with many varieties, but usually the ability of the mites to survive and reproduce on an abnormal host is limited.

Morphology These are tiny mites and disc-shaped, which are barely visible with the naked eye. All stages have stubby(粗短的) legs, some of which terminate in long setae(刚毛). The first two pairs of legs have roundish structures called ambulacra(吸垫). In female the posterior two pairs of legs lack ambulacra. The female are 0.3-0.5 mm long by 0.25-0.4 mm wide, and the male are 0.2-0.3 mm long by 0.15-0.2 mm wide.

Lifecycle The pattern of development in *Sarcoptes* is as follows:

Egg-> larva-> protonymph (前若虫)->tritonymph (后若虫) ->adult

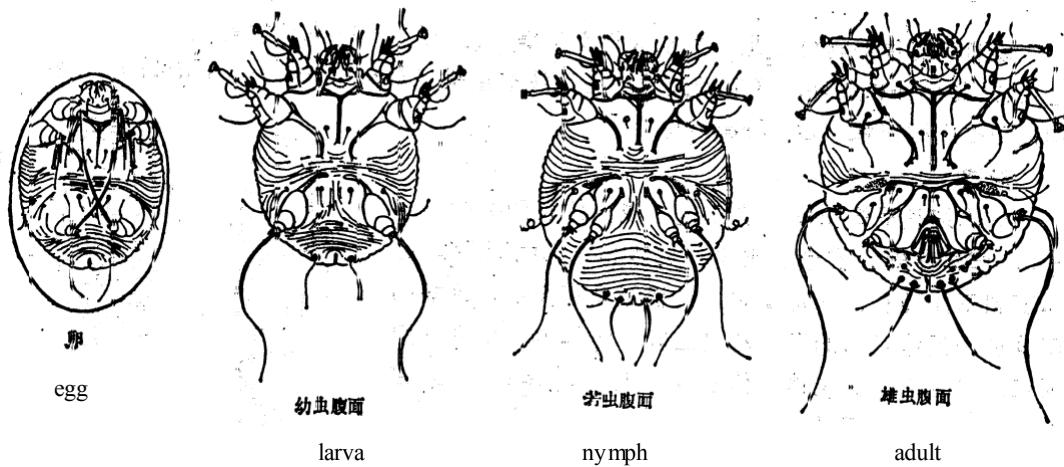


Fig VII- II -10 The development stages of SCAB MITE

Transmission from one host to the next take place through close contact or contamination of the environment, and any of the stages is capable of establishing an infection. Entrance into the skin is accomplished by the mite secreting saliva onto the unbroken skin; the cells of the skin are lysed and the mite then eats its way into and burrows along under the keratinized(角质层) layers of the skin. The female burrows into the skin and lays eggs in a sinuous Tunnel(隧道), which she forms as she oviposits. The eggs hatch in 3 to 5 days releasing the larval stage. The larva still live in the

tunnel or enter a new tunnel, and molts to the protonymph and the tritonymph stage. The larvae have 3 pairs of legs and nymphs have 4 pairs of legs. The tritonymph lasts from 3 to 4 days, and then molts to reach the adult.

The female lays from one to four eggs a day, and lives about 5-6 weeks; a female lays from 40 to 50 eggs in lifetime. The male die after mating with female.

Pathogenesis The female mite selects places on the body where the skin is thin and wrinkled, between fingers, wrists, elbows, feet, penis, scrotum, buttocks and axillae. The mite can cause more severe skin reactions, such as itching and allergic reactions. The irritation and hypersensitivity seen to result from excretions, which the female deposit in the skin as they burrow and oviposit. Secondary bacterial infections may also occur, probably as a result of scratching.. In young children whose skin is soft and tender, they may be found burrowing on the face and other parts of the body.

Diagnosis Determining whether a person has been invaded by the mites is based on the following:

- 1) Clinical signs and symptoms;
- 2) Finding the mites in the skin.

Sinuous tracks in the skin, inflammation, itching are all indicators of scab mites. Later in the infection, crusty patches are seen. The crux (结痂) of the matter is finding the mites in the skin, but it is necessary to scrape the skin somewhat nevertheless.

Scraping are examined under a compound microscope for mites, parts of mites, eggs and fecal pellets.

Control The transmission of the disease is accomplished by direct contact with the infected person or with their clothing or bedding. For scabies control, the acaricides can be applied to skin after a hot, soapy bath. All clothing and bedding should also be laundered.

The acaricides include 10% Brimstone ointment(硫磺软膏) etc.

