Toxoplasmosis

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Learning Objectives

1. To understand the importance of the different modes and vehicles of transmission.
2. To know the importance of serological tests in the diagnosis of different forms of toxoplasmosis and their interpretation in pregnancy.
3. To review the preventive measures which are needed in pregnancy and in immunocompromised hosts.

Introduction

Toxoplasma gondii is an apicomplexan protozoan parasite and is responsible for the cosmopolitan zoonotic infection of toxoplasmosis. The members of the family Felidae like cats are the only known definitive hosts of *T. gondii*. The life cycle of *T. gondii* is completed within a wide variety of hosts, especially in all warm-blooded animals along with its two reproductive phases – sexual and asexual. The sexual reproductive phase occurs only in domestic cats or the wild Felidae family members, while the asexual reproductive phase of the parasite occurs in both intermediate (birds or mammals) and final or definite (domestic cats) hosts. *T. gondii* has three major genotypes – type I, type II and type III. All of these genotypes vary in pathogenicity in the hosts and their prevalence. It is generally asymptomatic in immunocompetent individuals, or it may manifest as flu-like symptoms and other non-specific clinical signs. Humans acquire *T. gondii* through ingestion of undercooked meat, drinking contaminated water, transplantation of a contaminated organ and contact with feline faeces. In humans, *T. gondii* is frequently associated with congenital infection and abortion. Infections of *T. gondii* are usually minor and self-limiting but severe in case of immunocompromised patients, including HIV-infected individuals, in whom it can cause encephalitis. The control of toxoplasmosis is dependent on accurate diagnosis which determines the therapeutic options. However, available options for toxoplasmosis chemotherapy are limited.

History

The word "toxoplasma" comprises two words, i.e. "toxon" and "plasmid", both originating from the Greek; the former word means "bow"

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and the latter means "form". Therefore, the original Greek meaning of the word "Toxoplasma" is a bow-shaped organism. *T. gondii* is a member of the Apicomplexa, which is an assorted group of several parasitic protozoans such as *Babesia*, *Cyclospora*, *Cryptosporidium*, *Isopora* and *Plasmodium*. The organism was first identified in Tunis in 1908, isolated from a common gundi (*Ctenodactylus gundi*). Thereafter, Splendore discovered the same parasite in Brazil, which was isolated from a rabbit. After thorough microscopic study of several tissues and experimental studies in 1909, Nicolle and Manceaux recommended the present term *T. gondii* after considerable microscopic analysis of several tissues and experimental studies.

Six clades of *T. gondii* have been featured by seeking knowledge of population genetic structure studies which indicates the origin of diverse isolates from rare ancestral lineages. It has been delineated that *T. gondii* appeared first in South American felids and then expanded through migratory birds and mostly through the transatlantic slave trade culture that involved migration of domestic cats, mice and rats. The first observance of human infections was made in the 1920s in a series of cases of congenital diseases characterised by choroidoretinitis, hydrocephalus and encephalitis. After the advent of the HIV pandemic in the 1980s, toxoplasma encephalitis due to reactivation of latent infection came to light.

Taxonomy

The genus *Toxoplasma* belongs to the subfamily, Toxoplasmatinae; the family Sarcocystidae; the order Eucoccidiorida; the subclass Coccidiasina; and the class Conoidasida in the phylum Apicomplexa.

Toxoplasma gondii (Nicolle &Manceaux, 1908) is the only species in the genus *Toxoplasma*.

Genomics and Proteomics

In 2003, the initial output of the *T. gondii* genome sequencing effort was accomplished. The Toxoplasma Genome Consortium undertook 10 X shotgun genome sequencing and annotation of the type II strain ME49 in partnership with the University of Pennsylvania and the Institute for Genomic Research (TIGR) that resulted in a draft version of the 80 Mb genome sequence. The type II ME49 strain was the first to be sequenced, which was followed by the other two strains, GT1 and VEG, as well as chromosomes Ia and Ib of the RH strain. The ME49 strain chromosome map was utilised as a template to build the chromosomes for the GT1 and VEG strains. The genomic sequences of the three strains are between 61 and 64 Mb in size in the most recent release of ToxoDB (version 5). A recent version of the genome annotation for the ME49 strain, as well as a brand new genome annotation for the GT1 and VEG strains, has been released. *T. gondii* has an estimated number of genes of 8102 for the ME49 strain, 8145 for the GT1 strain and 7945 for the VEG strain.

The rapid development and implementation of *T. gondii* proteome analysis has been aided by the sequencing and annotation of the parasite's genome. The methods of host cell invasion, the structure and composition of apical organelles, the organisation of the cytoskeleton and the "entire" proteome of tachyzoites have all been studied. The tachyzoite has been the subject of proteome research since it is *Toxoplasma*'s active, infectious stage. No significant data has been reported to date on the other life cycle stages. Most proteome investigations have employed type I strain RH tachyzoites because they have essentially little bradyzoite differentiation in vitro under typical growth conditions. The first large-scale proteomic study of *T. gondii* tachyzoites revealed over 1000 *Toxoplasma* proteins. Advances in mass spectrometry have enabled the use of high- and medium-throughput proteomics approaches to study various aspects of protein functions. These include analysis of subproteomes, analysis of post-translational modifications and identification of macromolecular complexes.

The Parasite Morphology

T. gondii exists in three forms: the trophozoite/tachyzoite, the bradyzoites and the sporozoites. All these three forms are necessary for infections. These stages undergo sexual (*gametogony*) or asexual (*schizogony*) reproduction, depending on the host. While the trophozoite and bradyzoite stages are represented by the schizogony, the sporozoite stage is formed by either gametogony or sporogony. All three forms can occur in domestic cats as well as in other felines which are the definitive hosts of these parasitic forms and provide sustenance to both the schizogony and gametogony, while on the other hand, out of the three forms, two forms, i.e. trophozoites and bradyzoites, also exist in other warm-blooded animals including birds and humans, which are the intermediate hosts for them.

