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Research Article

A Review Toxoplasmosis

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Abstract: Toxoplasma gondii is an intracellular protozoan infecting humans and animals. The term toxoplasmosis refers to the clinical or pathological disease caused by Toxoplasma gondii and T. gondii infection for an asymptomatic primary infection or persistence of the parasite in tissues. When considering toxoplasmosis in the differential diagnosis of a patient's illness, it is important to keep in mind that emphasis should not be placed on whether the patient has or has not been exposed to cats. Transmission of parasites essentially occurs without knowledge of the patient and may be unrelated to direct exposure to a cat (e.g., by ingestion of vegetables or water contaminated with oocysts or ingestion of undercooked meat contaminated with cysts). Serologic investigation of a cat to establish whether it is a potential source of the infection should be discouraged; the prevalence of T. gondii antibodies among cats in a given locale is usually similar to their prevalence in humans. Seropositivity in the cat does not predict shedding of oocysts. For clinical purposes, toxoplasmosis can be divided for convenience into five infection categories, including those (Abbasi *et al.*, 2003) acquired by immunocompetent patients, (Abgrall *et al.*, 2001) acquired during pregnancy, (Abgrall *et al.*, 2001) acquired congenitally; and (Afonso *et al.*, 2009) acquired by or reactivated in immunodeficient patients, and including (Afonso *et al.*, 2009; Windal, 2015) ocular infections. In any of the above mentioned categories, clinical presentations are not specific for toxoplasmosis, and a wide differential diagnosis must be considered. Furthermore, diagnostic methods and their interpretations may differ for each clinical classification.

Keywords: Toxoplasma gondii, Immunity, Protozoan, Seropositivity, immunocompetent.

INTRODUCTION

Infection with the protozoan parasite Toxoplasma gondii has a worldwide distribution. This obligate intracellular parasite can infect humans as well as virtually all warm-blooded animals, including mammals and birds. Since its first description in the gondi, a rodent from North Africa, by Nicolle and Manceaux in 1908 (Nicolle & Manceaux, 1908), the parasite was progressively recognized as the agent of a widespread zoonosis. However, its entire life cycle was definitively understood only in the late 1960s (Dubey, *et al.*, 2002; Hutchison *et al.*, 1969), with the discovery of the central role of the cat as a definitive host harboring the sexual parasitic cycle and spreading oocysts through feces. In the same period of time, it was classified in the coccidian subclass (Frenkel *et al.*, 1970), phylum Apicomplexa, and the infectivity of the three parasitic stages (tachyzoite, cyst, and oocyst) was well characterized.

The true importance of toxoplasmosis in humans remained unknown until the first reports of cases of congenital toxoplasmosis (Schwartzman *et al.*, 1948). The history of clinical toxoplasmosis and the wide spec- trum of this disease revealed over the years were reviewed by Weiss and Dubey in 2009 (Weiss & Dubey, 2009). The growing role of Toxoplasma infection in immunocompromised patients was acknowledged in the mid-1970s, and the concept of the reactivation of infection was thereafter extensively explored by immunologists. During the last decade, the development of new genotyping tools and the multi- plication of field studies have led to breakthroughs in the comprehension of the phylogenetic evolution of T. gondii in the world (Mercier *et al.*, 2011), and recent advances in our knowledge of the particular virulences associated with some genotypes have been achieved (Saeij *et al.*, 2007).This review will cover information on toxoplasmosis, epidemiology, diagnostic and parasite genotypes.

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BIOLOGY OF THE PARASITE

Three Parasitic Stages

There are three infective stages of T. gondii: a rapidly dividing invasive tachyzoite, a slowly dividing bradyzoite in tissue cysts, and an environmental stage, the sporozoite, protected inside an oocyst. These infective stages crescent-shaped cells, approximately 5 µm long and 2µm wide, with a pointed apical end and a rounded posterior end. They are limited by a complex membrane, named the pellicle, closely associated with a cytoskeleton involved in the structural integrity and motility of the cell. They possess a nucleus, a mitochondrion, a Golgi complex, ribosomes, an endoplasmic reticulum, and a multiple-membrane-bound plastid-like organelle called the apicoplast, the result of a possible acquisition by the parasite via a secondary endosymbiosis of a free-living red alga (Roos *et al.,* 1999). As for other members of the phylum Apicomplexa, they concentrate in their apical part a specialized cytoskeletal structure (the conoid, involved in cell invasion) and numerous secretory organelles (rhoptries [ROPs], dense granules, and micronemes). More details were reported elsewhere previously Henriquez, 2008; Dubey *et al.,* 1998; Weiss & Kim, 2007).

Tachyzoites are the dissemination form. They are able to invade virtually all vertebrate cell types, where they multiply in a parasitophorous vacuole. Bradyzoites result from the conversion of tachyzoites into a slow-dividing stage and form tissue cysts. These cysts are more or less spheroid in brain cells or elongated in muscular cells. They vary in size from 10 µm for the younger cysts, containing only two bradyzoites, to up to 100 µm for the older ones, containing hundreds or thousands of densely packed bradyzoites. The cyst wall consists of a limiting membrane presenting numerous invaginations and an underlying layer of electron-dense granular material (Ferguson, 2004). Bradyzoites have a latent metabolism, well adapted to long-term survival. Cysts remain intracellular throughout their life span. The death of the host cell may trigger the disruption of the cyst wall and the consequent liberation of bradyzoites. The resistance of bradyzoites to the acid pepsin (1- to 2-h survival into pepsin-HCl) allows their transmission through ingestion.

Sporozoites are located in mature oocysts. Oocysts are 12- to 13-µm ovoid structures that after sporulation contain two sporocysts, each containing four sporozoites. The oocyst wall is an extremely robust multilayer structure protecting the parasite from mechanical and chemical damages. It enables the parasite to survive for long periods, up to more than a year, in a moist environment (Mai *et al.,* 2009).

Life Cycle of T. gondii

T. gondii is a tissue-cyst-forming coccidium functioning in a prey- predator system that alternates between definitive (sexual reproduction) and intermediate (asexual replication) hosts. It is unique among this group because it can be transmitted not only between intermediate and definitive hosts (sexual cycle) but also between intermediate hosts via carnivorism (asexual cycle) or even between definitive hosts. The parts of the sexual and asexual cycles and transmission dynamics in a given environment vary according to physical characteristics and according to the structures of Sexual reproduction occurs only in felids (domestic and wild cats). After the ingestion of cysts present in tissues of an intermediate host, the cyst wall is destroyed by gastric enzymes. Bradyzoites settle within enterocytes, where they undergo a self-limiting number of asexual multiplications, characterized by the development of merozoites within schizonts (Dubey, 1998). This first step is followed by sexual development, with the formation of male and female gametes (gametogony) (Ferguson, 2002). After fertilization, oocysts formed within enterocytes are liberated by the disruption of the cell and excreted as unsporulated forms in cat feces. The process of sporogony occurs after a few days in the external environment. It implies a meiotic reduction and morphological changes leading to the formation of a sporulated oocyst with two sporocysts, each containing four haploid sporozoites. The shedding of oocysts begins 3 to 7 days after the ingestion of tissue cysts and may continue for up to 20 days. Infected cats can shed more than 100 million oocysts in their feces (Dubey & Frenkel, 1972, Jones & Dubey, 2010). They can infect a wide range of intermediate hosts, virtually all warm-blooded animals, from mammals to birds, when ingested with food or water. Oocysts are also infective for cats although less efficiently.

Within intermediate hosts, the parasite undergoes only asexual development. After oocyst ingestion, sporozoites are liberated. They penetrate the intestinal epithelium, where they differentiate into tachyzoites. Tachyzoites rapidly replicate by endodyogeny both intermediate and definitive host populations (Afonso, *et al.,* 2009). Inside any kind of cell and disseminate throughout the organism. As a result of the conversion from tachyzoite to bradyzoite, tissue cysts arise as early as 7 to 10 days postinfection and may remain throughout life in most hosts, predominantly in the brain or musculature.

Upon the ingestion of these tissue cysts by an intermediate host through raw or undercooked meat, cysts are ruptured as they pass through the digestive tract, causing the release of bradyzoites. The bradyzoites will infect the intestinal epithelium of the new host and differentiate back into the rapidly dividing tachyzoite stage for dissemination throughout the body. In addition, if the acute phase occurs during pregnancy,

the parasite can cross the placenta and infect the fetus (congenital transmission). A role for this vertical transmission in maintaining high levels of infection in some species has been suggested (Duncanson *et al.,* 2001).

Mechanism of Cell Invasion

T. gondii is remarkable in its ability to invade a wide variety of host cells. Invasion is an active process relying on parasite motility and the sequential secretion of proteins from secretory organelles, the micronemes, the rhoptries, and the dense granules.

Attachment to the host cell membrane is a prerequisite for invasion. It requires the calcium-dependent secretion of adhesins from micronemes, such as the microneme protein MIC2, which recognize host cell receptors and promote parasite reorientation and attachment. Cell invasion relies on a complex interaction between the host cell surface and the parasite, a process called gliding motility, an intricate linear motor system promoted by actinmyosin interactions and dynamic rearrangements of the parasite cytoskeleton (Carruthers & Boothroyd, 2007). Entry is a rapid process (15 to 30 s) distinct from currently known host endocytic events. Toxoplasma forms a tight association between its apical end and the host cell membrane, called the moving junction. This moving junction moves from the apical end to the posterior end of the parasite, leading to the in- ternalization of the parasite into a parasitophorous vacuole (PV). The establishment of this moving junction around the invading parasite requires the distribution over the entire surface of the parasite of an apical membrane antigen (AMA1), also secreted by micronemes, and the secretion of rhoptry (ROP) neck proteins (RONs) inserted into the host cell membrane (Dubremetz, 2007). The formation of the nascent parasitophorous vacuole membrane (PVM) requires the secretion of proteins from the ROPs. In recent years, a major role for the ROP2 family proteins has been recognized. Of these proteins, ROP18 is associated with the cytosolic face of the PVM and exerts protein kinase activity, which has a profound effect on parasite growth and virulence (El Hajj *et al.,* 2007), and ROP16 is able to manipulate host gene expression, affecting interleukin secretion (Laliberte & Carruthers, 2008).

