Toxoplasma

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Abstract

Toxoplasmosis is a zoonotic infection caused by the protozoon *Toxoplasma gondii* affecting animals worldwide. *T. gondii* has a facultative heteroxenous life cycle with felids as definitive hosts and a wide range of mammalian and avian species as intermediate hosts. Infectious stages are oocysts present in felid feces, bradyzoites forming tissue cysts, and tachyzoites. While two major clonal lineages (type II and III) dominate clinical and natural isolates in Europe and North America, other parts of the world, like Brazil and Argentina, are dominated by non-clonal or other clonal *T. gondii* lineages, representing a greater genetic diversity. Different animal species show a variable degree of susceptibility to *T. gondii* infection and to the development of clinical signs. Humans are considered susceptible, and about one third of the human population in the world is estimated to be infected. Toxoplasmosis is an important abortive disease in small ruminants. It is mainly asymptomatic in cattle and chicken, but the latter is an epidemiological sentinel, and isolation of viable parasites from this source is frequent. Infected pigs can show reproductive failure, and, in addition, their tissues are considered, together with infected small ruminant tissues, a relevant source of human infection. Cats and dogs can show neuromuscular disease mainly associated with other immunosuppressive conditions, such as viral infec-

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tions. Some species like New World monkeys, lemurs, Pallas' cats, slender-tailed meerkats, and some Australian marsupials are highly susceptible to fatal generalized toxoplasmosis. General control measures are presented, focusing on the prevention of human toxoplasmosis.

6.1 Morphology, Life Cycle, and Host-Pathogen Interactions

6.1.1 Morphology and Life Cycle

The protozoan *Toxoplasma gondii* is an intracellular parasite which can infect mammals and birds. As other apicomplexan parasites, *T. gondii* shows a banana-shaped eukaryotic cell with a complete apical complex. It was detected initially in a hamster-like rodent (*Ctenodactylus gundi*) and due to its shape was designated as *Toxoplasma* (*toxo*, arc; *plasma*, life), while *gondii* is a deformation of the host species name. Each protozoon measures about 3–6 μm by 0.5–2 μm. They can parasitize virtually any nucleated cell. *T. gondii* is a cyst-forming coccidian from the order Eimeriorina and the unique species of the genus *Toxoplasma*.

T. gondii has a facultative heteroxenous life cycle with felids as definitive hosts and a wide range of mammalian and avian species as intermediate hosts (Dubey et al. 1998).The term facultative refers to the facts that transmission can occur among intermediate hosts, without a definitive host, and that felids can also act as intermediate hosts. In domestic cats and wild felids as definitive hosts, the parasites reproduce by asexual and sexual multiplication in intestinal cells. This is known as intestinal or entero-epithelial life cycle. Macro- and microgametes are formed—gametogony—by differentiation, and, as a result of gamete fusion, immature oocysts are produced and excreted with feces. The prepatent period can vary from 3 to 18 dpi in accordance with the infective stage ingested—tissue cysts and oocysts, respectively. Oocysts are spherical and measure 10–12 μm in diameter (Fig. 6.1). In the environment, under suitable conditions of temperature and humidity, oocysts undergo a division—sporogony or post-zygotic division—producing sporulated or mature oocysts which contain two sporocysts with four sporozoites. The regular zoites of *T. gondii* are haploids, and the formed zygote undergoes a meiotic division. At this stage, recombination of genetic material among sporozoites in formation can take place. Oocysts can remain viable for periods up to 18 months, especially with regular to high humidity (Dubey 1998b).

In intermediate hosts, including cats and humans, the parasites reproduce asexually, and an extraintestinal cycle takes place. If an intermediate host becomes infected by oocyst ingestion, sporozoites are released following oocyst wall disruption by enzymatic digestion. The zoites enter host cells inside a parasitophorous vacuole, which derives from the host cell membrane and components secreted by the protozoans. Then, *T. gondii* multiplies asexually in the host cell by endodyogeny—merogony—producing merozoites. Merogony takes place within any nucleated cell, including macrophages, and, initially, consists of a rapid division producing