Trophozoites/Tachyzoites

The term "Tachyzoite" (*tachos* = speed in Greek), previously called "trophozoite" (*trophicos* = feeding in Greek), was coined by Frenkel. It is the rapidly multiplying form which occurs intracellularly in the intermediate hosts and also extracellularly in the definitive host. Endodyozoites and endozoites were other terms used for tachyzoites. Various aggregated tachyzoites are called groups, clones or terminal colonies.

The tachyzoite is about 2 by 6 μm in size and appears as crescent-shaped, having a rounded and pointed posterior and anterior (conoidal) end (Fig. 1). Ultrastructurally, the tachyzoite comprises a number of cell organelles which include micronemes, mitochondrion, rhoptries, endoplasmic reticulum, Golgi complex and a multiple-membrane-bound plastid-like organelle (a Golgi adjunct or apicoplast) apart from a number of inclusion bodies. The nucleus is central in position with a prominent nucleolus.

Tachyzoites are the dissemination form. They have the ability to invade all cell types of the vertebrate and can divide in a parasitophorous vacuole. Tachyzoites enter the host cells either by phagocytosis or by penetration. Once inside the cell, the tachyzoite becomes ovoid in shape and comes to lie inside a parasitophorous vacuole. Both the multiplication and invasion rates vary and are mostly dependent on the *T. gondii* strain and the type of host cells.

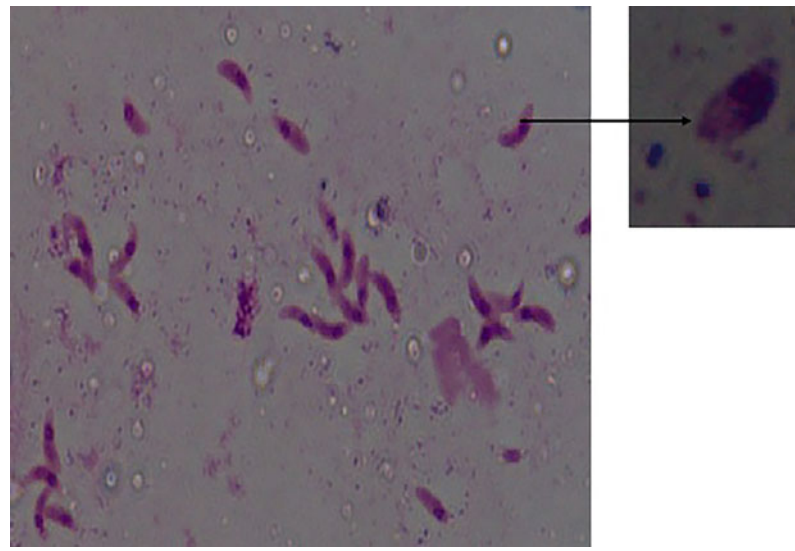

Fig. 1 Tachyzoites of *Toxoplasma gondii*, stained with Giemsa, smear was made from peritoneal fluid obtained from a laboratory-inoculated mouse. (Image courtesy: Department of Medical Parasitology, Postgraduate Institute of Medical Education and Research, Chandigarh, India)

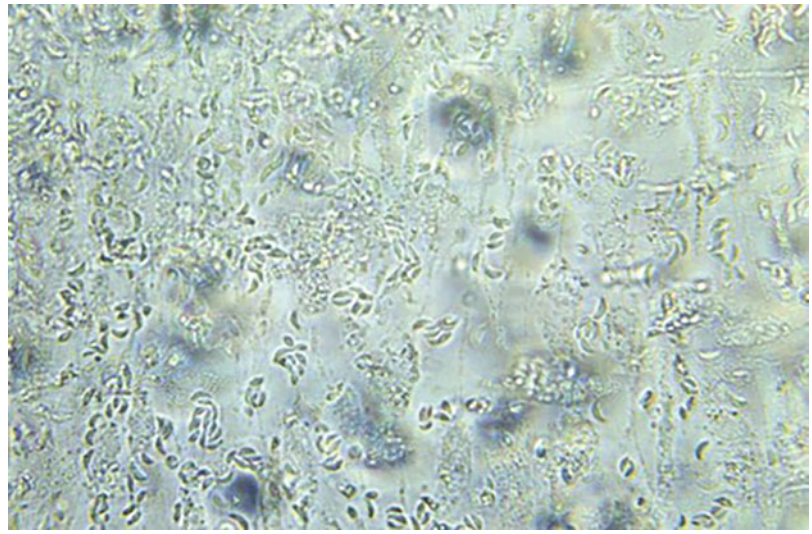

Fig. 2 Photomicrograph of a tissue sample showing a darkly stained, *Toxoplasma gondii* tissue cyst, which contained numbers of spherical-shaped bradyzoites (Courtesy: PHIL; CDC/ Dr Green)

Bradyzoite/Tissue Cyst

The term "bradyzoite" (*brady* = slow in Greek) was also coined by Frenkel to describe the organism that can divide slowly inside the tissue cyst and is also called cystozoite. Tissue cysts remain intracellular and expand, as the bradyzoites inside them multiply by *endodyogeny*. These tissue cysts have a thin (0.5 μm) and elastic wall that can accommodate hundreds of bradyzoites (Fig. 2). There is variation in the size of the tissue cysts, i.e. younger tissue cysts can be small at 5 μm in diameter and can only contain two bradyzoites, whereas the older ones may have hundreds of bradyzoites. Tissue cysts are found to be spheroidal in the brain and rarely as large as 70-μm in diameter, whereas intramuscular cysts are mainly elongated and can be 100-μm in size. Tissue cysts grow in visceral organs, such as the kidneys, lungs and liver, but are mainly found in the muscular and neural tissues, including the cardiac and skeletal muscles, brain and eyes. Intact tissue cysts are mostly harmless and can persist lifelong without inducing any inflammatory response inside the host.