Besides ROP proteins, dense granular proteins also contribute to the formation of the PVM during the first hour following invasion. Most host transmembrane proteins are stripped from the PVM during the invasion process; this process modifies biochemical characteristics of the PVM and prevents fusion with lysosomes or any cytoplasmic vesicle. Dense-granule secretions also support the development of a complex network of membrane tubules that develop from the PVM and extend into the vacuolar lumen (Mercier *et al.,* 2005). This network is supposed to have a role in developing exchanges between the parasite and the host cell, bringing in nutrients from the host cell cytosol or exporting proteins or lipids from the parasite toward the PVM or the host cell. The PVM is also closely associated with host cell mitochondria, which contribute to parasite metabolism. Within the PV, tachyzoites divide during a 6- to 9-h cycle, by a process of endodyogeny, leading to the formation of two daughter cells within each mother cell. They exit the cell usually after 64 to 128 parasites have accumulated in the PV (Black & Boothroyd, 2000). Egress from the cell is an active process dependent upon a rise in the calcium concentration after the release from intracellular stores (Sibley, 2010).

PREVALENCE AND IMPORTANCE OF TOXOPLASMOSIS IN THE WORLD

Prevalence of Infection in Humans

It is generally assumed that approximately 25 to 30% of the world's human population is infected by Toxoplasma (Montoya & Liesenfeld, 2004). Actu- ally, the prevalences vary widely between countries (from 10 to 80%) and often within a given country or between different com- munities in the same region (Pappas *et al.,* 2009). Low seroprevalences (10 to 30%) have been observed in North America, in South East Asia, in Northern Europe, and in Sahelian countries of Africa. Moderate prevalences (30 to 50%) have been found in countries of Central and Southern Europe, and high prevalences have been found Latin America and in tropical African countries.

As for animals, many factors can affect seroprevalence in humans. Climatic factors affecting the survival of oocysts in the environment and, hence, infection rates in meat-producing animals play a major role. Higher prevalences are classically observed for tropical countries with a humid and warm climate, and conversely, lower prevalences are found for arid countries or for colder countries, but anthropogenic factors explain a large part of the variations in human seroprevalence, including dietary habits (method of cooking meat, hand washing, kinds of meat or vege- tables consumed, and vegetable cleaning, etc.); economic, social, or cultural habits; quality of water; and sanitation coverage. Seroprevalence increases with age, but the rate of acquisition of infec- tion in relation to age varies according to the country and socio-economic level. Near-maximal seroprevalence may be reached in childhood in populations living under poor-hygiene conditions, probably linked to telluric or waterborne contamination by oocyst ingestion. This points toward water as an important source of human infection in areas where humans use unfiltered surface water for consumption and probably also in areas where there is contact with freshwater, for instance, for recreation (Ertug *et al.,* 2005; Jones & Dubey, 2010). As an example, in a city located in the northern Rio de Janeiro state (Brazil), the age-adjusted seroprevalence was 84% for the group of the lower socioeconomic level, compared to seroprevalences of 62% and 23% for

the groups of the middle and upper socioeconomic levels, respectively (Bahia-Oliveira *et al.*, 2003). Most persons (up to 84%) in the population of the lower socioeconomic level were infected by the age of 15 years, whereas infection was acquired mostly after the age of 20 years in the population of the upper socioeconomic level (from about 20% for the age group of 20 to 29 years to 70% for the age group of 40 to 49 years). In a multivariate risk factor analysis, this was attributed to differences in water supply, with the poorest populations living in areas supplied with unfiltered water. These different patterns of Toxoplasma acquisition according to socioeconomic levels may be more relevant in underdeveloped tropical countries, but in the United States, Toxoplasma infection was also considered an infection associated with poverty (Hotez, 2008). The overall seroprevalence (U.S. and foreign-born individuals combined) was higher among non-Hispanic black persons and Mexican Americans than among non-Hispanic white persons (Jones *et al.*, 2007). Logically, increased socioeconomic levels, together with an improvement of hygienic conditions, changes in farming systems, the consumption of frozen meat, and the feeding of cats with sterilized food, have led to a continuous decrease of the seroprevalence in most industrialized countries over the last decades. In the United States, a national survey found a decrease in the age-adjusted T. gondii prevalence in U.S.-born persons aged 12 to 49 years, from 14.1% in 1988 to 1994 to 9% in 1999 to 2004 (Jones *et al.*, 2007). In France, the seroprevalences in pregnant women were about 80% in the early 1960s, around 66% in the 1980s, 54% in 1995, and 44% in 2003, while at the same time, the average age of pregnant women in- creased (Villena *et al.*, 2010). This declining seroprevalence has been observed in all areas where it was studied in Europe. For example, in The Netherlands, the seroprevalence decreased from 35.2% in 1995 to 1996 to 18.5% in 2006 to 2007 in women of reproductive age (Hofhuis *et al.*, 2011).

HOW DO HUMANS BECOME INFECTED?

The majority of horizontal transmissions to humans is caused either by the ingestion of tissue cysts in infected meat or by the ingestion of soil, water, or food contaminated with sporulated oocysts derived from the environment or, less frequently, directly from feline feces. The relative importance of transmissions via tissue cysts versus oocysts in a given population is unknown, except in the case of outbreaks with a well-defined source of infection. Until now, only risk factor studies gave an indication of the predominant route of transmission in a given population. However, in these epidemiological studies, risk factors for infection remained unexplained in 14 to 49% of cases (Cook *et al.*, 2000, Jones, *et al.*, 2009). Persons may be unaware of their exposure or may have difficulty recalling specific risks that occurred. The recent discovery of a sporozoite or oocyst-specific protein, which elicited antibody production and differentiated oocyst- versus tissue cyst-induced experimental infection in pigs and mice, may help to solve this problem (Hill *et al.*, 2011). Serum antibodies to the sporozoite protein were detected in humans within 6 to 8 months of an initial oocyst-acquired infection. Therefore, this serological assay could be useful for detecting exposure to oocysts in the early months after T. gondii infection and could be useful for epidemiological studies.

Infection through Cysts

Consumption of meat (i) Type of meat. Any meat from warm- blooded animals and birds has been traditionally considered a major source of Toxoplasma infection in Western countries. The risk associated with the type of meat (lamb, pork, and beef, etc.) varies among different countries according to local eating habits and according to the prevalence in meat-producing animals. In a multicenter study in Europe, meat consumption was estimated to be responsible for 30 to 63% of cases of infection, while soil contact represented 6 to 17% of cases (Cook *et al.*, 2000). In the United States, a recent case-control study showed an elevated risk for T. gondii infection in persons eating raw ground beef (adjusted odds ratio [aOR], 6.67; attributable risk [AR], 7%); eating rare lamb (aOR, 8.39; AR, 20%); eating locally produced cured, dried, or smoked meat (aOR, 1.97; AR, 22%); or working with meat (aOR, 3.15; AR, 5%) (Jones, *et al.*, 2009). Outbreaks due to the consumption of undercooked meat have been described. These outbreaks generally involved only a few patients (2 to 20 persons) (Windal, 2015).

A quantitative assessment of the risk of Toxoplasma in food for consumers is hampered by the lack of data on the number of tissue cysts resulting in infection of humans, the distribution and the number of cysts in the different muscle sites in various hosts, as well as their infectivity in commercial meat products. One recent survey of meat from commercial markets (pork, chicken, and beef) in the United States suggested a low risk, perhaps owing to meat treatment processes, which could reduce the viability of cysts (Dubey *et al.*, 2005). This may not be the case in countries where lamb and sheep are the most consumed meats (Berger *et al.*, 2009, 162).

(ii) Cyst resistance. Tissue cysts remain infectious in refrigerated carcasses (1°C to 6°C) or minced meat for up to 3 weeks. Freezing alone is not a reliable means of rendering all tissue cysts noninfective: cysts have remained viable for >11 days at —7°C. However, the deep-freezing of meat at —12°C or lower for at least 3 days is usually efficacious to kill cysts, although it may depend on the thickness of the piece of meat (Dubey, 1988).

Tissue cysts are usually killed immediately by heating to 67°C. The survival of tissue cysts at lower

temperatures depends on the duration of cooking. Tissue cysts remain viable at 60°C for about 4 min and at 50°C for about 10 min (Dubey *et al.,* 1990). Cooking for a prolonged period of time may be necessary un- der household conditions to achieve the temperatures that are required to kill all tissue cysts of Toxoplasma in all parts of the meat. Some tissue cysts will remain infectious after cooking in a microwave oven, possibly due to an uneven heating of the meat. However, in a U.S. case-control study (Jones *et al.,* 2009), microwave cooking of meat was associated with a reduced risk of recent T. gondii infection. This was explained by the fact that microwave cooking is often associated with reheating already-cooked meat or with defrosting or cooking frozen meat.

Commercial procedures of curing with salt, sucrose, or low-temperature smoking may kill tissue cysts, but the survival time of tissue cysts varies greatly with the concentration of the salt solution and the temperature of storage. Salting does not necessarily kill tissue cysts in homemade pork sausages. Under laboratory conditions, solutions containing 2% sodium chloride or 1.4% potassium or sodium lactate are effective within 8 h of injection for the killing of T. gondii tissue cysts in pork loin (Hill *et al.,* 2006). Other food treatment processes, such as gamma irradiation at a dose of 1.0 kGy and high pressure (300 mPa), were found to be efficient for killing tissue cysts in meat, but some treatment procedures are barely applicable for meat destined for human consumption (Lindsay *et al.,* 2006).