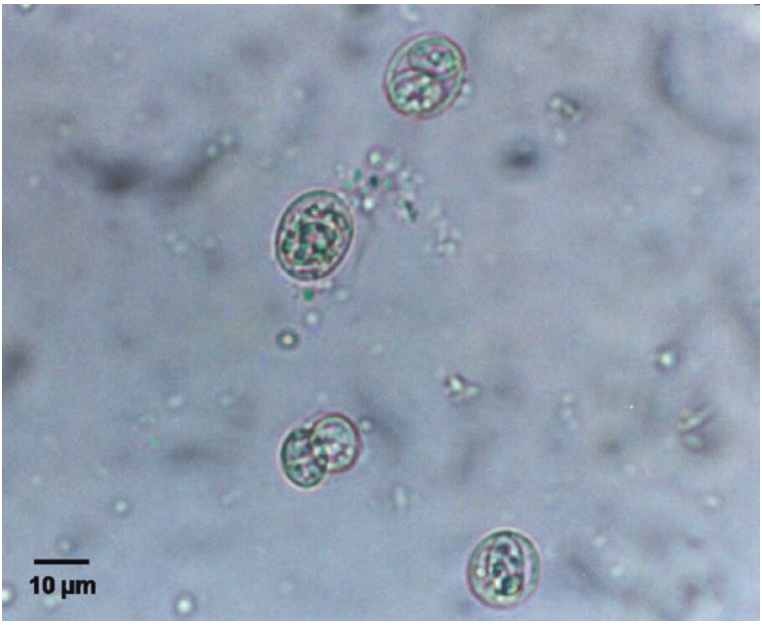

Fig. 6.1 *Toxoplasma gondii* oocysts in a cat stool sample. Note three sporulated oocysts with two sporocysts each and one immature/unsporulated oocyst. 400×

host cell disruption. Merozoites that result from this accelerated division are named tachyzoites, from the Greek *tachos*: speed. After dividing rapidly, tachyzoites enter cells, mainly of the neuromuscular system, and multiply slowly forming bradyzoites—*brady*: slow, in Greek—within tissue cysts. The brain, liver, lungs, skeletal muscle, and eyes are the main sites of appearance of tissue cysts. Cysts are spherical or elongated, limited by a cyst wall, and measure about 70 or 100 µm in diameter in brain and skeletal muscles, respectively (Figs. 6.2 and 6.3).

6.1.2 Infection Routes and Host-Pathogen Interactions

One of the principal sources of infection for both definitive and intermediate hosts is the ingestion of tissue cysts. It can take place through the consumption of raw or not well-cooked tissues, cannibalism, or scavenger behavior and can affect carnivores, omnivores, or scavenger animals (Tenter et al. 2000).

Another important route of infection is the ingestion of mature oocysts present in food, soil, water, and pastures, contaminated with infected cat feces. All animal species can be infected through this route, but it is considered particularly relevant for herbivores (Tenter et al. 2000). Oral infection with mature oocysts or with tissue cysts containing bradyzoites is known as horizontal infection.

A special infection route of *T. gondii* is the transplacental or vertical transmission through the passage of tachyzoites from mother to offspring. This process can

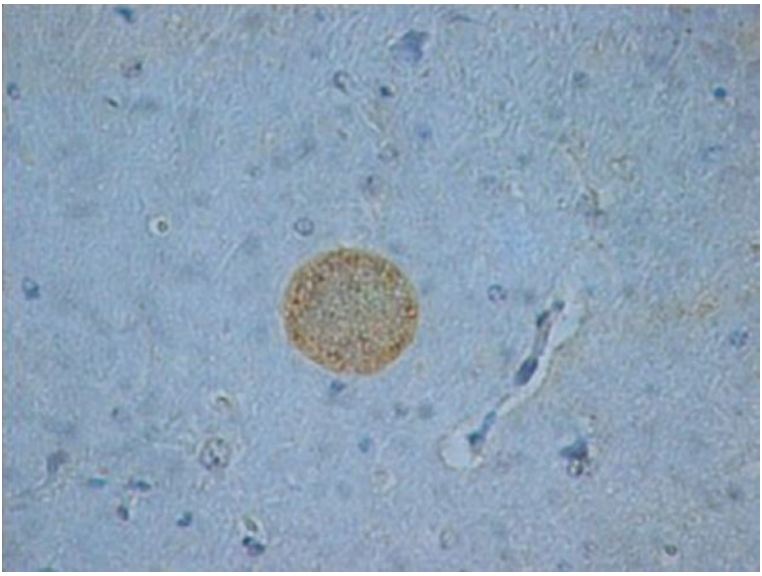

Fig. 6.2 Tissue cyst of *Toxoplasma gondii* in the brain. Positive specific immunohistochemistry stain. 400×