Bradyzoites differ slightly from tachyzoites in structural appearance. The nucleus is located at the posterior end in bradyzoites, while it is centrally positioned in tachyzoites. The bradyzoites usually have electron-dense rhoptries (Fig. 3), whereas tachyzoites have labyrinthine ones. Bradyzoites are more resistant to proteolytic enzymes in comparison to tachyzoites, and this explains why cats have a longer prepatent period if fed with tachyzoites in comparison to ingestion of bradyzoites. After the definitive host ingests the tissue cysts, the cyst wall gets ruptured by the proteolytic enzymes present in the small intestine and stomach. Thereafter, the released bradyzoites invade intestinal epithelial cells and start generating numerous *T. gondii*.

Sporozoites

Sporozoites are found in mature oocysts. Oocysts have ovoid structures and are mainly 12 to 13 μm in size. After sporulation oocysts have two sporocysts, each accommodating four sporozoites. The wall of the oocyst has a multilayered structure which shields the parasite from any chemical and mechanical damages and enables it to persist in a moist environment for a longer period (>1 year). When definitive hosts like cats and other felines become infected by the ingestion of either oocysts or tissue cysts, the parasites begin further development in the host's intestinal epithelial cells, where both the schizogony and gametogony take place. Therefore, the

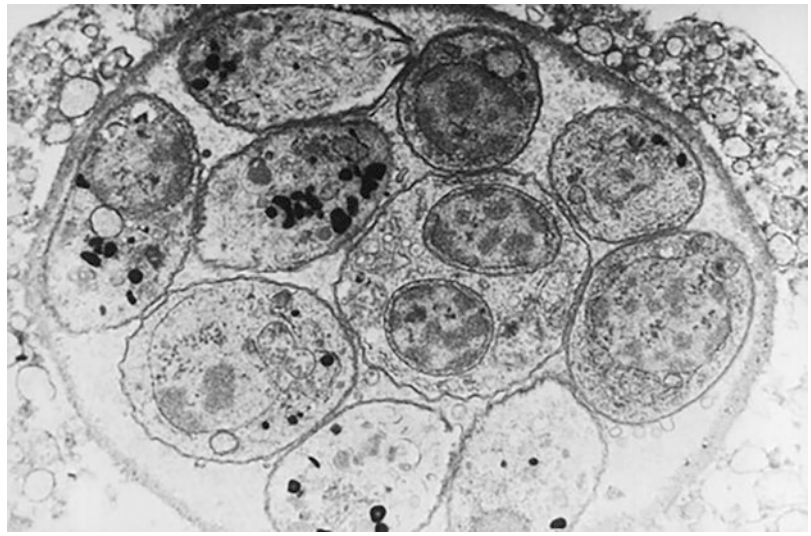

Fig. 3 This transmission electron microscopic (TEM) image reveals some of the ultrastructural details displayed by a *Toxoplasma gondii* tissue cyst, within which bradyzoites could be seen developing (Courtesy: PHIL; CDC)

definitive hosts shed millions of oocysts each day in faeces, but the freshly passed oocysts are not infectious. They become infectious only after development in water or in the soil for a few days according to the availability of temperature and aeration.

Ultrastructurally, the sporozoite is the same as the tachyzoite, but with fewer rhoptries, micronemes and amylopectin granules. They are $2 \times 6–8$ μm in size with a subterminal nucleus.

All the forms of *T. gondii*, i.e. trophozoite, bradyzoite and sporozoite, are crescent-shaped, but ultrastructurally, they vary in the size of inclusion bodies and in certain organelles. Often all of these three forms have similar numbers of rhoptries but the appearance of these is different in each stage.

Cultivation of Parasites

There are various cell lines, such as transformed cell lines (HeLa, CHO, Vero, LM, MDBK, 3 T3, etc.), and culturing techniques which are being employed to maintain tachyzoites in vitro. Tachyzoites are obligate intracellular forms multiplying every 6–9 h depending on the strain. Once the host cell reaches a count of 64–128 parasites, the cells burst with the release of a fresh batch of tachyzoites which infect new healthy cells.

T. gondii strains do not grow uniformly in all of the cell lines. Human foreskin fibroblast cells (ATCC CRL-1634™) are best suited to maintain *T. gondii*. Dulbecco's Modified Eagle Medium (DMEM) and RPMI 1640 medium, with added growth factors like glutamine and foetal bovine serum along with antibiotic supplementation, have also been found to be alternative satisfactory media. Since low CO_2 and high pH can affect the parasite's growth, culture media should be incubated at pH 7.2 in an atmosphere of 5%. All work should be carried out in a biosafety level 2 laboratory as many *T. gondii* strains are extremely virulent and can easily penetrate any human tissue.

Laboratory Animals

Guinea pig was the first animal model established by Markham in the year 1937 for studying toxoplasmosis. Later, in 1951, Hogan produced the first animal model for ocular toxoplasmosis in rabbits using intracarotid injection, and Frenkel managed an intraperitoneal injection in a hamster in 1953, following the same lineage. Thereafter, nonhuman primates, cats, dogs and pigs have

been explored. Toxoplasmosis can be identified in an experimental model by looking for *T. gondii* cysts in biopsies using a particular colour reaction and immunohistochemistry or by the PCR method.