DEMODICIDAE MITE(蠕形螨)

Demodicidae mite belong to Family Demodecidae. *Demodex spp* are all parasites of mammals. They cause a disease usually called demodectic mange or demodicosis (蠕螨病) .

Members of the genus have a high degree of both host and site specificity. Human have two species, *D. folliculorum* (毛囊蠕形螨), which lives in hair follicles, and *D. brevis* (皮脂蠕形螨), which is found in sebaceous glands (皮脂腺) .

Morphology *Demodex spp.* are elongate and have four pairs of stubby legs. The mouthparts are not apparent and the hysterosoma(末体) is quite long.

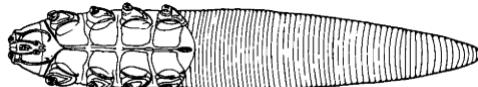


Fig VII-II-11 Adult of *Demodicidae mite*

Life cycle The mites live in hair follicles, sebaceous gland, and sweat gland depending on the species. The follicles or glands may become packed with mites. Transmission between hosts is by close bodily contact. The pattern of development is as follows:

Egg-> larva-> protonymph (前若虫)->nymph (若虫) ->adult

The life cycle probably require about a half month. The female live more than 4 months, and the male will die after mating.

Diagnosis and control The presence of the mite can be determined by gently squeezing (挤) the skin and looking for the mite in the exudates (渗出物). They are seen mostly on the face in oily areas, such as around the nose, or in the eyebrows and eyelashes which may be plucked and examined under a microscope.

The acaricides include 10% Brimstone ointment(硫磺软膏) etc.

III CLASS INSECTA (昆虫纲)

Insects comprise an important part of the biological world in all biomes. In this section, we discuss those insects that are parasitic or are vectors of disease agents.

MORPHOLOGY

The insects share with the other members of the phylum Arthropoda a) a segmented

exoskeleton (外骨骼) with jointed legs, b) an open circulatory system with a dorsal heart, and c) paired, ventral nerve chords. They are differentiated from other members of the phylum by having a) three distinct body segments: the head, thorax, and abdomen, b) a single pair of antennae (触角), and c) three pairs of legs. Wings are present in most adults and they arise as extensions of the body wall on the meso-thorax (中胸) and metathorax (后胸). The legs all have the same parts, starting at the body: coax (基节), trochanter (转节), femur (股节), tibia (胫节) and tarsi (跗节).

Ecto-morphology The main features are as follows:

- 1) Head: a pair of compound eye, a pair of antennae, three types of mouthparts(chewing 咀嚼式, sucking 刺吸式 and sponging type mouthparts/mopping type mouthparts 吸式口器).
- 2) Thorax(胸): it consists of prothorax, mesothorax and metathorax; the thorax bears three pairs of legs and two pairs of wings.
- 3) Abdomen: 11 segments; the ecto-reproduction organ(外生殖器) also locate in the part.