Infection related to solid-organ transplantation. As T. gondii tachyzoites can invade all nucleated cells, cysts can be found in virtually any organ. Therefore, in solid-organ transplantation (SOT), Toxoplasma infection can be transmitted through a cyst-containing organ from a donor (D) with infection acquired in the distant past to a nonimmunized recipient (R). However, certain organs are more likely to harbor persistent cysts than others. Muscles commonly sustain parasite encyst- ment; thus, heart transplant patients are at a higher risk for organ-related toxoplasmosis than are liver, lung, or kidney transplant patients. Toxoplasmosis was recognized early as an infectious complication in heart transplant patients (Ryning *et.al.,* 1979), which motivated the implementation of large retrospective studies in several countries from 1980 onwards. However, the incidence of acquired toxoplasmosis in case of a mismatch (D+/R—) is variable, since it depends largely on the prevalence of toxoplasmosis in the country of study and on the use of chemoprophylaxis after transplantation. In retrospective studies, the incidence can vary from 9 to 56% when the patients benefit or not from a chemoprophylaxis scheme, respectively, indicating that prevention is efficient. In a recent multicenter retrospective study including 22 patients with acquired toxoplasmosis within a median time of 92 days post transplantation, mismatched transplants were documented for 9 patients, and the donor's serology was unknown for 8 other negative recipients (Fernandez-Sabe N, *et al.,* 2011). Twelve of 22 cases were heart transplant patients. The incidence of donor acquired toxoplasmosis is less frequent in other SOT patients, and only 9 and 16 cases were reported for liver and kidney mismatched patients, respectively, supported by solid serologic evidence. A case of disseminated toxoplasmosis following small bowel transplantation was also described, but the serostatus of the donor was unknown, making the source of infection uncertain (Campbell *et al.,* 2006).

Infection through Oocysts Survival of oocysts in the environment. As highlighted by previous epidemiological studies, environmental conditions are important for oocyst survival. Moist conditions can increase oocyst survival during long periods of heat, which likely accounts for the high prevalences in tropical countries of South America and Africa. In Colombia, a correlation was found between the mean amount of rainfall and the incidence of congenital toxoplasmosis (Gomez-Marin *et al.,* 2011). Even in a country with a temperate climate, such as France, the risk of infection in cats was shown to increase when the weather was both warm and moist, or moderate and less moist, reflecting the influence of climatic conditions on the prey population and oocyst survival (Abgrall *et al.,* 2001).

Despite the low prevalence (<1% in most studies) and short duration of oocyst shedding by cats, the burden in the environment may be very high (Dabritz *et al.,* 2007). A single cat may shed more than 100 million oocysts, which are nonsporulated. These oocysts need between 1 and 5 days to mature and become infective for other hosts, which explains why direct contact with cats is not thought to be a major risk for human infection. In the United States, an increased risk associated with exposure to kittens was limited to respondents who had 3 or more kittens, thus more likely to be infected through the shedding of oocysts after primary infection (Jones *et al.,* 2009). Oocysts are able to sporulate within 2 to 3 days in different types of commercial cat litter and occasionally remain viable for 14 days (Dubey *et al.,* 2011). Unsporulated oocysts lose their capacity to sporulate, and, hence, to become infective, after freezing at —6°C during 7 days or after exposure to 37°C for 1 day. Once sporulated, oocysts are resistant to harsh environmental conditions. They remain viable in a moist environment for more than a year. Under laboratory conditions, sporulated oocysts can survive storage at 4°C for up to 54 months. They survive freezing at —10°C for 106 days and heating at 35°C and 40°C for 32 days and 9 days, respectively. How- ever, they are killed within 1 to 2 min by heating to 55°C to 60°C (Dubey, 2010), conditions easily obtained when cooking vegetables. The wall of sporulated oocysts is highly impermeable and, there-

fore, very resistant to disinfectants (Dumetre & Darde, 2003).

Contamination of water. Oocysts can remain viable for long periods of time in water and can resist freezing and moderately high water temperatures. They are not killed by chemical and physical treatments currently applied in water treatment plants, including chlorination and ozone treatment (Dumetre *et al.,* 2008). Outbreaks associated with the contamination of reservoirs supplying water, such as those described for the Greater Victoria area of British Columbia, Canada (Aramini *et al.,* 1999); in Santa Isabel do Ivai, Brazil (De Moura *et al.,* 2006); or in Coimbatore, India (Balasundaram *et al.,* 2010), involved a large number of patients. The epidemics were preceded by peaks of heavy rainfall and turbidity in the implicated reservoirs. Smaller epidemics were described after the drinking of raw surface water in remote tropical areas (Benenson *et al.,* 1982; Demar *et al.,* 2007). Freshwater runoff from urban centers next to seashores may contaminate seawater. Toxoplasma oocysts can remain viable for extended periods of time in seawater (Lindsay & Dubey, 2009). Shellfish are filter feeders that concentrate T. gondii. Oocysts remained viable and were detected in various species of shellfish under natural conditions (Esmerini *et al.,* 2010; Miller *et al.,* 2008; Putignani *et al.,* 2011). The consumption of oysters, clams, and mussels has been shown to be a risk factor for acquiring Toxoplasma infection in the United States (Jones *et al.,* 2009).

The detection of Toxoplasma in water is difficult, and no standardized methods are available. The methodology is based on the experience gained from other coccidians, such as Cryptospo- ridium, and involves the concentration of oocysts using centrifugation, filtration, immunomagnetic separation, or flocculation of large volumes of water (Dumetre & Darde, 2007; Shapiro *et al.,* 2010). Different PCR methods have been proposed (Sotiriadou & Karanis, 2008). In France, a survey found Toxoplasma DNA in 7% of raw surface water samples. Well water was PCR positive in 9% of samples in this French study (Villena *et al.,* 2004) and also in 13 to 27% of samples in Poland, depending on the depth of the well (Sroka *et al.,* 2010). A positive correlation was observed between the consumption of unboiled well water and the presence of Toxoplasma antibodies, especially for farms with poor-hygiene conditions sur- rounding shallow wells.

Contamination of soil, vegetables, and fruits. Contact with soil was identified as a strong risk factor in a European multicenter case-control study, and 6 to 17% of primary infections in humans were attributed to this risk factor (Cook *et al.,* 2000). A.U.S. study showed that the detection of antibodies against Toxoplasma was 2fold higher in a population with positive Toxocara antibodies, suggesting a common exposure to contaminated soil (Jones *et al.,* 2008). The risk of acquiring Toxoplasma infection after soil contact or ingestion is particularly high for children. Toxoplasma oocysts were isolated in as many as 32% of school play grounds in a Brazilian study (Dos Santos *et al.,* 2010).

Contaminated water and soil may act as vehicles for the transfer of oocysts to vegetables and fruit for human consumption, although there are few data available to confirm this. In several risk factor or case-control studies, the eating of unwashed raw vegetables or fruits was associated with an increased risk of primary infection (Berger *et al.,* 2009; Kapperud *et al.,* 1996; Liu *et al.,* 2009). Experimentally, T. gondii oocysts can adhere to berries, especially raspberries, and can be recovered by bioassays in mice (Kniel *et al.,* 2002), but there has been no report of the detection of Toxoplasma on fruits or vegetables under nonexperimental conditions.

Infection through Tachyzoites

Food-borne contamination. Outside its host cell, the tachyzoite is a fragile stage, easily destroyed by digestive enzymes (10-min survival in pepsin-HCl). It is also very sensitive to environmental conditions and is usually killed rapidly outside the host. Therefore, the horizontal transmission of Toxoplasma via tachyzoites is probably not important from an epidemiological point of view. However, tachyzoites were suggested to be the cause of rare cases of acquired toxoplasmosis in humans after the consumption of unpasteurized goat's milk (Tenter *et al.,* 2000). The drinking of unpasteurized goat's milk was found to be a risk factor in an epidemiological survey (Jones *et al.,* 2009), suggesting that tachyzoites may enter the host by the penetration of mucosal tissue.

Congenital infection. When primary infection is acquired by a pregnant woman, tachyzoites can colonize placental tissues during the dissemination process and from there can gain access to the fetal compartment in about 30% of cases. The frequency of vertical transmission increases with the gestational age at maternal infection. At the beginning of pregnancy, the transplacental pas- sage of tachyzoites is a rare event, but the consequences for the offspring are heavy. The immune control of placental infection is probably a key event in the occurrence of congenital infection (Pfaff *et al.,* 2007), but advances in the comprehension of the pathophysiological process remain to be achieved. Congenital infection is the most important part of the disease burden due to Toxoplasma infection in humans. Clinical manifestations of congenital toxoplasmosis first motivated research on the parasite and its pathophysiology and epidemiology. However, the factors influencing congenital transmission are still poorly known, apart from the term of pregnancy at the time of maternal infection and, of course, the immune status of the mother.

The observation of a decreasing seroprevalence of toxoplasmosis in industrialized countries has complex consequences for the risk of acquisition of Toxoplasma infection during pregnancy. At first glance, a reduced seroprevalence increases the percentage of pregnant women susceptible to primary infection and, hence, to congenital transmission to their fetuses. However, the lower level of circulation of the parasite in the environment diminishes the global risk of acquiring infection during pregnancy. A national surveillance system was implemented in France in 2007, which aims to collect data from all cases of congenital toxoplasmosis through data transmitted by laboratories certified for prenatal diagnosis or implicated in neonatal serological diagnoses. This net- work reported 272 cases of congenital toxoplasmosis in 2007 (Villena *et al.,* 2010). If these data can be considered exhaustive, they can allow an evaluation of the overall prevalence of congenital toxoplasmosis in France, 3.3 per 10,000 live births (Villena *et al.,* 2010), which is nearly the prevalence reported in Brazil (1 per 3,000 live births) (Neto *et al.,* 2000) but 3-fold higher than that estimated in a pilot study in Massachusetts (1 per 10,000 live births) (Guerina *et al.,* 1994).

Transmission through injection. Fourteen cases of laboratory contamination of a parenteral origin have been described (Herwaldt, 2001). Most cases were attributed to needlestick injuries or scratching while manipulating tachyzoites from the RH strain.