Among various laboratory animals, the most common are mice, rabbits, pigs and nonhuman primates, which are used for testing the efficacy of any drugs against *T. gondii* infection. Rats are partially resistant to infection by *T. gondii*. The type of laboratory animal has a significant impact on the infection's prognosis. Mice are the most regularly utilised animals in studying the efficacy of drugs. However, in the case of congenital toxoplasmosis, rats and sheep are found to be more relevant. Furthermore, the mouse strain, parasite strain (virulence and lethality versus non-lethality), route of infection (oral versus intraperitoneal) and size of parasite inoculum are the main factors that determine intensity of infection. In animal models, coinfection with various microbes has been explored to simulate a similar situation to immunocompromised hosts, which is a prevalent feature in immunocompromised individuals, particularly during AIDS. In an attempt to explain the pathogenicity of *T. gondii* in hosts with virus-induced immunodeficiencies, experimental models of dual infections were created. Mice infected with *T. gondii* and the retrovirus LP-BM5, which causes murine acquired immunodeficiency syndrome (MAIDS) in mice, and cats infected with *T. gondii* and the feline immunodeficiency virus (FIV) are more vulnerable to primary acquired toxoplasmosis; however, reactivation of chronic infection is not always detected. *T. gondii* was found to be related to other opportunistic pathogens in various experimental models of concurrent infections. In immunocompromised rats, infection with *Pneumocystis carinii* and *T. gondii* was obtained, and this model was utilised to test the efficiency of combined prophylaxis against both diseases.

Further, use of genetically immunodeficient animal models clearly illustrates the role of immunity as a major adjunctive factor in the management of acute infection. These models, on the other hand, are more difficult to create and standardise, but they are meant to closely mimic the characteristics of clinical diseases and to help researchers better comprehend the complicated interactions between infections and host defence.

Life Cycle of *Toxoplasma gondii*

Hosts

Definitive Host

Cats and other felines.

Intermediate Host

Humans and other mammals like sheep, goat, pig, cattle and mice.

Infective Stages

1. Oocysts from ingestion of food or water or vegetables contaminated with cat faeces
2. Tissue cysts containing bradyzoites in undercooked meat (goat, sheep, pork, etc.) from herbivores that have ingested cat faeces

Transmission of Infection

The life cycle of *T. gondii* is completed within a wide variety of hosts, especially in all warm-blooded animals along with its two reproductive phases – sexual and asexual (Fig. 4). While the sexual reproductive phase occurs only in domestic cats or the wild Felidae family members, the asexual reproductive phase of the parasite occurs in both intermediate (birds or mammals) and final or definitive (domestic cats) hosts. During different periods of its life cycle, individual parasites convert into various cellular stages, which include the tachyzoites, bradyzoites (found in tissue cysts) and sporozoites (found in oocysts).

Asexual Cycle

When the intermediate host ingests the tissue cyst or oocyst, the parasites first invade the intestinal epithelial cells. Inside these cells, the parasites differentiate into the rapidly dividing tachyzoites. *T. gondii* have two phases of asexual development. During the acute stage, the first phase,

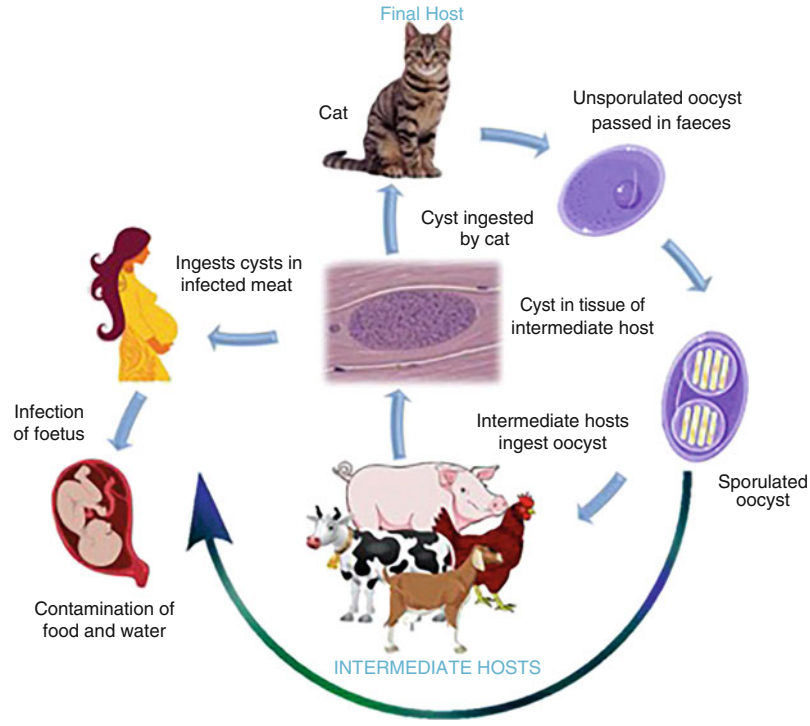

Fig. 4 Life Cycle of *Toxoplasma gondii*

tachyzoites replicate quickly in several discrete varieties of host cells. Inside host cells, the tachyzoites continue to multiply inside the parasitophorous vacuoles formed during entry into the cell. Ultimately the host cell ruptures, releasing the tachyzoites, which can disseminate to any organ of the body including the brain.

During the chronic stages of infection, pressure from the host's immune system causes tachyzoites of the last-generation stage to convert to bradyzoites to form tissue cysts. Tissue cysts in tissues such as brain and muscle tissue form approximately 7–10 days after initial infection. The tissue cysts are principally formed in the brain, eye and the striated muscles and can persist for a long time. Cysts usually range in size between 5 and 50 μm in diameter.

Inside the tissue cyst, gradual multiplication of bradyzoites (or cystozoites) by endodyogeny takes place. They are immediately infectious. Sometimes, in certain intermediate hosts, they may persist lifelong. Tissue cysts undergo lysis with release of the bradyzoites, which transform into tachyzoites that again infect healthy cells and form the tissue cyst.

Sexual Cycle

When a definitive host like a cat consumes a tissue cyst, the bradyzoites convert into merozoites inside intestinal epithelial cells. The merozoites start multiplying asexually by endodyogeny followed by repeated cycles of endopolygeny. During the final stages of this asexual cycle, gamogony and resulting oocyst formation occur. The unsporulated oocysts are then released by the intestinal epithelial cells and pass out with the faeces of the animal.