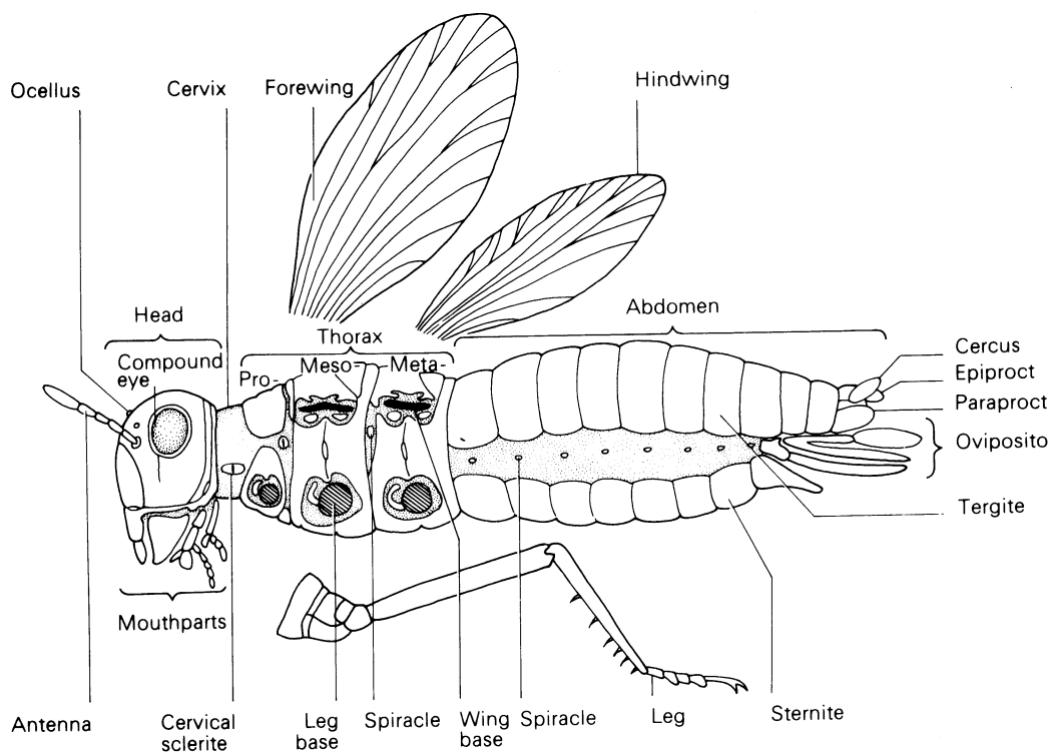


Fig VII-III-1 Generalized adult, winged insect(from Romoser and Stofollano, 1994)

DEVELOPMENT AND METAMORPHOSIS (发育和变态)

Complete metamorphosis Some species of insects belong to complete metamorphosis arthropod, including mosquito, fly, sandyfly and flea etc. The pattern of the development is as

follows:

Egg->larva->pupa/chrysalis->adult

The larva and adult have differences in morphology and life habits; There is the pupa stage in the life cycle.

Incomplete metamorphosis

Some species of insects belong to incomplete metamorphosis arthropod, such as louse, bug(臭虫), and cockroach etc. The pattern of the development is as follows:

Egg->nymph->adult

The larva/nymph stage is similar to the adult in morphology and life habits, but the sexual organ still undeveloped; there is no pupa stage in their life cycle.

IMPORTANT MEDICAL INSECTS(重要的医学昆虫)

Order Diptera(双翅目) Mosquito (蚊), fly (蝇), sandyfly (白蛉) etc

Order Siphonaptera(蚤目) Flea(蚤)

Order Blattaria(蜚蠊目) Cockroach(蟑螂)

Order Hemiptera(半翅目) bug(臭虫)

MOSQUITO (蚊)

The mosquitoes belong to *Family Culicidae*(蚊科), and an important medical arthropod. The family Culicidae contains more than 3500 described species that divided into three subfamilies: Anophelinae(按蚊亚科), Culicinae(库蚊亚科), and Toxorhynchitinae(巨蚊亚科). Among the m *Anopheles*(按蚊), *Culex*(库蚊) and *Aedes*(伊蚊) are the most common species of mosquitoes.

Morphology Adults of mosquitoes are generally 1.6 to 12.6 mm long, consists of the head, thorax, and abdomen .

1) Head There are a pair of compound eye ,and a pair of antennae, and a pair of maxillary palp/palpus (触须) . The mouthparts are a long proboscis(喙), which belong to sucking type mouthparts, adapted for sucking blood and plant juices; the females have mandibles (上颚), but the males usually lack them and connot take blood, only plant juices. The antennae (触角) are long and plumose(轮毛) in the males, but the female have only a few sparse (稀疏的) hairs. Antennae divide into 15 segments, first one is called scape(柄节), second called torus(梗节), third to fifteen

segments called flagellum (鞭节) .

2) Thorax. The thorax is broader than the head. It contains prethorax(前胸), mesothorax (中胸) and metathorax (后胸) , each part has a pair of leg, fore legs, middle legs, and hind legs . The metathorax has a pair of halters(平衡棒). There is a pair of wing, the scales on the wings are on the veins(纵脉) and the margins (缘脉) .

3) Abdomen. It contains 11 segments, only 8 are visible. The last 3 segments usually modified into external reproduction organ.

Larvae are aquatic (水生的) , and have a well-defined head, thorax, and abdomen; an air tube (呼吸管) arises so-called gills (呼吸器) arises on the anal segment, but these are actually osmoregulatory (渗透调节的) organs.