The risk of transmitting infection through a blood transfusion is theoretically possible if the donor has recently acquired a Toxoplasma infection and is parasitemic at the time of blood sampling. Similarly, a risk associated with bone marrow is possible if the donor is parasitemic at the time of collection. However, the maximal duration of dissemination of tachyzoites through the blood flow is barely known for humans; it may depend on the parasite strain and on the host immune response. Parasite DNA was detected in 9 out of 17 patients during 5 weeks following acute toxoplasmosis with lymphadenopathy (Guy & Joynson, 1995). In a mouse model, it was also shown by PCR that parasitemia was detected during 3 weeks after oral infection (Paugam *et al.,* 1995).

POPULATION STRUCTURE OF T. GONDII

Genotypes and Their Geographic Distribution

Studies of genotypes of T. gondii began in the early 1990s and at first relied on isoenzyme analysis (Darde *et al.,* 1992, Darde *et al.,* 1988) and on a few PCR- restriction fragment length polymorphism (RFLP) markers (Howe & Sibley, 1995). Genotyping was later refined by the addition of new PCR-RFLP markers (Zhang & Dubey, 2006) and by microsatellite analysis (Ajzenberg *et al.,* 2010, Ajzenberg *et al.,* 2005). The sequencing of selectively neutral introns was proposed to be a better tool for phylogenetic studies (Khan *et al.,* 2007), whereas microsatellites are better adapted to population genetic structure and outbreak investigations (Demar *et al.,* 2007; Mercier *et al.,* 2010; Weiss & Dubey, 2009).

Despite the presence of a sexual cycle and a worldwide distribution, the population structure of this parasite was initially described as being highly clonal and exhibiting a low genetic diver- sity. This was the conclusion of genetic studies of isolates from Europe and the United States, which grouped these isolates into three major multilocus genotypes, types I, II, and III, equivalent to clonal lineages, stable in time and space (Ajzenberg *et al.,* 2002; Darde *et al.,* 1992; Darde *et al.,* 1988; Howe & Sibley, 1995; Sibley & Boothroyd, 1992). This simple clonal structure is accompanied by a low level of genetic divergence among the three lineages. However, multilocus and multichromosome genotyping of isolates from other continents revealed a much more complex population structure with a greater genetic diversity, likely reflecting a history of more frequent genetic exchanges and genetic drift (Ajzenberg *et al.,* 2004; Lehmann, et *al.,* 2004). The majority of isolates from South America, Africa, or Asia do not fit into the three major lineages. The clustering of these genotypes led to the description of new haplogroups, some of them largely distributed over continents, being considered other successful clonal lineages (Mercier *et al.,* 2010 ; Pena *et al.,* 2008). Ongoing efforts are aimed at gathering data from analyses with different markers (PCR-RFLP, microsatellites, and sequencing of introns) to establish a consensus nomenclature for these haplogroups, which may be useful for basic biology as well as for clinical studies. Up to now, 12 haplogroups (including the 3 initially described lineages, types I, II, and III) have been described (Khan *et al.,* 2011; Khan *et al.,* 2007), based on sequence-based analyses, but these haplogroups are not totally homogenous, and more resolutive markers revealed sub clustering that may be associated with geographical origins and phenotypic characteristics. There still remain truly atypical and highly diverse isolates with many unique polymorphisms which cannot be clustered into one of these haplogroups (Mercier *et al.,* 2011).

From Northern Europe (Jokelainen *et al.,* 2011) to Southern Europe (De Sousa *et al.,* 2006), the population structure of T. gondii is markedly clonal, with a pre- dominance of strains belonging to the type II lineage. In France, type II strains represent more than 90% of isolates from both humans and animals (Ajzenberg *et al.,* 2002; Aubert *et al.,* 2010, Halos *et al.,* 2010). Two other clonal lineages are occasionally (type III) or exceptionally (type I) found in Europe. Type III may be more frequently encountered in Southern Europe (Waap *et al.,* 2008). The isolation of atypical strains which do not fit into these 3 major lineages is rare in Europe and likely suggests contamination by non-European strains either during residence abroad or after the consumption of imported

food (Dardé *et al.,* 2007; Pomares *et al.,* 2011). In North America, the population structure appeared similar to that observed in Europe, with a predominance of type II strains (Howe & Sibley, 1995), but recent data suggest a higher prevalence of atypical strains in North America in wild as well as in domestic animals (Dubey *et al.,* 2008) and another clonal haplogroup (haplogroup 12) close to type II (Khan *et al.,* 2011). South America is an area with a high level of diversity for T. gondii. Although additional clonal lineages, known as the Br I to IV haplogroups, may be common and endemic in Brazil, it is clear that frequent genetic exchanges have generated a wide variety of different genotypes (Pena *et al.,* 2008). Eighty-eight genotypes (defined with 11 PCR-RFLP markers) have already been identified from a variety of animal hosts in Brazil, and new genotypes are continuously being identified in different animal species, indicating an extremely high level of diversity of T. gondii in the population (Pena *et al.,* 2011), whereas type II seems to be very rare in South America (Dubey *et al.,* 2006). The high level of genetic diversity observed in this continent is maxi- mal in the wild Amazonian area, with many unique polymorphisms (Ajzenberg *et al.,* 2004). In an Amazonian country such as French Guiana, the interpenetration of anthropized and wild rainforest environments leads to hybridization between strains that may represent a potential risk for human health (Mercier *et al.,* 2011). In Africa, a clonal population structure consisting of additional common clonal lineages known as the Africa 1 to 3 haplogroups, coexisting with type II and III lineages, has been described (Velmurugan *et al.,* 2008). In Asia, the first re- ports from China, Sri Lanka, and Vietnam (Dubey *et al.,* 2007) revealed a more limited genetic diversity than in South America, with some genotypes being common to both areas. In China, a clonal lineage seems to be widespread across the country (Zhou *et al.,* 2010).

Genotypes and Virulence

Experimental virulence is usually defined with the mouse model after the intraperitoneal inoculation of a given number of tachyzoites. Type I isolates are highly virulent, leading to the death of mice less than 10 days after the inoculation of <10 tachyzoites, while type II or III strains are considered avirulent strains, allowing survival after the inoculation of >103 tachyzoites. Isolates from other clonal lineages or from atypical strains range from the highly virulent to the intermediate or non-virulent phenotype, according to differences in the combination of genes that they have inherited (Darde, 2008; Grigg & Suzuki, 2003). Genotypes with a majority of type I alleles are usually more virulent (Mercier *et al.,* 2010).

The mouse-virulent strains display several characteristics that may explain the rapid dissemination of the parasite and the higher tissue burden observed for mice and other susceptible hosts: enhanced migration across polarized epithelia or across the extracellular matrix, higher rates of the ex vivo penetration of the lamina propria and submucosa (Barragan & Sibley; 2002), and, in cell culture, higher growth rates and lower rates of interconversion from tachyzoites to bradyzoites (Saeij *et al.,* 2005). Experimental crosses between strains with different virulence patterns facilitated the identification of several poly morphic genes coding for secreted factors of Toxoplasma associated with differences in the expression of virulence in mice (Reese *et al.,* 2011; Saeij *et al.,* 2006, Taylor *et al.,* 2006). These key virulence factors are secretory proteins discharged from apical organelles, the rhoptries. The proteins of this rhoptry family (ROP5, ROP16, and ROP18) exert kinase or pseudokinase activity. They are injected directly into the host cell and play a role during the process of parasite invasion or in the induction of interleukin-12 (IL-12) secretion by mouse macrophages (Robben *et al.,* 2004). Although these biological and genetic data demonstrate the different intrinsic properties of the different strains, the expression of this virulence in a given host species is a more complex trait which depends on several host and parasite characteristics. Different host species are more or less susceptible. The genetic background of a given species, as demonstrated for different mouse or rat strains, may also influence the expression of virulence (Cavailles *et al.,* 2006).

The expression of virulence in humans is a complex phenomenon due to many other factors that could influence the pathogenicity of a given strain: other parasitic factors (infectious stage and inoculum), the genetic background of the host, and overall immune status (Maubon *et al.,* 2008). Toxoplasma gondii is usually considered an opportunistic parasite in humans, and any analysis of the relationship between genotype and pathogenicity should consider these different factors. This explains why the role of the strain is still a matter of debate, especially when the host is immunocompromised. Strains isolated from patients are mainly the strains circulating in a given country, and the same type of strain can be responsible for different outcomes. For example, type II strains were involved in 96% of consecutive cases of congenital toxoplasmosis in France (Ajzenberg *et al.,* 2002), in 85% of immunocompromised patients who acquired Toxoplasma infection in Europe (Dardé *et al.,* 2007), and in 73% of cases of ocular disease in France (Fekkar *et al.,* 2011). In immunocompromised patients, the conclusion of a study of 85 patients (HIV and non-HIV immunodeficient patients) was that the genotype of the infecting strain had no influence on the clinical manifestation (cerebral or extracerebral) or clinical outcome (Dardé *et al.,* 2007), indicating that immune status is responsible for virulence expression in these patients.

However, several direct and indirect arguments plead for an influence of the strain on clinical severity. In immunocompetent patients, severe toxoplasmoses

with multiorgan failure were linked to atypical strains acquired from the Amazonian rainforest (Carme *et al.,* 2002). Occasional reports of such severe cases due to atypical strains have come from other countries (De Salvador-Guillouet *et al.,* 2006), sometimes after the consumption of infected food (Pomares *et al.,* 2011). The high rate of occurrence of acquired ocular toxoplasmosis in Southern Brazil (21% in individuals over 13 years of age) has been attributed to the genotypes circulating in this region (De-la-Torre *et al.,* 2007; Khan *et al.,* 2006). In cases of congenital toxoplasmosis, the strain was likely to play a role in the different outcomes observed by a comparative prospective cohort study of congenitally infected children in Brazil and Europe (Gilbert *et al.,* 2008). In France, where systematic diagnoses of congenital toxoplasmosis were performed, type II isolates were found in all different aspects of congenital disease, from lethal infection to latent toxoplasmosis, classically depending on the term of pregnancy during which the infection was acquired. On the other hand, the few atypical isolates detected in this country were observed only for severe cases of congenital toxoplasmosis (De-la-Torre *et al.,* 2010). The possibility of reinfection by a different strain is another consequence of this genetic diversity, raising the new concept that immunity against one strain may not be completely protective against another one, as shown for a case of reinfection with an atypical strain leading to severe congenital toxoplasmosis (Elbez-Rubinstein *et al.,* 2009).