Pathogenesis and Pathology

In most cases human acquire toxoplasmosis mainly by ingesting tissue cysts which are present in infected meat or oocysts present in food which is contaminated with cat faeces. After ingestion, bradyzoites from tissue cysts or sporozoites from oocysts are released and enter intestinal epithelial cells and start multiplying. The tachyzoites so formed spread to the regional lymph nodes from where they may be carried to various organs through the lymphatics or blood. Necrosis of the

lymph nodes and affected organs is the hallmark of infection. The vulnerable organs include the adrenals, eye and heart. There is no toxin production by *T. gondii*, and necrosis occurs because of intracellular multiplication of tachyzoites.

In AIDS patients, there is reactivation of latent infection leading to opportunistic infection. The encephalitis, a main lesion of toxoplasmosis, is distinguished by necrosis in the tissue of these patients, which mainly leads to multiple abscesses.

Immunology

Both innate and adaptive immune responses play significant roles in toxoplasma infection. This comprises systematic and well-coordinated cellular interactions between the parasite, enterocytes, monocytes, dendritic cells (DC), macrophages, NK cells and neutrophils. The human cellular response to *T. gondii* infection is highly dependent on cell type and the infecting strain of *T. gondii.* In healthy humans and animals, *T. gondii* infection is asymptomatic because the host's innate and adaptive immunity suppresses the parasite's initial multiplication and eliminates the majority of the parasites. When a *T. gondii* tachyzoite infects monocytes, it triggers innate immune responses such as the generation of proinflammatory cytokines, which triggers adaptive immunological responses mediated by T and B cells. Activation of adaptive immunity also induces cell-autonomous immune responses in infected cells, causing *T. gondii* stage change into a bradyzoite (a form that develops slowly but evades host immunological responses) that eventually leads to chronic infection.

T. gondii trigger innate immunity, the initial line of defence for the host, which responds quickly and recognises pathogens via pattern recognition receptors (PRRs), such as TLRs, NOD-like receptors and C-type lectins. Ligand detection by PRRs triggers the production of proinflammatory cytokines such as TNF-, interleukin-1 beta (IL-1), IL-6 and IL-12 and plays a key part in the subsequent cascade of events. *T. gondii* activates innate immunity, and *T. gondii* produces a robust CD4 T cell response resulting in IFNγ production in acute as well as chronic stages of infection. CD8+ T cells may act as effector cells during *T. gondii* infection, while the CD4+ T cells provide the necessary help in maintenance of these cells. Depletion of both CD4+ and CD8+ T cell populations has been shown to result in the reactivation of latent toxoplasmosis and, as a result, infection in animals vulnerable to toxoplasma encephalitis. Additionally, antibodies generated during toxoplasma infection can destroy the parasites. Parasite-specific IgM, IgA, IgE and IgG2 antibodies can be detected in patients with toxoplasmosis and serve as important tools in distinguishing recent from past or chronic infections.

Infection in Humans

Toxoplasmosis symptoms vary based on parasite characteristics such as strain virulence and inoculum size, as well as host immune status and genetic background. The three genotypes of *T. gondii* differ in virulence and epidemiological pattern of occurrence.

T. gondii infects a huge percentage of the world's population but rarely leads to a clinically significant disease. Asymptomatic infection with *T. gondii* is seen most often with development of latent infection and formation of tissue cysts. Sometimes mild symptoms may appear as lymphadenopathy, which is the most remarkable clinical feature. Severe manifestations may occur such as encephalitis, sepsis or myocarditis but these are found to be rare in immunocompetent humans. However, some individuals are at high risk for fatal or life-threatening toxoplasmosis. These individuals include foetuses, newborns and immunologically impaired patients where *T. gondii* can lead to dangerous complications like encephalitis, chorioretinitis, congenital infection and neonatal mortality and postnatally acquired toxoplasmosis in immunocompetent humans.

Ocular toxoplasmosis can be a result of infection acquired either postnatally or during the prenatal period. The symptoms such as retinitis and retinochoroiditis manifest later in life.

Congenital toxoplasmosis is the most deadly form of toxoplasmosis and is caused by *T. gondii* transplacental contamination of the foetus during pregnancy. The severity of the disease is mostly determined by the gestational age at the time of transmission. Infection of the foetus during the first trimester of pregnancy can result in serious damage to the foetus, but later trimesters have less severe foetal disease. Early-stage infections can cause anaemia, chorioretinitis, jaundice, seizure and hydrocephalus in the foetus. Sensorineural deafness, microcephaly, mental retardation, visual deficiency and slow development are late consequences of congenital toxoplasmosis.

In immunocompromised individuals, an earlier acquired latent infection of *T. gondii* gets reactivated, which commonly manifests as encephalitis. Toxoplasma encephalitis and disseminated toxoplasmosis are commonly seen in patients with Hodgkin's disease, those on immunosuppressive therapy or bone marrow or other organ transplant patients. *T. gondii* is an important opportunistic pathogen in AIDS patients causing severe encephalitis and death in over 30% of these patients.

Infection in Animals

Infected domestic cats remain asymptomatic with no clinical disease. Nevertheless, clinical signs may appear with the intensity of infection that include fever, anorexia, ocular inflammation, lethargy, abdominal discomfort, pneumonia and central nervous system distress. Kittens are more vulnerable to clinical infection, and feral domestic cats are at a higher risk of infection as compared to indoor cats.

Domestic dogs can be infected with *T. gondii*, but clinical infection occurs less commonly than subclinical disease. However, clinical signs of the disease involve respiratory, neuromuscular or gastrointestinal systems and sometimes prove fatal. Stray dogs are supposed to be at higher risk, and mostly become infected by eating uncooked infected meat.