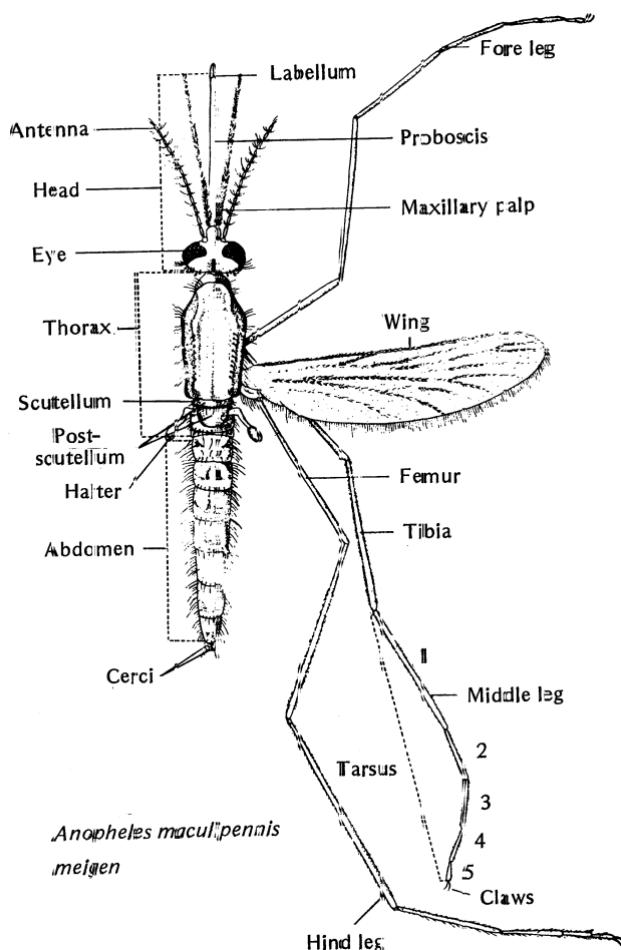


Fig VII-III-2 A diagram of a female Anopheline mosquito

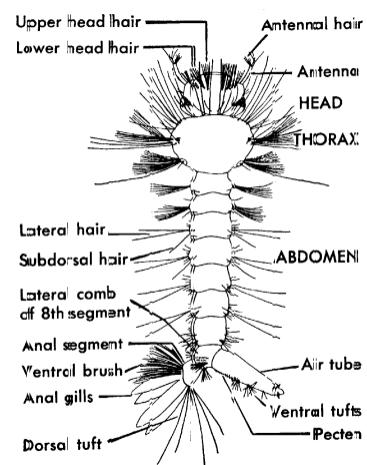


Fig VII-III-3 A diagram of a mosquito larva

Life cycle The complete life cycle contains eggs, larva, pupa and adult. All mosquitoes require water for the development of the larvae and pupae, but the adult live in land.

1) Egg Eggs are laid in or near water but never in open water. Females respond to a number of environmental stimuli in choosing places to deposit their eggs. *Aedes* lay their eggs in damp or tree-hole etc, whereas *Anopheles* lays its eggs in the fresh water, e.g., rice field. *Culex* lay their eggs in different type water, e.g., sewage etc. Under the proper conditions, eggs develop and hatch quickly, often within 2-3 days.

Anopheles eggs is boat-shaped, have a pair of lateral floats, laid single and float on the water surface.

Culex eggs is cylindrical or ovoid in shape and no float. They are laid stuck together in “egg’ rafts”.

Aedes eggs is olive-shaped, no float. They are laid single on humid soil or the bottom of water(cans, contains).

2) Larva Most mosquitoes larvae require food for the development. After 4 times of molting, the larva develop to fourth stage larva, which is the last one of larva before the pupa, and it typically does not feed but rather prepares itself to become a pupa.

3) Pupa The head and thorax of the pupa are fused to a cephalothorax. The pupa is free swimming but nonfeeding and usually lasts only two to three days and emerge to the adult.

4) Adult The adults emerge(羽化) from the pupa at the surface of the water; Female are not ready to take a blood meal until one to three days after emergence. This is the beginning of the phase called the gonotrophic cycle. During this time the ovarian follicles develop. The females are then ready to mate, and it takes place in swarms of males. Males may mate several times, but females mate only one. The next phase, host seeking, last for 3 to 10 days, during which time they seek hosts, take a blood meal, and then rest somewhere while the eggs develop. Egg laying take place over about a three days period. The gonotrophic cycle(生殖营养周期), except for copulation, is then repeated, and one female may have as many as five cycles of egg laying. It should be noted that in biological transmission of disease agents of all sorts, the female is infected at one feeding, lays eggs, and then must take another blood meal for transmission to occur.