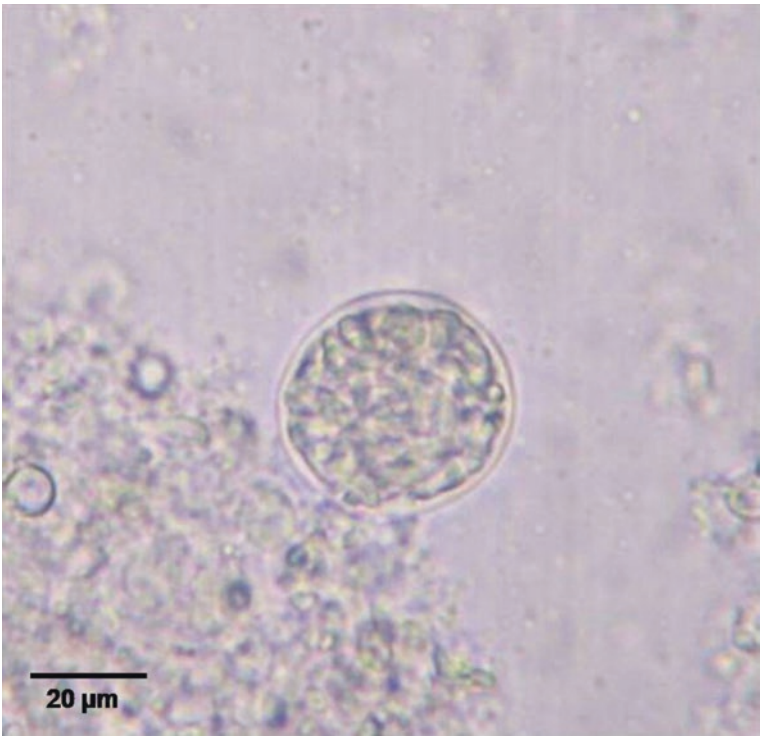

Fig. 6.3 *Toxoplasma gondii* tissue cyst from a mouse brain homogenate. Unstained. 400×

maintain the infection within a population, even without contact with definitive hosts (Hide 2016). Additionally, depending on the gestational age, animal species, and parasite strains, vertical transmission can result in abortion or cause neurological, muscular, and/or ocular disorders in the fetus. Other potential routes for the transmission of tachyzoites, which are labile in the environment, include the ingestion of contaminated raw milk or colostrum, as well as receiving organ transplants or blood transfusion from an infected individual (Tenter et al. 2000).

Natural infections of hosts, both definitive and intermediate, are usually asymptomatic, but most infected animals become chronic carriers of tissue cysts. Following asexual tachyzoite reproduction, cell death and focal necrosis occur. Frequently, necrosis and inflammation are reduced with the acquisition of immunity, which controls tachyzoite division. However, immunity has little or no effect on tissue cysts and bradyzoites, allowing the chronic stage of infection to develop. A protective immune response against *T. gondii* infection is mainly dependent on the host cellular immunity and is closely related to the effect of cytokines such as interferon gamma (IFN-γ) and different lymphocyte population products. On the other hand, antibodies are not useful to prevent parasite multiplication, and an overexpressed humoral response can be associated with high parasite levels (Tenter et al. 2000). In chronically infected animals under immune-compromised conditions, proliferation of *T. gondii* can be reactivated. This particularity, where bradyzoites, which normally divide slowly, can reinitiate a rapid asexual division, is known for only a few protozoans (Dubey 1998a). It can be useful for parasite isolation, making protozoan multiplication possible in mice or cell culture, using tissues containing viable tachyzoites or bradyzoite-containing cysts as starting material. Once divided as tachyzoites, it is possible to maintain the parasite by mice passages or cell culture in several cell lines.