CLINICAL FEATURES OF TOXOPLASMOSIS IN HUMANS

Pathogeny and development of the immune response during the course of infection following the ingestion of cysts or oocysts, the respective excysted forms, bradyzoites or sporozoites, rapidly invade the small intestinal epithelium, where they convert into tachyzoites. The acute early steps of intestinal infection of humans are not well characterized, but the establishment of infection probably relies on the intrinsic properties of the parasites. First, the high motility of tachyzoites and cell interactions between the parasite protein MIC2 and the host intercellular adhesion molecules (ICAM-1) could be used for paracellular crossing. Moreover, the active invasion of the apical side of the epithelial cell could be followed by egress from the basolateral side (transcellular traversal) (Lambert & Barragan, 2010). Whatever the early scenario comprising or not an initial multiplication of tachyzoites in the intestinal epithelium, they further cross the intestinal barrier and invade monocyte cells in contact with the lamina propria, which are key cells for the dissemination of Toxoplasma through the blood flow toward all organs, using them as Trojan horses to cross biological barriers (Barragan & Hitziger, 2008; Bierly *et al.,* 2008), as shown with a murine model of infection by intracellular fluorescent parasites (Unno *et al.,* 2008). This peculiar capacity to actively invade all nucleated cells, including professional phagocytic cells, contributes to the complexity of the host-parasite interactions through the direct modulation of the host immune response.

The cellular and soluble effectors involved in the immune response against T. gondii have been extensively studied in the last 2 decades. It was recognized early that a T helper 1 (Th-1) immune response driven by gamma interferon (IFN-µ) and interleukin-12 (IL-12)-producing cells is essential for the control of the parasite burden. The fine regulation of immune effectors and their signaling pathways were reviewed recently by Miller et al. (Miller *et al.,* 2009). Briefly, following the ingestion and transepithelial transfer of parasites, there is a local release of chemokines by infected cells, leading to the attraction of cells of the innate immunity. Neutrophils are attracted to the infected foci early to phagocytose free parasites and contribute to reducing parasite burdens (Bliss *et al.,* 2001). Other phago- cytic cells, such as dendritic cells (DCs) and macrophages, play a pivotal role in the initiation of innate immunity, as they are the major sources of IL-12 as well as IL-18, thus promoting natural killer (NK) and NKT cell activation (Iwasaki *et al.,* 2004), with both cell types producing IFN-µ in large quantities (French *et al.,* 2006). Moreover, DCs and macrophage cells can present parasite antigens associated with major histocompatibility complex (MHC) class II antigens and costimulatory molecules and further prime T cells (Combe *et al.,* 2006). In addition, DCs and NK cells can also interact directly, with this dialog resulting in the mutual activation and amplification of IL-12 and IFN-µ synthesis, respectively. Classically, the release of IFN-µ can trigger macrophage activation to synthesize tumor necrosis factor alpha (TNF-a), thus being responsible for an amplification loop. The further recognition of parasite antigens by pattern recognition receptors (PRRs) leads to an exacerbation of phagocytic activity with an enhanced production of reactive oxygen species (ROS) and nitric oxide (NO) species and tryptophan starvation through 2-3-indole-amine dioxygenase (IDO) activation (Pfefferkorn, 1984).

However, this potent machinery has two limitations. The first limitation resides in the negative counterpart of a strong Th-1 immune response, which may overwhelm its goal and be respon- sible for severe inflammation, resulting in intestinal tissue damage or even the death of the susceptible host, as shown with a murine C57BL/6 model (Liesenfeld, 2002). Thus, there is a need for down regulating effectors, a role devoted at least partially to IL-10 and transforming growth factor þ (TGF-þ), which modulate macrophage activation (Mosser, 2003). Such a deleterious effect of an acute Th-1 immune response is also well known in the setting of primary acquired infection during pregnancy and can result in fetal loss, since IFN-µ destabilizes the Th-2 microenvironment necessary for maternal- fetal tolerance. Thus, the complexity of the

maternal-fetal interface is magnified by Toxoplasma infection, and the role of the placenta in the immunomodulation process is probably essential for the maintenance of gestation after maternal infection (Pfaff *et al.,* 2007; Robert-Gangneux *et al.,* 2011).

On the other hand, despite the powerful host cell effectors described above, recent data provided mechanistic details on how Toxoplasma surrounds the host immune system, which makes it a successful parasite persisting lifelong in host tissues. It is now recognized that the parasite rhoptry protein ROP16 can rapidly process into the host cell nucleus, where it interferes with signaling pathways of host immune responses, particularly through the phosphorylation of the STAT3 and STAT6 transcription factors (Saeij *et al.,* 2007), leading to the downregulation of IL-12 production by macrophages and, subsequently, of IFN-µ (Denkers *et al.,* 2004). Interestingly, this capacity is not shared by all strains but is devoted to type I and III isolates (Saeij *et al.,* 2006). This could partially explain the greater severity usually observed for infections due to strains harboring type I alleles. Moreover, type II strains, which do not exert this capacity to repress the host response, induce a rapid immune response, limiting parasite growth, thereby ensuring the survival of both the host and parasite and resulting in bradyzoite conversion and the encystment of the parasite for persistence. At the same time, it was shown that Toxoplasma can also inhibit apoptotic mechanisms of the infected cell by antagonizing caspase 8 (Villena *et al.,* 2010) and interfering with the NF-nB pathway (Laliberte & Carruthers, 2008), thus ensuring both protection against the rapid clearance of intracellular tachyzoites by macrophages and the long-term survival of bradyzoites in the cysts. In the immunocompetent host, the occasional rupture of individual cysts is considered to be responsible for the continuous stimulation of the immune response, which ensures a dynamic control of the cysts.

Toxoplasmosis in Immunocompetent Subjects

Primary acquired infection is asymptomatic in more than 80% of cases of immunocompetent subjects in European countries or North America (Montoya & Liesenfeld, 2004). In the remaining cases, patients may experience fever or cervical lymphadenopathy, sometimes associated with myalgia, asthenia, or other nonspecific clinical signs. Lymphadenopathy and asthenia may persist for several weeks, mimicking infectious mononucleosis, especially since monocytosis can be observed on blood smears. A study conducted in the United States showed that only 48% of mothers who gave birth to congenitally infected infants could recall clinical signs suitable with toxoplasmosis during their pregnancy (Boyer *et al.,* 2005). More rarely but not exceptionally, toxoplasmic chorioretinitis with visual impairment may reveal primary infection (Montoya & Remington, 1996), although it was previously thought that ocular toxoplasmosis was the result of congenital infection. Indeed, in a retrospective study by Delair et al., 100 out of 425 (23.5%) consecutive cases of ocular toxoplasmosis were attributed to acquired toxoplasmosis (Delair *et al.,* 2008).

In fact, it is now recognized that the severity of infection may depend on the genotype of the strain. Indeed, as stated above, the severity of infection is low in Western European countries and North America, where type II strains predominate (Howe & Sibley, 1995), but much higher in other parts of the world, such as South America (Demar *et al.,* 2007) or Africa, where other genotypes circulate (Khan *et al.,* 2006; Mercier *et al.,* 2010; Vallochi *et al.,* 2005). In particular, several studies have shown higher incidences and severities of chorioretinitis in Brazil (Gilbert *et al.,* 2008) or Colombia, both dur- ing primary infections of immunocompetent subjects and in con- genitally infected infants. The strain genotype could also be an explanation for the high proportion of retinochoroiditis (19 of 100 cases with proven acute infection) in an outbreak in Victoria, British Columbia, Canada, where an atypical cougar isolate was suspected to be the cause (Bowie *et al.,* 1997), and for the 100-fold-higher incidence of ocular toxoplasmosis in patients born in Africa than in patients born in Britain (Gilbert *et al.,* 1995). Moreover, in the British Columbia outbreak, 20% of patients had recurrent episodes of retinochoroiditis (Burnett *et al.,* 1998). Such strains with atypical genotypes can also be responsible for severe or lethal infections in immunocompetent subjects, which may take the form of pneumonitis, myocarditis, meningoenceph- alitis, or polymyositis. Data collected through the Resource Biological Center of the Centre National de Référence de la Toxoplasmose (Limoges, France) showed that among the few severe or lethal infections that occurred from 2007 to 2010 in France, 7 of 10 were related to atypical genotypes acquired in French Guiana (Carme *et al.,* 2002).

Toxoplasmosis in Immunocompromised Patients

Contrasting with the setting of Toxoplasma infection in immunocompetent subjects, toxoplasmosis is always life threatening in immunocompromised patients, whatever the strain, yet the host immune background is of prime importance. Various factors responsible for profoundly impaired cellular immunity can lead to severe toxoplasmosis, among which are HIV infection and immunosuppressive therapies. Patients are more commonly at risk for disease reactivation resulting from cyst rupture than for a newly acquired infection, but the risk may differ among categories of patients. In transplant patients, severe or disseminated toxoplasmosis can result from either a reactivation of latent infection in the recipient or infection from a cyst-containing organ from a seropositive donor given to a seronegative recipient (Botterel *et al.,* 2002; Martina *et al.,* 2011; Rogers *et al.,* 2008), a situation where heart transplants carry the highest risk (Gallino *et al.* , 1996 ; Sanchez *et al.,* 2011). A reactivation of a chronic

infection may occur in the recipient irrespective of the type of graft, but the risk is closely related to the duration and degree of immunosuppression, with hematopoietic stem cell transplant (HSCT) patients being most at risk (Derouin *et al.*, 1992; Derouin & Pelloux, 2008; Roemer *et al.*, 2001). In HIV-infected patients, the incidence of toxoplasmosis is closely related to CD4+ T cell counts, with an increasing risk when the count falls under 100 cells/µl. Toxoplasmic encephalitis (TE) is the most predominant manifestation of the disease in these patients and can lead to various symptoms, ranging from headache, lethargy, incoordination, or ataxia to hemiparesis, loss of memory, dementia, or focal to major motor seizures, usually associated with fever (Luft & Remington, 1992). The incidence of TE has decreased since the use of highly active antiretroviral therapy (HAART) (Jones *et al.*, 2002), as was shown with a French cohort, where the risk was divided by 4 and fell from 3.9 to 1.0 cases per 100 person-years (Abgrall *et al.*, 2001).