Toxoplasmosis is common in sheep, goats, pigs and chickens as intermediate hosts; however, horses and cattle are found to be resistant to the disease. In sheep, congenital infection causes stillbirth and preterm lamb loss. Infected lambs usually survive with normal growth, but because of its consumption, it represents a public health issue. Toxoplasmosis in adult goats is more intense than in sheep, and congenital infection leads to death of kids pre- or post-birth. Pigs may get infected with *T. gondii*, by ingestion of oocysts, congenitally by tachyzoite transplacental transmission and through intake of meat having *T. gondii* bradyzoite tissue cysts. Adult pigs barely show any clinical signs, but the meat of these infected pigs is the major source of human infection. Toxoplasmosis in young pigs proves fatal and they often die without participating in the human food chain. *T. gondii* infection in animals occurs mainly by environmental exposure to the oocysts, and roaming of outdoor domestic cats is found to be a risk factor for infection in various farm animals.

Epidemiology and Public Health

Toxoplasmosis is a significant public health problem worldwide. About one-third of the world population are estimated to be exposed to this parasite. Toxoplasmosis is usually more prevalent in moist, warm and low-altitude regions. This fact is associated with longer viability of *T. gondii* speculated oocysts in warm and humid areas. About 8–22% of the US population are infected, and a similar percentage of infected population is also estimated for the United Kingdom. In Central America, South America and continental Europe, estimates of infection range from 30 to 90%. Europe, in particular, has been found to have a wide range of prevalence, ranging from 10% in Iceland to 63% in Poland.

Toxoplasmosis can be classified as a One Health disease as it significantly affects the health of various creatures (humans, domestic animals, wildlife) and ecosystems and is viewed as a serious threat for all those who depend on animal resources. *T. gondii* infection in food-producing animals has become an important public health issue, as a source for human toxoplasmosis by

transmission of the parasite via pork and wild boar meat and meat products. Infected cats are a major contributor to environmental contamination. The presence of cats in the environment has been linked to greater *T. gondii* seropositivity in pigs (19%) and wild boars (23%), respectively, around the world. These infections have serious consequences that affect mortality and standard of life.

T. gondii have three archetypal clonal lineages. Diverse aberrant genotypes have been seen in the Americas and China numbering 189, and most of these come under genotypes 1 through 5. There is no dominant genotype reported in the Southern Hemisphere; however, a few genotypes are found in the Northern Hemisphere, particularly genotypes 1 (type II clonal), 2 (type III) and 3 (type II variant), which include most isolates, and these are mainly found in Europe. In North America, genotypes 2 to 5 (4 and 5, jointly known as type 12 and prevalent in wildlife) are common. In Africa, genotypes 2 and 3 predominate, whereas genotypes 9 and 10 are very common in China. Several genotypes are linked with intense virulence in humans and wildlife. Clonal lineages 1–4 are most abundant, with highly similar multilocus genotypes, a high degree of linkage disequilibrium and infrequent recombination. Type II strains, which are avirulent in mice, have been identified as the cause of more than 70% of human cases of toxoplasmosis in the United States and Europe as shown primarily in France. Type I, recombinant and atypical strains, has been associated with a higher frequency of ocular toxoplasmosis and severe toxoplasmosis in immunocompetent patients.

Diagnosis

The diagnostic methods mainly employed are serologic tests, molecular methods (PCR, RT-PCR), histological demonstration and bioassay. Other less preferred methods that help in detection are a toxoplasmin skin test, antigenaemia and antigen analysis in body fluids and antigen-specific lymphocyte alteration.

Microscopy

Tachyzoites detection in any histological sections from biopsies specifies an acute infection. Chronic toxoplasmosis can be confirmed through the detection of bradyzoites containing tissue cysts in histological samples. Stains such as haematoxylin and eosin as well as Wright stain are usually used for encysted tachyzoites and bradyzoites demonstration (Figs. 5 and 6). Immunoperoxidase staining has been found to be sensitive and specific, uses antisera to *T. gondii* and has been used successfully to detect *T. gondii* in the brain of AIDS patients.

Animal Inoculation

It is assessed as the gold standard for detection of *T. gondii* infection. Secretions, excretions, body fluids, lymph nodes and muscle and brain tissues are possible specimens, and mice and cats are the common animals which can be used. IFN-γ knockout mice are preferred, due to high sensitivity, or normal mice may be immunosuppressed by administrating dexamethasone. The tachyzoites are found in peritoneal cavity of mice after 6–10 days of inoculation.

Serodiagnosis

Serologic tests serve as the primary choice for diagnosis. Chronic infection is diagnosed by the serological detection of antibodies produced against parasite-specific antigens. For this, IgG and IgM ELISA assays in combination format are mostly used.

IgM antibodies are mainly traceable around 1 week post-infection and remain for several months or years. This makes IgM antibodies detection insufficient while confirming acute infection. Moreover, IgA antibodies are produced before IgM and remain for only several months, so they are considered to be a satisfactory and initial marker of acute infection. Also, IgG antibodies provide information about the