The relation between host immune response and *T. gondii* evasion mechanisms has allowed this protozoan to develop chronic infections and to survive in a large diversity of hosts worldwide. *T. gondii* can thrive within dendritic cells and macrophages and, using the migratory properties of these cells, can disseminate inside the host (Blader and Saeij 2009; Lang et al. 2007; Melo et al. 2011; Saeij et al. 2006; Tait and Hunter 2009). Dendritic cells are the most efficient producers of IL-12 during infections, and, additionally, they induce IFN-γ secretion by Th1 lymphocytes and NK cells, generating a protective cellular immune response (Miller et al. 2009). Innate immune responses begin upon contact of parasite structural proteins—considered key virulence factors in the acute stage—with toll-like receptors (TLR) of dendritic cells or macrophages. For example, profilins interact with TLR 11, and glycophosphatidylinositols (GPIs) associated with parasite surface proteins, such as SAG1 or SAG2, and heat shock protein (HSP) 70 interact with TLR2 and TLR4 (Karsten et al. 1998; Pollard et al. 2008). ROP5, ROP16, ROP18, and dense granule (GRA) proteins, that are involved in penetration into host cells, are also important in the pathogenesis of infection, because the contact with TLR induces the activation of nuclear factor κβ (NFκβ) and the secretion of pro-inflammatory cytokines (Blader and Saeij 2009). Different expression levels of ROP5, ROP16, and ROP18 have been related to *T. gondii* genotypes and linked to the virulence of isolates, since they avoid mechanisms of innate immune response facilitating the invasion

and proliferation of the parasite due to interaction with the STAT3—signal transducers and activators of transcription—and STAT6 and Irga6 and ATF6-beta, respectively (Blader and Saeij 2009; Fentress et al. 2010; Fleckenstein et al. 2012).

6.2 Diagnostics and Epidemiology

6.2.1 Diagnosis

There are many diagnostic methods to detect *T. gondii* infection in domestic animals. Although the clinical signs of *T. gondii* in placental cotyledons of sheep and goat are considered characteristic, most signs are unspecific, making it important to confirm the diagnosis using direct or indirect methods (Ortega-Mora et al. 2007). Moreover, as mentioned previously, most infected animals do not develop clinical signs. Direct methods identify the parasite or parts of them, such as parasite DNA, and include the presence of compatible histopathological lesions and/or identification of *T. gondii* by immunohistochemistry, detection of oocysts in feline feces, isolation of the parasite, and identification of *T. gondii* by polymerase chain reaction (PCR) and genotyping. Indirect diagnostic methods, on the other hand, generally detect an immune response generated by contact with the parasite. Among them, detection of specific antibodies to *T. gondii* is useful for immunological and epidemiological examinations.

6.2.1.1 Histopathology

Observation of tissues infected with the acute stages of multiplication—tachyzoites—frequently evidences multifocal nonsuppurative inflammation. Chronic infection is evidenced by the observation of tissue cysts frequently surrounded by no inflammatory reactions. Tissue cysts should be differentiated from those produced by other cyst-forming coccidia. The wall of *T. gondii* cysts is <1 μm wide, while the wall of the cysts of the related protozoon *N. caninum* is thicker than 3 μm. Also, *T. gondii* tissue cysts lack septum or trabecular inner structures, which is distinctive of cysts from *Sarcocystis* spp. Final identification of the presence of parasites in tissues and lesions can be achieved by specific immunohistochemistry. In addition, polyclonal- or monoclonal-specific antibodies allow to identify groups of zoites—meronts—or free tachyzoites, as well as to confirm the identity of tissue cysts (Uggla et al. 1987).

6.2.1.2 Copro-Parasitological Techniques

Identification of oocysts in feline feces can be achieved by flotation techniques, using high-density liquids like sucrose, sodium chloride, or zinc sulfate solutions. Oocysts measure around 10–12 μm of diameter and are morphologically indistinguishable from *Hammondia hammondi* oocysts. Differentiation can be carried out by specific molecular or bioassay methods (Schares et al. 2008). Microscopic examination has a sensitivity of approximately 100 oocysts per gram of feces.

6.2.1.3 Isolation

Parasite isolation is not carried out for routine diagnosis but is useful to identify viability and type of parasites infecting different hosts. Bioassays in mice and cats and cell culture are the applied methods. Mice can be inoculated subcutaneously or intraperitoneally with homogenized tissues or orally with suspensions of oocysts. Most virulent strains of *T. gondii* are lethal for mice at 3–12 dpi. At necropsy, it is possible to recover large amounts of tachyzoites from peritoneal washes or lung homogenates. These fluids are the optimal inocula for isolation in cell culture. Inoculated mice, even in the absence of clinical signs, should be checked at 3–4 weeks' post-infection for the presence of specific *T. gondii* antibodies. Cats are regularly fed with suspected tissues, and their feces observed by sucrose flotation technique to detect oocysts. Several cell lines are appropriate for in vitro growth of the parasite; the most frequently used are the VERO cell line derived from kidney epithelial cells from African green monkey and the primary cell line HFF, derived from human foreskin fibroblasts (Saadatnia et al. 2010).