Table VII-III-1 Some recognition features in the adults of *Anopheles* (按蚊), *Culex* (库蚊) and *Aedes* (伊蚊) are as follows:

features	<i>Anopheles</i>	<i>Culex</i>	<i>Aedes</i>
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1. color	Dust	Brown	Black
2. palpus(触须)	The palpus is same as proboscis in long	The palpus of the female is shorter than proboscis in long, but the palpus of the male is longer than proboscis	The palpus of the female is shorter than proboscis in long, but the palpus of the male is same as proboscis
3. wings	With white and black spots	Without white and black spots	Without white and black spots
3. legs	With or without white rings	Without white rings	With white rings
4. sitting posture	There is a angle between the body and the resting surface	There is a parallel between the body and the resting surface	There is a parallel between the body and the resting surface

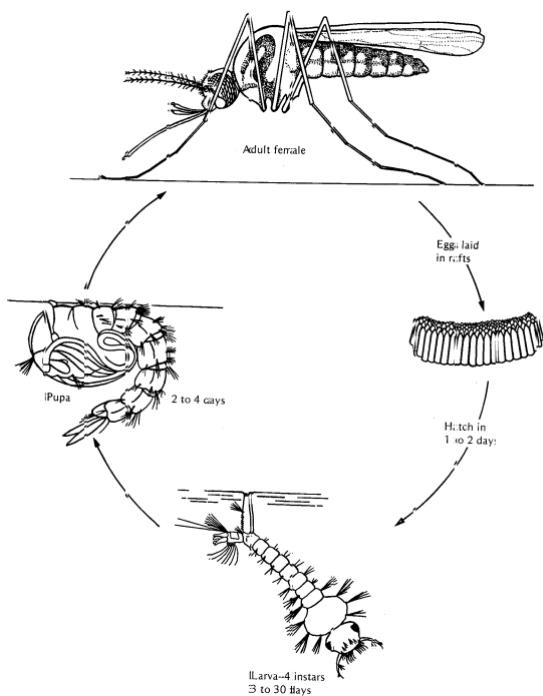


Fig VII-III-4 The life cycle of mosquito

Ecology Breeding sites and the behaviors of sucking blood of mosquitoes related with the importance of disease-transmission.

1) Breeding habits There are five type of breeding sites, paddy field/rice field (稻田型), slowly flow water (缓流型), jungle or forest areas (丛林型), dirt water (污水型), and container

water (容器型) . Breeding sites is the place where the females lay eggs and breed larvae. The selecting of breeding sites vary with the species of mosquitoes.

Paddy field type of breeding sites include rice fields, marsh and pond, in which water areas are large, water is clean and still. The breeding sites is suitable to *Anopheles sinensis* (中华按蚊) , *Anopheles anthropagous* (嗜人按蚊) , and *Culex tritaeniorhynchus* (三带喙库蚊) etc.

Slowly flow water type includes stream and irrigation with clean and slowly flow water, which is the breeding site of *Anopheles minimus* (微小按蚊) commonly.

Jungle type includes mountain stream, stone cave and spring pond, in which *Anopheles dirus* (大劣按蚊) is found usually.

Dirt water type includes dirt water pit, sewer, fecal pit, which are the breeding sites of. *Culex pipiens pallens* (淡色库蚊) , *C.p. quinquefasciatus* (致倦库蚊) .

Container type includes water vats, jars, bamboo container, tree-hole etc, which are the breeding sites of *Aedes albopictus* (白纹伊蚊) and *A.aegypti* (埃及伊蚊) .

2) The behaviors of sucking blood Only females of mosquitoes suck blood. The females also feed on plant fluids, but they require a blood meal after mating. Some mosquitoes prefer to suck humans blood, another mainly feed on animals.

3) Resting sites of the adults After sucking blood, female need to find a place for blood digestion and maturation of the ovaries. *Anopheles dirus* and *A.anthropagous* prefer to rest inside of house(called endophilic type); some mosquitoes such as *Anopheles sinensis* rest inside of house for a while, then fly to outdoor for blood digestion and maturation of the ovaries(Called half endophilic type); *Anopheles dirus* rest outdoor for blood digestion and maturation of the ovaries(Called half exophilic type).The period from feeding blood to laying eggs is called gontrophic cycle(生殖营养周期), the times of spending gontrophic cycle is called physiological age(生理龄期)。

4) Flying and activity Mosquitoes' activities have relationship with temperature, humidity, light and wind. a) Most Anopheles are crepuscular or nocturnal in their activity. Their feeding blood and oviposition normally occur in the evening, at night or in the early morning; b) Many Culex bite humans and other animals at night; c) Aedes usually bite humans during the day or early morning. Mosquitoes commonly disperse within less than 2 km, and only fly a few hundred meters from their breeding sites. But modern transport can spread mosquitoes to thousands miles away.

5). Over winter In the winter, mosquitoes don't suck blood and hide in warm place such as inside of house; The ovary don't develop. Commonly, the stage of mosquitoes for over winter is the adult, but in Aedes the stage for over winter is eggs, and in *Anopheles minimus* hibernation(越冬) stage is larva. In sun-tropic and tropical areas, the average month temperature is over 10°C, there is no hibernation for mosquitoes.