Other organs can be involved, either because they are target organs for encystment and thus are subsequent potential sites for cyst reactivation or because they are secondarily infected following the dissemination of parasites from an initial reactivation site. After the brain, the most frequently involved organs are the lungs, the eyes (Rabaud *et al.*, 1994), and the heart, resulting in myocarditis, but the isolation of Toxoplasma from many other sites, such as liver, pancreas (Hofman *et al.*, 1993), bone marrow, bladder (Rabaud *et al.*, 1994), lymph nodes, kidney, spleen, and skin (Arnold *et al.*, 1997), has been documented. Pulmonary or disseminated toxoplasmosis is seen mostly in transplant patients, who develop rapidly progressive infection and a massive dissemination of parasites (Botterel *et al.*, 2002; Derouin *et al.*, 1992; Patrat-Delon *et al.*, 2010). Less frequently, toxoplasmic retinochoroiditis may occur independently of any other signs of evolutive infection and must be distinguished from other infectious etiologies, in particular eye lesions due to cytomegalovirus (CMV), HIV, or syphilis.

Congenital Toxoplasmosis

Classically, congenital infection results from primary acquired maternal infection during gestation. The frequency of vertical transmission and the severity of fetal damage depend on the stage of pregnancy when maternal infection occurs. The placenta plays a main role in the process, as it is both a natural barrier which is supposed to protect the fetus and a target tissue for parasite multiplication (Abbasi *et al.*, 2003). In fact, the placental barrier is more efficient at the beginning of gestation, leading to the passage of parasites in less than 10% of cases during the first trimester, but becomes more permeable throughout pregnancy, allowing parasite transmission in around 30% of cases in the second trimester and 60 to 70% of cases in the third trimester and even more close to the time of delivery (Dunn *et al.*, 1999). The severity of fetal infection is inversely correlated, since neonates are asymptomatic in more than 80% of cases when infected during the third trimester of gestation (Desmonts & Couvreur, 1974). However, when transplacental transmission occurs during the first trimester, the consequences for fetal development are heavy, often leading to severe abnormalities or to abortion. Parasite multiplication induces necrosis foci and strong inflammation, leading to major abnormalities in the brain and eye tissues. It can induce the destruction or profound remodeling of the white substance. Infected necrotized foci may block the aqueduct of Sylvius, resulting in hydrocephalus of lateral ventricles. These foci further calcify and can be detected by transfontanellar echography or cranial X-ray. Major sequelae include mental retardation, seizures, microcephalus, hydrocephalus, deafness, and psychomotor deficiency (Remington *et al.*, 2001). Eye lesions are also more severe in early pregnancy, where microphthalmia, cataract, increased intraocular pressure, strabismus, optic neuritis, and retinal necrosis can be observed (Delair *et al.*, 2011; Roberts *et al.*, 2001), as can uveitis and retinochoroiditis, possibly leading to blindness if retinal lesions affect the macula. During the second trimester, fetal infection can be of variable severity. Echographical ultra sounds may reveal areas of hyperechogenic mesentery, hepatosplenomegaly, or cerebral calcifications. Clinical manifestations at birth may include epilepsy, anemia, thrombocytopenia-induced petechiae, rash, hepatic disorders, pneumonitis, or retinochoroiditis (Remington *et al.*, 2001). In a prospective European study, intracranial lesions detected at birth were associated with serious neurologic disorders in about 30% of cases (Cortina-Borja *et al.*, 2010). Among the data from 272 cases col- lected in 2007 through the French Surveillance Network (Villena *et al.*, 2010), 11 cases resulted in the termination of pregnancy owing to cerebral lesions or fetal death, and 87% of live-born infants were asymp tomatic. The remaining 13% of cases had intracranial calcifica tions (14 cases), hydrocephalus (3 cases), and/or retinochoroiditis of variable severity (12 cases).

Retinochoroiditis is a common feature that can be observed whatever the time of maternal infection. Its particularity resides in its frequently delayed clinical expression after birth. During a longitudinal U.S. study including 25 infants who were not treated in utero or during their first year of life, Phan et al. (Phan *et al.*, 2008) observed that 72% of these infants developed new eye lesions during a mean follow-up time of 5.7 years. Another prospective study of 102 infants who benefited from antenatal and postnatal treatment showed that 78% were asymptomatic during a median follow-up time of 7.8 years (Berrebi *et al.*, 2010). A recent European cohort study showed that the risk of developing eye lesions by 4 years of age was highest for children with serious neurologic sequelae at birth but also significantly increased for those with intracranial

lesions or hepa- tosplenomegaly (Freeman *et al.,* 2008). Conversely, children without retinochoroiditis detected by 4 months were at a low risk of developing eye manifestations by 4 years of age. In any event, the question of long-term pathogenicity may differ according to prevention protocols, and probably according to the strain genotype, as retinal lesions are more extensive in congenitally infected Brazilian infants (Vasconcelos-Santos *et al.,* 2009). Indeed, a comparative prospective cohort study of congenitally infected children in Brazil and Europe showed that, independently of treatment, Brazilian children had a 5-times- higher risk than European children for developing eye lesions, and their lesions were larger, more multiple, more recurrent, and more likely to impair vision (Gilbert *et al.,* 2008).

Although the vast majority of congenital infections results from primary acquired infection during pregnancy, parasite transmission can occur in rare instances in immunocompetent, previously immunized women who are reinfected with Toxoplasma during gestation (Elbez-Rubinstein *et al.,* 2009; Gavinet *et al.,* 1997; Hennequin *et al.,* 1997; Kodjikian *et al.,* 2004). A recent case benefited from genotyping, which revealed that reinfection was due to an atypical strain which was responsible for severe congenital toxoplasmosis, raising the temptation to take primary prevention measures even in previously immunized pregnant women, particularly in cases of travel to areas where atypical genotypes circulate (Elbez-Rubinstein *et al.,* 2009). A reactivation of past infection in HIV-infected women can also lead to congenital transmission, as shown by several case reports (Lindsay & Dubey, 2011).

Other exceptional cases of vertical transmission following maternal infection in the 2 months before conception have been described (Desmonts *et al.,* 1990; Marty *et al.,* 1991), but in most cases, the immune background of the mother could explain the prolonged dissemination of the parasite and thus further placental colonization and transmission. Another concept which has emerged from the French experience of the systematic screening of pregnancies for >20 years is that parasite transmission can be delayed, since few asymptomatic neonates born to mothers with periconceptional infection were diagnosed with congenital infection after birth despite negative pre- natal screening results (Robert-Gangneux *et al.,* 2009). Indirect arguments also support this hypothesis, since in a study by Romand et al. (Romand *et al.,* 2001), the sensitivity of prenatal diagnosis was lower in early pregnancy than in mid-pregnancy, suggesting that vertical transmission may be delayed for some women infected in early pregnancy (Thulliez, 2001). Thus, in rare instances, parasites could persist in the placenta and proceed into the fetal compartment only at the end of gestation, which could explain why neonates may not have any clinical or radiological signs in utero and at birth.

DIAGNOSTIC METHODS

The diagnosis of T. gondii infection or toxoplasmosis may be established by serologic tests, amplification of specific nucleic acid sequences (i.e., polymerase chain reaction [PCR]), histologic demonstration of the parasite and its antigens (i.e., immunoperoxidase stain), or by isolation of the organism (Remington, *et al.,* 2006).

Serologic Tests

The use of serologic tests for demonstration of specific anti- body to T. gondii is the initial and primary method of diagnosis. Different serologic tests often measure different antibodies that possess unique patterns of rise and fall with time after infection (Montoya & Remington, 1995). A combination of serologic tests is usually required to establish whether an individual has been most likely infected in the distant past or has been recently infected.

A panel of tests (the Toxoplasma Serological Profile [TSP]) consisting of the Sabin-Feldman dye test (DT) (Sabin & Feldman, 1948), double sandwich IgM ELISA (Naot & Remington, 1981), IgA ELISA (Stepick-Biek *et al.,* 1990), IgE ELISA (Pinon *et al.,* 1990; Wong *et al.,* 1993), and AC/HS test (Dannemann *et al.,* 1990) has been used successfully by our group to determine if serologic test results are more likely consistent with infection acquired in the recent or more distant past (Liesenfeld *et al.,* 2001; Montoya & Remington, 1996). The AC/HS test is interpreted as previously described (Dannemann *et al.,* 1990) by comparing IgG titers obtained with formalin-fixed tachyzoites (HS antigen) with those obtained with acetone-fixed tachyzoites (AC antigen).

The TSP has been successfully used in the setting of toxoplasmic lymphadenitis (Montoya & Remington, 1995), myocarditis (Montoya *et al.,* 1997), polymyositis (Montoya *et al.,* 1997), and chorioretinitis (Montoya & Remington, 1996) and during pregnancy (Liesenfeld *et al.,* 2001). For sera with positive results in IgG and IgM tests, the discriminatory power of the TSP to differentiate between recently acquired infection and chronic infection is probably superior to any other single serologic test.