6.2.1.4 PCR and Genotyping

Identification of different gene fragments by molecular methods—mainly PCR and real-time PCR—has become a frequent method for the specific diagnosis of *T. gondii*. The main diagnostic targets include a 529 base pair (bp) repeat segment, the B1 gene, and the 18S rRNA gene. As mentioned previously, the genome of a single zoite is haploid with 14 chromosomes. The mentioned target genes are present in multiple copies in the genome and have been identified as specific and sensitive enough for detection. The 529 bp repeat is present in 200–300 copies in the genome and has been found to be the most suitable region for specific diagnosis (Su et al. 2006). A real-time PCR targeting fragments of the 529 bp repeat has shown a detection limit lower than 1 zoite per assay (Lin et al. 2000). A disadvantage of the technique is related to its costs that include DNA extraction kits, PCR solutions, specialized equipment, and a well-designed laboratory structure, which is particularly relevant to avoid false-positive results. Once specific *T. gondii* DNA is identified in tissues with an acceptable concentration, it is possible to proceed with the typing of the protozoa. Genotypes relate to virulence or adaptation to different hosts and geographical areas and can be studied by restriction fragment length polymorphism of a selected region amplified by nested polymerase chain reaction (nPCR-RFLP), microsatellite analysis, or sequencing different target genes. Analysis of 9–12 different genetic markers distributed in the *T. gondii* genome by nPCR-RFLP is the most widely used technique (Su et al. 2006). Once the genotype has been established by this method, microsatellite or sequencing analysis can be applied to identify phylogenetic relations among similar RFLP haplotypes (Su et al. 2010). This type of studies allows associations with the outcome of clinical signs and the identification of potential sources of infection, contributing essential information for epidemiological studies (Su et al. 2010).

6.2.1.5 Serological Tests

Modified agglutination test (MAT), indirect fluorescent antibody test (IFAT), and enzyme-linked immunosorbent assay (ELISA) are the most used serological methods for toxoplasmosis in animals. For human diagnosis, on the other hand, the reference assay is the Sabin-Feldman dye test, although different results have been obtained in animal species (Dubey 2010b). Its disadvantage is that living tachyzoites must be used as antigen. Briefly, tachyzoites of a reference strain—RH or BK—maintained in the laboratory are allowed to react with the test serum in the presence of complementary factor and methylene blue. The presence of specific antibodies is verified by blue staining of tachyzoites, as the dye enters the parasite through holes produced on the membrane by antigen-antibody reactions. Blue and white tachyzoites are counted after exposure to serum dilutions to express titers of antibodies against *T. gondii*. Diagnostic methods need to be validated for each animal species in different geographical regions, since they can show variable sensitivity and specificity according to the antigen characteristics and the protocols used. Antibodies can be detected in most infected animals from 15 days post-infection. Detection of seroconversion with 3- to 4-week intervals is recommended to determine the evolution of infection. Serological tests in cats are useful to identify infection, mainly when they act as intermediate hosts, since oocyst shedding generally occurs before the peak of IgG antibodies, when they act as definitive hosts. On the other hand, a seropositive cat probably has already shed oocysts. When an animal has compatible clinical signs with *T. gondii* infection and shows a significant increase of serological titers after two consecutive determinations, an acute infection can be assumed (Dubey 2010b). A similar conclusion can be drawn from aborted dams if seroconversion occurs, indicating active or reactivated infection during pregnancy. Considering the type of placenta in pigs and ruminants, detection of antibodies in fetal fluids indicates transplacental infection, because there is no *in uterus* passage of maternal antibodies (Dubey et al. 1987). However, gestational age is an important factor to analyze, because fetuses are immunocompetent from the second or third stage of gestation (Tizard 2009) and younger fetuses are unable to generate antibodies, remaining serologically negative.