6) Seasonal distribution Each species of mosquitoes acquires a set of favorable environmental conditions for its development. The phenomenon that population density of the mosquito varies with the environmental conditions is called seasonal distribution. The seasonal distribution has closed relationship with temperature, humidity and rainfall. The seasonal distribution in mosquitoes is closed relationship with the seasonal distribution of the arbo-diseases.

7) Longevity(寿命) In tropical areas, the adult mosquitoes may live on average about two to 3 weeks; in temperate areas, the adult may live on four to five weeks or longer; the males have a shorter lifespan than the females.

Mosquito and diseases

1) Direct harm to humans Biting by mosquitoes can cause irritation, or allergic reaction.

2) Transmission of diseases As vectors, mosquitoes can transmit lots of arbo-diseases.

Arbo-disease	Mosquito	Epidemic area
1. malaria	<i>Anopheles sinensis</i>	Plain areas
	<i>A. anthropophagus</i>	Mountain or hilly areas in South China
	<i>A. minimus</i>	Mountain or hilly areas in South China
	<i>A. dirus</i>	Jungle areas of Hainan island
2. Japanese B encephalitis	<i>Culex tritaeniorhynchus</i>	Paddyfield
3. Filariasis	<i>Culex pipiens pallens</i> <i>C.p. quinquefasciatus</i>	As a vector of Filariasis bancrofti in North areas of Yangtse river
	<i>Anopheles sinensis</i> <i>A. anthropophagus</i>	As a vector of filariasis malayi
4. Dengue fever	<i>Aedes aegypti</i> <i>A.albopictus</i>	Tropical areas In China: Hainan, Guangdong etc

Control It include the larva control and adult control.

- 1) Larva control Chemical control, e.g., insecticide is placed in the water; biological control, e.g., predators(天敌), disease agents; source reduction or habitat management.
- 2) Adult control Insecticides(direct action and residual action); Personal protection.

FLY (蝇)

Fly belong to Order Diptera(双翅目), there are more than 1500 species. The medical important species of fly include *Muscidae* (蝇科) , *Calliphoridae* (丽蝇科) , *Sarcophagidae* (麻蝇科) and *Oestrodiae* (狂蝇科)。

Morphology The fly measure 5-10 mm long and are dust-gray or black color, some species have metallic color.

1) Head: a pair of compound eyes and three single eyes, a pair of antenna; the mouthparts is lapping type or sponging type, a few species have sucking type mouthparts.

2) Thorax: The thorax bears three pairs of legs and a pair of wings. Each leg terminates has a pair of claws (爪) and a pair of pupvilli (爪垫). These specific structures can carry the pathogens.

3) Abdomen: There is external reproduction organ, which can be as a specific feature for identifying the species of fly.

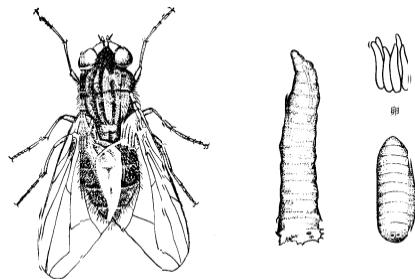


Fig VII-III-5 Development stages of fly: adult, pupa, larva and egg

Life cycle It belong to complete metamorphosis, the development stages contains eggs, larvae, pupae and adults.

Ecology Females oviposit in wet, decaying organic material. The usual sites are garbage cans, feces, or other decaying material. Most species of fly (e.g, housefly) are non sucking blood species. They have dirt habits of feeding indiscriminately (不加区分) on both excreta and foods, and excreting and regurgitating (吐出) their partially digested meals over food. These specific

eating behaviors are main causes of transmitting diseases by flies.

Flies and diseases Flies affect human health by mainly mechanical transmission of diseases. For example, housefly can carry the agents of diseases by its pads, hairy legs, body bristles and mouthparts, or by ingesting the pathogens and then deposition with feces.

1) **Mechanical transmission:** intestinal dysentery, e.g. cholera, typhoid fever, bacterial dysentery, amebic dysentery etc.

2) **Biological transmission:** Trypanosomiasis (锥虫病) is transmitted by tsetse flies (采采蝇).

3) **Myiasis(蝇蛆病):** The disease caused by the parasitism of fly larva, cutaneous myiasis, intestinal myiasis, urogenital (泌尿生殖系统) myiasis and eye myiasis.

Control The main methods of control are as follows:

- 1) Source reduction by environmental modify
- 2) Insecticide use.
- 3) Physical methods: in house, barns, milking parlors, screening to keep flies out is the first line of defense.