IgG antibodies. The most commonly used tests for the measurement of IgG antibody are the DT, the ELISA, the IFA, and the modified direct agglutination test (Thulliez *et al.,* 1986). In these tests, IgG antibodies usually appear within 1– 2 weeks of acquisition of the infection, peak within 1– 2 months, decline at various rates, and usually persist for life. When two different compounds (i.e., acetone and formalin) are used to fix parasites for use in the agglutination test, a "differential" agglutination test (also known as the

"AC/HS test") results due to the fact that the different antigenic preparations vary in their ability to recognize sera obtained during the acute and chronic stages of the infection. This test has proved useful in helping to differentiate acute from chronic infections (Dannemann *et al.,* 1990) but is best used in combination with a panel of other tests (e.g., the TSP).Recently, a number of tests for avidity of Toxoplasma IgG antibodies have been introduced to help discriminate between recently acquired and distant infection (Hedman *et al.,* 1989; Jenum *et al.,* 1997; Liesenfeld *et al.,* 2001). The functional affinity of specific IgG antibodies is initially low after primary antigenic challenge and increases during subsequent weeks and months by antigen-driven B cell selection. Protein-denaturing reagents including urea are used to dissociate the antibody-antigen complex.

IgM antibodies. IgM antibodies may appear earlier and decline more rapidly than IgG antibodies. The most commonly used tests for the measurement of IgM antibody are double- sandwich or capture IgM-ELISA kits (Naot & Remington, 1981) , the IFA test, and the immunosorbent agglutination assay (IgM-ISAGA; available from bioMe´rieux) (Remington *et al.,* 2006). False-positive results due to rheumatoid factor and antinuclear antibodies in some IgM-IFA tests are not detected by the most commonly used commercial double-sandwich or capture IgM-ELISAs (Naot & Remington, 1981). Despite the wide distribution of commercial test kits to measure IgM antibodies, these tests often have low specificity, and the reported results are frequently misinterpreted (Wilson *et al.,* 1997).An IgM test is still used by most laboratories to determine if a patient has been infected recently or in the distant past, and because of the hurdles posed in interpreting a positive IgM test result, confirmatory testing should always be performed.

In patients with recently acquired primary infection, T. gondii– specific IgM antibodies are detected initially, and in most cases, these titers become negative within a few months. However, in some patients, positive T. gondii– specific IgM titers can still be observed during the chronic phase of infection (Liesenfeld *et al.,* 1997). Some in- vestigators have reported that IgM antibodies can be detected as long as 12 years after the acute infection (Bobic *et al.,* 1991). The persistence of these IgM antibodies does not appear to have any clinical relevance, and these patients should be considered chronically infected. Further complicating the interpretation of a positive IgM test result is the fact that several methods for its detection still may result in a relatively high frequency of false-positive results (Wilson *et al.,* 1997). As is true for IgM antibodies to the parasite, IgA antibodies may persist for many months or more than a year. For this reason, they are of little additional assistance for diagnosis of acute infection in the adult. In contrast, the increased sensitivity of IgA assays over IgM assays for diagnosis of congenital toxoplasmosis represents an advance in diagnosis of the infection in the fetus and newborn. In a number of newborns with congenital toxoplasmosis and negative IgM antibodies, the serologic diagnosis has been established by the presence of IgA and IgG antibodies (Stepick-Biek *et al.,* 1990). IgE antibodies. IgE antibodies are detectable by ELISA in sera of acutely infected adults, congenitally infected infants, and children with congenital toxoplasmic chorioretinitis (Pinon *et al.,* 1990; Wong *et al.,* 1993).

PCR

PCR amplification for detection of T. gondii DNA in body fluids and tissues has successfully been used to diagnose con- genital(Bobic *et al.,* 1991; Grover *et al.,* 1990) , ocular (Montoya *et al.,* 1999), and cerebral and disseminated (Brezin *et al.,* 1990; Dupouy-Camet *et al.,* 1993) toxoplasmosis. PCR has revolutionized the diagnosis of intrauterine T. gondii infection by enabling an early diagno sis to be made, thereby avoiding the use of more invasive procedures on the fetus. PCR has enabled detection of T. gondii DNA in brain tissue (Holliman *et al.,* 1990), cerebrospinal fluid (CSF) (Parmley *et al.,* 1992), vitreous and aqueous fluids (Johnson *et al.,* 1997), bronchoalveolar lavage (BAL) fluid (Roth *et al.,* 1992), and blood (Dupouy-Camet *et al.,* 1993) in patients with AIDS.

Histologic Diagnosis

Demonstration of tachyzoites in tissue sections or smears of body fluid (e.g., CSF or amniotic or BAL fluids) establishes the diagnosis of the acute infection. It is often difficult to dem- onstrate tachyzoites in conventionally stained tissue sections. The immunoperoxidase technique, which uses antisera to T. gondii, has proven both sensitive and specific: It has been used successfully to demonstrate the presence of the parasite in the central nervous system (CNS) of AIDS patients (Conley *et al.,* 1981). The im- munoperoxidase method is applicable to unfixed or formalin- fixed paraffin-embedded tissue sections (Conley *et al.,* 1981). A rapid, technically simple, and under-used method is the detection of T. gondii in air-dried, Wright-Giemsa stained slides of centrifuged (e.g., cytocentrifuge) sediment of CSF or of brain aspirate or in impression smears of biopsy tissue. Multiple tissue cysts near an inflammatory necrotic lesion probably establish the diagnosis of acute infection or reactivation of latent infection.

Isolation of T. gondii

Isolation of T. gondii from blood or body fluids establishes that the infection is acute. Attempts at isolation of the parasite can be performed by mouse inoculation or inoculation in tissue cell cultures of virtually any human tissue or body fluid.

Diagnosis of Specific Clinical Entities

The first step in pursuing the diagnosis of T. gondii infection or toxoplasmosis is to determine whether the individual has been exposed to the parasite. In essentially all cases, any of the tests for the detection of IgG antibodies reliably establish the presence or absence of the infection. In a small number of patients, IgG antibodies might not be detected within 2– 3 weeks after the initial exposure to the parasite; however, this is rare. In addition, rare cases of toxoplasmic chorioretinitis and toxoplasmic encephalitis in immunocompromised patients have been documented in patients with negative T. gondii– specific IgG antibodies.

The second step consists of establishing whether the patient has a recently acquired infection or an infection acquired in the more distant past. In general, a true-negative IgM test essentially rules out that the infection has been acquired in recent months. A positive IgM test is more difficult to correctly interpret. One must not assume that a positive IgM test result is diagnostic of recently acquired infection Confirmatory testing should be done for all cases for whom IgM test results are positive (Liesenfeld *et al.,* 1996). Serologic tests should not be considered useful for measuring response to therapy.

The third step is to establish whether the patient's condition or illness is due to toxoplasmosis (recently acquired infection or recrudescence of latent infection) or is unrelated to the infection.

Toxoplasmosis in the Immunocompetent Patient

The vast majority of cases of T. gondii infection in adults and children are asymptomatic (Remington, 1974). Lymphadenopathy is the most common manifestation in the 10%– 20% percent of other- wise immunocompetent individuals whose primary T. gondii infection is symptomatic. Less common presentations in these patients include, but are not limited to, chorioretinitis, myocar- ditis, and/or polymyositis (Montoya & Remington, 2000).

Tests for IgG and IgM antibodies should be used for initial evaluation of these patients. Testing of serial specimens obtained 3–4 weeks apart (in parallel) provides the best discriminatory power if the results in the initial specimen are equivocal. Negative results in both tests virtually rule out the diagnosis of toxoplasmosis. In rare instances early in infection, IgG antibodies may not be detectable, whereas IgM antibodies are present. This means there is need for both tests to be performed. Acute infection is supported by documented seroconversion of IgG and IgM antibodies or a greater than four-fold rise in IgG antibody titer in sera run in parallel. A single high titer of any immunoglobulin is insufficient to make the diagnosis since IgG antibodies may persist at high titers for many years and IgM antibodies may be detectable for .12 months. The TSP, performed on a single serum sample, is useful in determining the likelihood that the infection is acute. Characteristic histologic criteria and a TSP consistent with recently acquired infection establish the diagnosis of toxoplasmic lymphadenitis in older children and adults (Dorfman & Remington, 1973). Endomyocardial biopsy and biopsy of skeletal muscle has been successfully used to establish T. gondii as the etiologic agent of myocarditis and polymyositis in immunocompetent patients. Isolation studies and PCR have rarely proven useful for diagnosis in immunocompetent patients.

Ocular Toxoplasmosis

Toxoplasmic chorioretinitis may result from congenital or postnatally acquired infection. In both of these situations, le- sions may occur during the acute or latent (chronic) stage of the infection (Silveira *et al.,* 1988; Couvreur & Thulliez, 1996). Low titers of IgG antibody are usual in patients with active chorioretinitis due to reactivation of congenital T. gondii infection; IgM antibodies usually are not detected. When sera from such patients are examined by use of the DT, titers should be first determined with undiluted serum since in some cases, the conventional initial dilution of 1:16 may be negative. In most cases, toxoplasmic chorioretinitis is diagnosed by ophthalmologic examination, and empiric therapy directed against the organism is often instituted on the basis of clinical findings and serologic test results. In a number of patients, the morphology of the retinal lesion(s) may be non-diagnostic and the response to treatment is suboptimal. In such cases (unclear clinical diagnosis and/or inadequate clinical response), detection of a local and increased T. gondii antibody response in ocular fluids (immune load), demonstration of the parasite by isolation or histopathology, or amplification of T. gondii DNA (in both aqueous and vitreous fluids) have been used successfully to establish the diagnosis (Holland *et al.,* 1996 ; Diaz *et al.,* 1997).

Toxoplasmosis in the Immunodeficient Patient

In contrast to the relatively favorable course of toxoplasmosis in almost all immunocompetent individuals, immunologically impaired patients usually develop a dreadful and often life-threatening disease (Israelski & Remington, 1993). Immunocompromised patients at higher risk for toxoplasmosis include those with hematologic malignancies (particularly patients with lymphoma), bone mar- row transplant, solid organ transplant (including heart, lung, liver, or kidney), or AIDS.