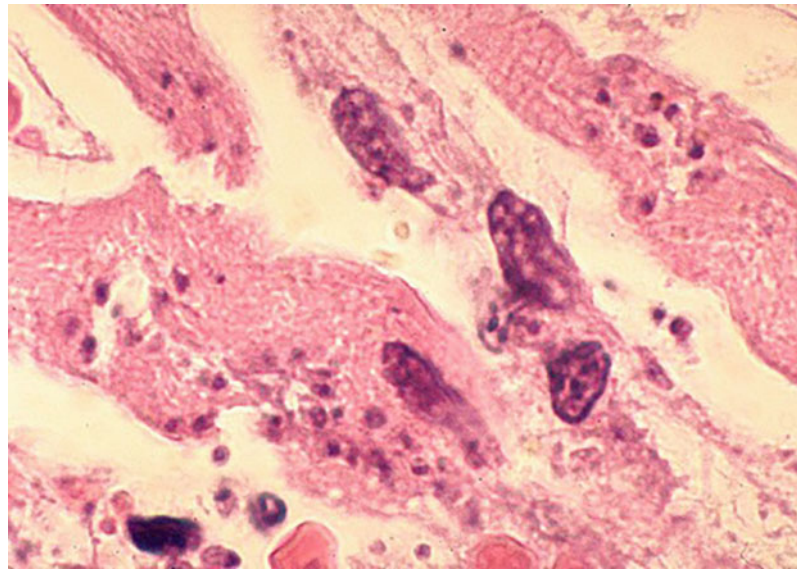

Fig. 5 This photomicrograph reveals some of the histopathology found in this cardiac tissue sample in a case of cardiac toxoplasmosis. The biopsy specimen was harvested from a patient with a fatal case of AIDS. Within the myocytes numerous *Toxoplasma gondii* tachyzoite can be seen. (Courtesy PHIL, CDC/ Dr. Edwin P. Ewing, Jr.)

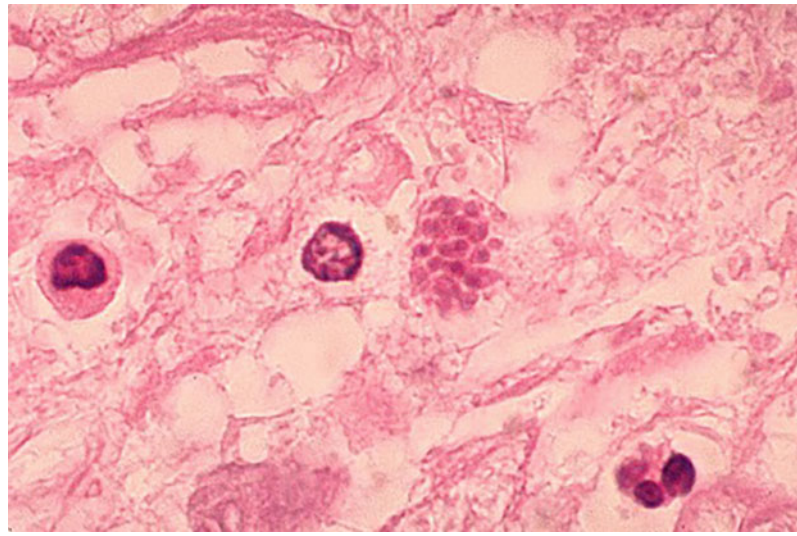

Fig. 6 This photomicrograph reveals some of the histopathology found in this brain tissue sample in a case of neuro-toxoplasmosis. The biopsy specimen was harvested from a patient with a fatal case of AIDS. A pseudocyst, containing numerous, *Toxoplasma gondii* tachyzoites is visible (Courtesy: PHIL, CDC/ Dr. Edwin P. Ewing, Jr.)

occurrence of infection, without revealing the timing of infection.

Although IgM indicates an acute infection, it may persist for a prolonged period following infection. Therefore, an IgG avidity test is usually done to distinguish between previous and current infections, because IgG affinity increases over time as it is obtained from the antigen-driven B-cell selection process. For confirmatory diagnosis, samples should be analysed in a reference laboratory where serological panel testing can be done comprising avidity testing; ELISA for IgA, IgM and IgE; the dye test used for IgG antibodies measurement and the differential agglutination test (Table 1). Other serological tests that can be employed are the latex agglutination test, the indirect haemagglutination test and indirect fluorescent antibody tests.

Molecular Diagnosis

T. gondii DNA detection through PCR in body fluids (bronchoalveolar lavage fluid,

Table 1 Serological tests used in toxoplasmosis

SN	Serological test	Principle	Remarks
1.	*Sabin-Feldman dye test (reference test)* IgG detection	Based on the inhibition of staining of live tachyzoites by antibody. Live tachyzoites from mice are incubated with patient's serum, and alkaline methylene blue solution is added. Antibodies will kill the tachyzoites and will not take up the dye and will appear colourless and thin or distorted	If less than 50% of the tachyzoites take up the stain, the test is considered positive Test is potentially hazardous and requires a high degree of technical expertise
2.	*Differential agglutination test:* IgG detection	Formalin-treated antigen (HS) and methanol-treated antigen (AC) used with a single sample. AC antigen is specific for membrane	To rule out recent infection. AC strong antibody response to AC in early infection, wanes after 6–12 months HS/AC$\geq$ 4: Infection has occurred more than 6 months earlier
3.	*Avidity test*: IgG detection	With increasing humoral response, there is increasing avidity of IgG. In early stage of infection, weak avidity antibodies are produced and in late stage those of strong avidity. Two parallel ELISA are run with untreated serum and one with serum treated with urea/guanidine/thiocyanate which dissociates Ag-Ab complex of weak avidity	High avidity index indicates infection in remote past Not always true since increase in avidity may be slow
4.	*IgM/IgA detection*	Uses cytosol antigens enriched with membrane antigen (P30, SAG 1) to enhance sensitivity	A titre of 1:256 in double sandwich IgM ELISA is considered diagnostic for recent acute infection IgA can be detected in serum or in aqueous or vitreous samples in case of ocular infections But IgM antibodies can persist for months to more than 1 year A negative or low titre of IgM does not exclude a positive diagnosis for cerebral toxoplasmosis since antibody production is suppressed in HIV infection
5.	*Western blot test:* IgG	Two samples tested in parallel: blood/CSF; blood/aqueous humour; maternal/neonatal blood	Additional bands in the second sample denote organ/neonatal infection

cerebrospinal fluid (CSF), vitreous and aqueous fluids, blood and brain tissues) has been employed for diagnosis of cerebral, ocular, congenital and disseminated toxoplasmosis. It is also successfully used for early detection of intrauterine *T. gondii* infection. For PCR amplification, B1 gene, 18S rDNA gene, 529-bp repeat element, GRA1, SAG1 and SAG2 are the target genes. Real-time PCR by targeting amplification of the B1 gene is the most recommended diagnosis technique for congenital toxoplasmosis in comparison to nested and conventional PCR. The LAMP assay has also been developed which targets the *T. gondii* oocyst wall protein (OWP) genes, 529-bp repetitive element, SAG1, B1, SAG2, GRA1 and 18S rRNA for testing medical and veterinary samples and water samples (Table 2).