SANDFLIES(白蛉)

Sandflies belong to *Order Psychodidae*, there are more than 500 species of sandflies in the world. In China, 40 species was reported, among them, *Phlebotomus chinensis* (中华白蛉指名亚种) and *Ph.c. longiductus* (中华白蛉长管亚种).

Morphology Adult sandflies are only 1.5-4 mm long, and yellow in color. They may be recognized by their hairy bodies and wings that are held erect over the body. They have short mouthparts(sucking type) and are pool feeders. They have a pair of relatively large black eyes. The antennae are long and relatively long and still-like legs. Humpback (驼背形), erect V shaped position of the wings at rest.

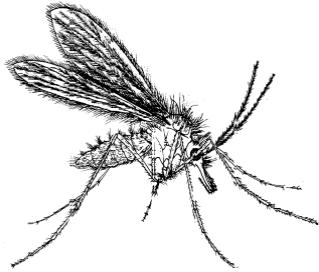


Fig VII-III-6 Adult sandfly

Life cycle It belongs to complete metamorphosis, the development stages contain eggs, larvae, pupae and adults.

1) Egg Eggs are laid in the crack of soil and the wall, or hole. Under suitable conditions, eggs hatch to larvae within 6 to 12 days.

2) Larva Larvae feed on organic material. There are four larval instars (中间形态).

3) Pupa Pupae neither feed nor do activities. After 6 to 10 days, they emerge into the adult stage.

4) Adult After emergence, the copulation appears to take place within 1 to 2 days. The gonotrophic cycle (生殖营养周期) requires about 6 days after feeding to develop ova. Females only mate with males one time within life time, male will die after mating, and females can live 2 to 3 weeks.

Ecology In China, the sandflies distribute in North areas of Yangtze River. Only females feed on blood, but both males and females take plant juices and nectar as a source of energy. The adult rest in house or outdoor. *Phlebotomus chinensis* (中华白蛉指名亚种) in plain areas usually rest in house, but in plateau areas of North China it is usually found outdoor, e.g., tree-hole. Their ability to fly are weak, and sandflies do not disperse more than 30 meters. The peak of the population density occurs in summer, the stage of hibernation(越冬) is the larva.

Sandflies and diseases Sandflies can transmit Leishmaniasis (利什曼病), sandfly fever (白蛉热), and Bartonellosis (巴尔通病)。

Control The main methods of control are as follows: a) Environmental modify, b) Insecticide use, b) personal protection.

FLEAS(蚤)

Fleas belong to Order Siphonaptera, are ecto-parasites of mammalian and birds. There are more than 2000 species in the world. In China, 454 species was reported, among them, only a few species are vectors of zoonoses.

Morphology The males measure 3 mm long, and females are shorter than the males. The body is brown-yellow color and covered with bristle(鬃).

Life cycle and ecology It belongs to complete metamorphosis, the development stages contain eggs, larvae, pupae and adults. The life span of the adult is about a year under favorable condition. Both males and females can take a blood meal, so they are equally important as vector of disease. Most species of fleas are not entirely host-specific, small mammalian e.g., rat are common host. The adults of fleas can jump about 20 cm vertically and 30 cm or more horizontally.

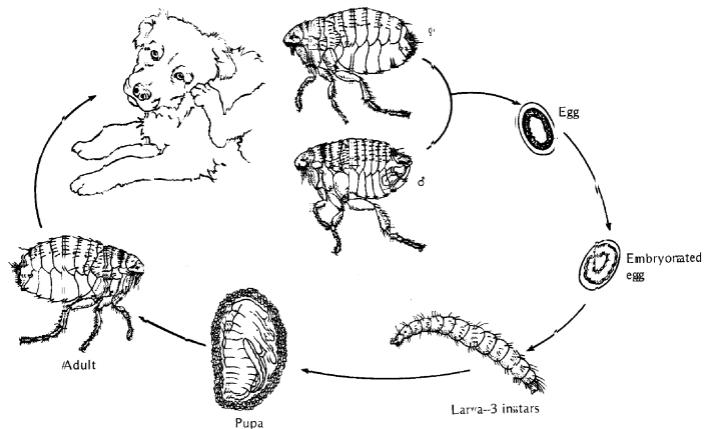


Fig VII-III-7 The life cycle of flea

Harm to humans Fleas can cause harms to humans by irritation, parasitism and transmission of diseases. Fleas frequently bite person on the ankles and legs, but at night a sleeping person may be bitten on other parts of the body.