Toxoplasmic encephalitis is the most common presentation of toxoplasmosis in immunocompromised patients (Israelski & Remington, 1993) and is the most frequent cause of focal CNS lesions in AIDS patients (Luft & Remington, 1992). It is unclear whether T. gondii penetrates the brain more easily than other organs or whether it is more difficult for the brain, as an immunologically privileged site, to eradicate the

organism during the initial acute infection and once residual infection has been established (Montoya & Remington, 1997). A wide range of clinical findings, including altered mental state, seizures, weakness, cranial nerve disturbances, sensory abnormalities, cerebellar signs, meningismus, movement disorders, and neuropsychiatric manifestations are observed in patients with toxoplasmic encephalitis (Liesenfeld & Wong, 1999). Other organs commonly involved in immunocompromised patients with toxoplasmosis are the lungs, eyes, and heart.

In the vast majority of immunocompromised patients, toxoplasmosis results from reactivation of a latent infection. In contrast, in heart transplant patients and in a small number of other immunocompromised patients, the highest risk of developing disease is in the setting of primary infection (i.e., a seronegative recipient who acquires the parasite from a seropositive donor via a graft) (Derouin *et al.,* 1986; Luft *et al.,* 1983). Because reactivation of chronic infection is the most common cause of toxoplasmosis in patients with malignancies or AIDS or in recipients of organ transplants (other than heart transplants), initial assessment of these patients should routinely include an assay for T. gondii IgG antibodies. Those with a positive result are at risk of reactivation of the infection; those with a negative result should be instructed on how they can prevent becoming infected.When toxoplasmosis is suspected in immunocompromised patients chronically infected with the parasite (those with documented positive T. gondii–specific IgG antibody prior to the onset of immunosuppression) additional serologic testing adds very little (or may be misleading) to the diagnostic evaluation (Luft *et al.,* 1986).

When clinical signs suggest involvement of the CNS and/or spinal cord, tests should include computed tomography or magnetic resonance imaging (MRI) of the brain and/or spinal cord. Neuroimaging studies of the brain should be considered even if the neurologic examination does not reveal focal deficits. Empiric anti–T. gondii therapy for patients with multiple ring enhancing brain lesions (usually established by MRI), positive IgG antibody titers against T. gondii, and advanced immunodeficiency (i.e., CD4 cell count of, 200 cells/mm3) is accepted clinical practice; a clinical and radiologic response to specific anti–T. gondii therapy is considered as supportive of the diagnosis of CNS toxoplasmosis. Brain biopsy should be considered in immunocompromised patients with presumed CNS toxoplas- mosis if there is a single lesion on MRI, a negative IgG antibody test result, or inadequate clinical response to an optimal treatment regimen or to what the physician considers to be an effective prophylactic regimen against T. gondii (e.g., trimethoprim-sulfamethoxazole) (Montoya & Remington, 2000). If T. gondii serologic and radiologic studies do not support a recommendation for empiric treatment or are inconclusive and if brain biopsy is not feasible, a lumbar puncture should be considered if it is safe to perform; PCR can then be performed on the CSF specimen. CSF can also be used for isolation studies, although it is uncommon for T. gondii to be isolated from CSF from immunocompromised patients. Of note, PCR examination of CSF can also be used for detection of Epstein-Barr virus, JC virus, or cytomegalovirus DNA in patients in whom primary CNS lymphoma, progressive multifocal leukoencephalopathy, or cytomegalovirus ventriculitis, respectively, have been considered in the differential diagnosis.

T. gondii Infection in Pregnancy

T. gondii infection acquired during pregnancy may result in severe damage or death of the fetus and long-term sequelae in offspring. Because congenital toxoplasmosis results almost solely in women who acquire the infection during gestation, it is critical to determine whether infection during pregnancy has occurred. The incidence of congenital toxoplasmosis in the off- spring of women infected prior to gestation has been shown to be extremely rare unless a woman is immunocompromised (e.g., receiving corticosteroids or immunosuppressive drugs or positive for human immunodeficiency virus. A negative IgM test result for a pregnant woman in the first 24 weeks of gestation with a low IgG test titer (i.e., DT, 1024) essentially places the acquisition of the infection prior to ges- tation. In the third trimester, a negative IgM test titer is most likely consistent with a chronic maternal infection but does not exclude the possibility of an acute infection acquired early in pregnancy; this is especially true in those patients who exhibit a rapid decline in their IgM titers during the acute infection. In such cases, the use of other serologic tests (e.g., IgA, IgE, AC/ HS, avidity) may be of particular help.

In contrast, a positive IgM test result requires further assessment with confirmatory serologic testing. A false positive IgM test result or its erroneous interpretation can be misleading and re- sult in unnecessary abortions (Liesenfeld *et al.,* 2001). Sixty percent of pregnant women with IgM results determined to be positive by non- reference laboratories were found to be chronically infected when tested at TSL-PAMFRI (Liesenfeld *et al.,* 2001). The potential pitfalls of relying solely on an IgM test as a discriminatory method to allow such distinction and the low reliability of commercial T. gondii– specific IgM kits when positive results are obtained have been reported by our group and others (Liesenfeld *et al.,* 1997; Wilson *et al.,* 1997).

Congenital Infection in the Fetus and Newborn

Prenatal diagnosis. Prenatal diagnosis of fetal infection is advised when a diagnosis of acute infection is established or highly suspected in a pregnant woman or an abnormality in the fetus suggests congenital toxoplasmosis. Methods to obtain fetal blood, such as

periumbilical fetal blood sampling, have been largely abandoned because of the risk involved for the fetus and the delay in obtaining definitive results with conventional parasitologic tests (Hohlfeld *et al.,* 1994).

Prenatal diagnosis of congenital toxoplasmosis is currently based on ultrasonography and amniocentesis. PCR on amniotic fluid for detection of T. gondii– specific DNA performed from 18 weeks onwards of gestation should be used in all cases of established acute maternal infection or cases with serologic test results highly suggestive of acute acquired infection during pregnancy (Hohlfeld *et al.,* 1994). In a recent report, the overall sensitivity of PCR on amniotic fluid was estimated to be 64%, the negative predictive value was estimated to be 87.8%, and specificity and positive predictive value were estimated to be 100% (Romand *et al.,* 2001); in this study, marked differences in sensitivity were observed depending on the gestational age at the time of the amniocentesis (Romand *et al.,* 2001).

Diagnosis in the newborn. Maternal IgG antibodies present in the newborn may reflect either past or recent infection in the mother. For this reason, tests for detection of IgA and IgM anti- bodies are commonly employed for diagnosis of infection in the newborn. Serum samples obtained from peripheral blood are preferred. Samples from umbilical cord should not be used as they may be contaminated with maternal blood. Demonstration of IgA antibodies appears to be more sensitive than detection of IgM antibodies for establishing infection in the newborn (Stepick-Biek *et al.,* 1990). T. gondii– specific IgA may be present when there is no T. gondii– specific IgM, and the converse may also occur. If IgA antibodies are detected in the newborn, the test should be repeated at ~10 days after birth to make certain that what is being measured is not contaminating maternal IgA antibodies. In addition, if the newborn has received a blood transfusion, serologic tests may measure exogenously administered rather than endogenous antibody.

Infants born to mothers chronically infected with T. gondii will have maternal Toxoplasma-specific IgG antibodies detected in their peripheral blood. In these infants, Toxoplasma serologic tests for IgM and IgA antibodies are usually negative, and efforts to detect T. gondii in body fluids or tissues (by isolation or PCR) should yield negative results. Follow-up serologic testing should be done on these patients until the IgG antibodies become undetectable. Maternally transferred IgG antibodies should dis- appear within the first 6–12 months of life. A negative T. gondii–specific IgG test result at 1 year of age essentially rules out congenital toxoplasmosis.

Additional diagnostic methods that have been used successfully to diagnose the infection in infants include direct demonstration of the organism by isolation of the parasite (e.g., mouse inoculation or inoculation in tissue cultures of CSF, urine, placental tissue, or peripheral blood) and amplification of T. gondii– specific DNA (e.g., PCR in CSF, peripheral blood, or urine) (Remington *et al.,* 2006). Although not clinically available, antigen-specific lymphocyte transformation and lymphocyte typing in response to exposure to T. gondii antigens has been used successfully to diagnose the congenital infection in infants >2 months of age (Krahenbuhl et al.,1972 ; Wilson *et al.,* 1980). Specific lymphocyte anergy to the organism may also occur in congenitally infected infants (McLeod *et al.,* 1990).

CONCLUSION

Advancements in the field of Toxoplasma studies have occurred in the past 20 years. There is need of more basic research in the field of molecular and cell biology employing technologies such as genomic and proteomic and if possible to involve imaging techniques. This means another review will be necessary to evaluate these advances. However, in the meantime, epidemiological studies have also gained more importance. The emergence of Toxoplasma as a waterborne disease in several countries has stimulated environmental research. An ecological and integrated approach was developed for a better understanding of the complex circulation of Toxoplasma between its multiple hosts and the environment and, finally, of the risk factors for human infection. Thanks to the isolation and genotyping of strains from various animal species in different continents which helped in the revelation of diversity of T. gondii. There is a need to still evaluate the practical consequences of these epidemiological and genetic advances for diagnosis strategies and for the management of human toxoplasmosis. Available tools for the biological diagnosis of toxoplasmosis allow diagnosis in most cases, but the development of other biomarkers should be helpful for the most difficult cases, for instance, for ocular or cerebral localizations. The diagnosis of T. gondii infection or toxoplasmosis can be established by serologic tests, PCR, histologic examination, or isolation of the parasite. T. gondii infection can be asymptomatic, and the clinical manifestations of patients with symptomatic toxoplasmosis are protean and nonspecific. The choice of the appropriate diagnostic method(s) and its (their) interpretation may differ for each clinical categories. Therefore in order to optimize the choice and handling of the specimens and their yield, reference laboratories should be contacted prior to diagnostic procedures.

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