A summary of all the diagnostic tests is provided in Table 3.

Treatment

Patients who are immunocompromised or who are immunocompetent but have severe or prolonged symptoms are frequently treated with pyrimethamine, sulfadiazine and folinic acid. Treatment lasts between 2 and 4 months, depending on the severity of clinical indications and symptoms. Trimethoprim/sulfamethoxazole,

Table 2 DNA target regions for detection of *Toxoplasma gondii* in various molecular methods

Molecular method for detection	DNA target regions
Conventional PCR	B1 gene, 529-bp repeat element, 18S rDNA gene, SAG1, SAG2 and GRA1
RT-PCR	B1 gene, 529-bp repeat element, 18S rDNA gene, SAG1
LAMP	B1, 529-bp repetitive element, SAG1, SAG2, GRA1, oocyst wall protein genes

Table 3 Laboratory diagnosis of toxoplasmosis

Diagnostic method	Target	Remarks
Microscopy of biopsy specimen by using haematoxylin-eosin or immunoperoxidase staining	Tachyzoites or tissue cysts	Invasive procedure
Animal inoculation in cats and mice	Tachyzoites are found in peritoneal cavity	Gold standard but not done routinely
Immunodiagnostics: Sabin-Feldman dye test, ELISA, avidity test, differential agglutination test (DAT)	IgM/IgG/IgA	Standard mode of diagnosis Dye test is the reference serological test Avidity test and DAT can be done to differentiate present from past infections
Molecular diagnosis: PCR, real-time PCR	B1 gene, 18S rDNA gene, 529-bp repeat element, GRA1, SAG1 and SAG2 genes	Highly useful for diagnosis of cerebral, ocular and disseminated toxoplasmosis. It is also used for early detection of intrauterine *Toxoplasma gondii* infection

on the other hand, is the same as pyrimethamine/sulfadiazine. Maintenance therapy is usually started after resolution of the acute phase and mainly consists of the same regimen as in the acute phase but at half dose. This regimen is followed for the rest of the patient's life or until the immunosuppression has resolved.

Prevention and Control

The prevention of toxoplasmosis can be done primarily by imparting health education related to this pathogen and disease and various precautionary measures to avoid personal exposure to the parasite. The infection can be prevented using precautionary measures such as the following: cooking meat to 66 °C throughout before eating; washing hands with detergent and water after touching meat; feeding cats cooked and dry/canned food instead of raw meat; keeping cats indoors and changing litter boxes daily and cleaning them with boiling water; flushing cat faeces down the toilet or burning it; and usage of gloves while gardening.

Currently no vaccine is available to protect humans and animals from congenital infections except for a live attenuated vaccine, Toxovax® (Intervet Schering Plough, Boxmeer, The Netherlands), available in New Zealand and Europe to prevent abortions in sheep.

Proper counselling and health education about risk factors can lower the incidence and probability of getting the infection, which is adapted by many countries to reduce incidence of congenital toxoplasmosis. Countries like Germany and Italy have reported surveillance of congenital toxoplasmosis. Health education may include instructing women about possible environmental exposure and ways to avoid it during pregnancy and receiving appropriate treatment without delay in case of acute infection.

Case Study

A 7-day-old newborn infant underwent a screening test and presented with good health with no symptoms. The mother of this newborn participated in a newborn toxoplasmosis survey conducted at the university hospital which

involved the detection of toxoplasmosis through filter paper screening and accordingly the survey and requirement of fresh blood collection after a few months were explained. The newborn during its first screening using filter paper tested negative for toxoplasmosis and was selected as negative control group. Six months later the second blood collection of both mother and baby was done.

The mother presented negative anti-*T. gondii* IgM and IgG results, while the 6-month-old infant tested positive for anti-*T. gondii* IgA and IgM and had low-avidity IgG and positive PCR assay, which was again confirmed through mouse bioassay and repeated PCR assay. To determine how such a young infant became infected, his mother was interviewed regarding epidemiological aspects. During this interview, she reported that she had given her 2-month-old baby a piece of undercooked beef to suck on. There was no house pets (cats or dogs) and the child mostly fed on breast milk and filtered water. Later on, the nursing infant presented with fever and swollen lymph nodes, which were confirmed as signs and symptoms of acquired toxoplasmosis. For treatment, sulfadiazine (100 mg/kg/day, every 12 h), pyrimethamine (1 mg/kg/day, once daily) and folinic acid (10 mg/day, every 3 days) were prescribed for 1 year with clinical follow-ups throughout his early childhood. The detection of acquired toxoplasmosis in a 6-month-old nursing infant is very rare. This report emphasises the importance of serological surveys for toxoplasmosis control in pregnant women and infants.

1. What is the significance of the above study?
2. How is the infant diagnosed about acquired toxoplasmosis?
3. Who is at risk for developing severe toxoplasmosis and what are the preventive measures?

Research Questions

1. How do we develop an experimental model which can exactly mimic the focal toxoplasma encephalitic lesions as found in immunocompromised humans?
2. Why is there variation in response to *T. gondii* infection by the different cell types in humans? What is the reason behind there being no unified defence strategy?
3. What are the challenges in the production of efficacious vaccines against toxoplasmosis?

Further Readings

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