The most serious disease, plague (鼠疫) is transmitted by flea. The pathogen of plague is *Yersinia pestis* (鼠疫杆菌), its natural hosts include Marmota (旱獭), Citellus (黄鼠) and Meriones (沙鼠) in China, the species of fleas include *Pulex irritans* (致痒蚤), *Xenopsylla cheopis* (印度客蚤). Flea-born epidemic typhus is also an important disease transmitted by fleas. The fleas are also intermediate hosts of *Dipylidium caninum* (犬复孔绦虫) and *Hymenolepi diminuta* (缩小膜虫).

壳绦虫).

Control The main methods of control are as follows: a) Environmental modify, b) Insecticide use, b) personal protection.

LICE(虱)

Lice are permanent ectoparasite. The parasitic lice of humans include 3 species: *Pediculus humanus*(head louse), *P.humannus corporis*(body louse) and *Pthirus pubis*(crab louse, pubis louse). *Pediculus humanus*(head louse), and *P.humannus corporis*(body louse) are called as human locus.

Morphology Adults are small, grayish and wingless insect with dorsoventrally flattened bodies. The head is rhombus in shape, the mouthparts is sucking type. There is a pair of five segmented antennae and a pair of conspicuous eyes in the head. Three pairs of legs are short and well developed. The pubic lice(耻阴虱) is generally smaller than *Pediculus*. Their bodies are broad with very large claws on the middle and hind legs.

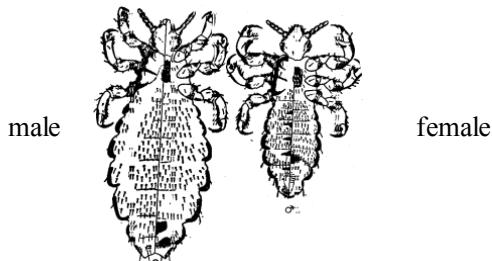


Fig VII-III-8 Adult of human lice

Eggs are oval, white and firmly attached to the hairs or the clothes.

Life cycle and ecology It belongs to incomplete metamorphosis, the development stages contains eggs, nymph and adults. Human lice parasitize on human, head lice live in the hairs, and its egg are laid on the root of the hairs. Body lice live in clothes, and pubic lice live in the density areas of body hairs such as pubic hairs etc. Both sexes of the adult lice take a blood meal at any time during the day or night.

Harms to humans Lice can cause pediculosis(虱病), which symptoms include cutaneous irritation, loss of sleep and psychological depression. Epidemic typhus(流行性斑疹伤寒), lice-born relapsing fever(虱传回归热), and trench fever(战壕热) can be transmitted by lice.

Control

Personal sanitation is important for prevention of the lice infestation. The measures for lice control include Physical and chemical measures, e.g., cutting off the hairs with eggs, washing and cleaning the hairs with sulfur soap, sterilizing and boiling clothes of infected person.

COCKROACHES (蟑螂)

Cockroaches belong to *Order blattaria* (蜚蠊目), there are more than 4000 species of Cockroaches. In our country, 168 species of cockroaches were reported. Among them, *Blattella germanica* (德国小蠊) and *Periplaneta Americana* (美洲大蠊) are the most common species in China.

Morphology Cockroaches range from 2 mm to 100 mm long, in generally 10-30 mm long. The bodies is soft and flattened dorsoventrally with chestnut brown or black in color. They have a chewing mouthparts much like a grasshopper(蝗虫), large eyes, and long flexible antennae. The forewing (前翅) are leathery and the hind-wings (后翅) membranous. A large pronotum (前胸板) covers the head..

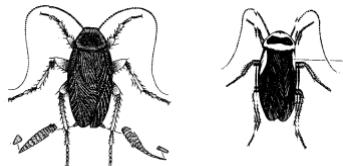


Fig VII-III-9 Adult of Cockroaches

Life cycle and ecology

Cockroaches belong to incomplete metamorphosis, the development stage contains eggs, nymph and adult. Females lay eggs which are attached to one another in packets. The eggs hatch giving rise to tiny, soft first instar nymphs. Development is slow with the time from egg to adult requiring several weeks or months at moderate temperatures. The adults prefer to rest and act at humidity and warm places. They have dirt habits of feeding indiscriminately (不加区分) on both excreta and foods, and excreting and regurgitating (吐出) their partially digested meals over food.

Harms to humans

Cockroaches are likely serve as mechanical vectors of a number of bacterial agents, especially enteric species. They have also been implicated as being paratenic hosts

of hookworm larvae. Cockroaches have also been implicated as causing asthma in Children.

Control In house and other building, control is attempted by the following measures:

- 1) Insecticide application.
- 2) Removal of any possible food sources.
- 3) Preventing migration of roaches from one dwelling to another.