In our laboratory, the IFAT technique is widely used. The presence of antibodies to *T. gondii* is detected using RH strain tachyzoites as antigen. For antigen preparation, tachyzoites obtained after 6–7 days in vitro cultured in 5% CO_2 are washed three times with phosphate-buffered saline (PBS), pH 7.2, by centrifugation at 500 g. Tachyzoites are treated with 1% formalin for 15 min at 4 °C, washed three times with PBS, and then fixed on the multi-spot areas of a glass slide. Slides can be stored at −20 °C until use. For detection of antibodies, sera are diluted with PBS and incubated with antigen for 30 min at 37 °C. At the end of incubation, slides are washed three times with PBS. Species-specific anti-immunoglobulin antibodies conjugated to fluorescein isothiocyanate are diluted and incubated as mentioned above and washed twice with PBS. Fluorescence is detected using an epifluorescence microscope (Fig. 6.4). Serological titers are expressed as the end dilution of sera where full fluorescence of tachyzoites is observed.

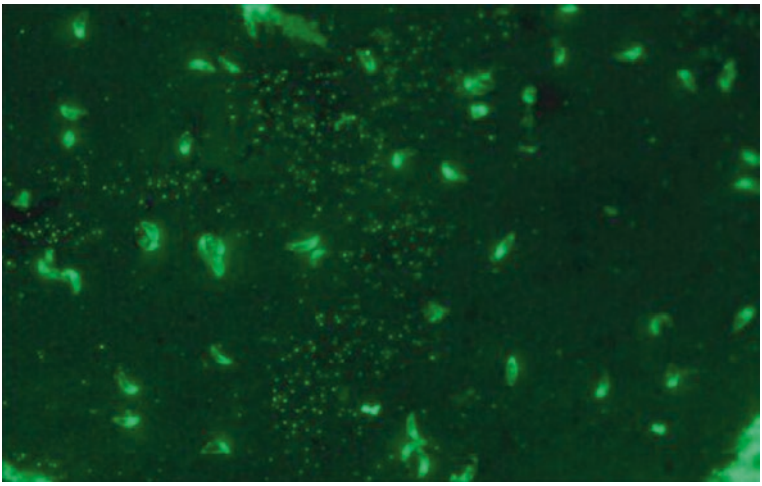

Fig. 6.4 Positive IFAT reaction using *T. gondii* RH strain as antigen. 400×

The ELISA test allows detection of IgM, IgG, and IgA antibodies and is as sensitive as the IFAT. There are different commercially available tests produced using whole antigens, native proteins, or recombinant proteins; thus a solid validation is required to use them (Dubey 2010b; Pardini et al. 2012).

The modified agglutination test (MAT) consists in a direct agglutination which uses 2-mercaptoethanol to treat the sera and to destroy nonspecific immunoglobulins and IgM. It is useful for serological diagnosis in different animal species, particularly in wild animals for which specific conjugates are not available. The antigen is prepared with whole *T. gondii* tachyzoites treated with formalin that are recognized by the test sera. The reaction is performed in microplates using different buffers and Evans blue staining. Diluted sera are incubated with the antigen preparation overnight at room temperature. In addition, the indirect agglutination test, in which antigenic fractions are adsorbed to particles, such as red blood cells or latex particles, can be used for prevalence studies. However, it is not advisable for serological diagnosis, due to its low sensitivity (Dubey 2010b). Immunoblotting is not commonly employed as routine diagnosis but can be useful to confirm results of other serological tests. Since there is no serological method considered as a gold standard, measuring antibodies by two tests or using a widely validated test is advisable for prevalence studies and for a proper diagnosis in different animal species.

6.2.2 Epidemiology

Toxoplasmosis is a worldwide distributed disease affecting a wide range of hosts. Different animal species show different degrees of susceptibility to the parasite and a variety of clinical signs. Humans are considered susceptible to *T. gondii* infection, and about one third of the human population in the world is estimated to be

asymptomatic carriers of the parasite (Dubey 2010b). In addition to congenital transmission, humans can acquire the parasite by the ingestion of undercooked or raw tissue cyst-containing meat mainly from pigs, small ruminants, and birds, as well as food or vegetables contaminated with oocyst-infected felid feces.

Serological studies of *T. gondii* in small ruminants are useful to evaluate the infection status of herds and individual animals and to relate it with the occurrence of abortions. They are also important to identify possible sources of infection for other hosts. Antibodies to *T. gondii* have been detected in goats worldwide with prevalence rates between 17 and 71% (Deng et al. 2016; Faria et al. 2007; Garcia-Bocanegra et al. 2013; Iovu et al. 2012; Lopes et al. 2013; Mancianti et al. 2013; Stormoen et al. 2012; Tzanidakis et al. 2012). The relative importance of the vertical transmission in the epidemiology of goat toxoplasmosis is under study. Also in sheep, antibodies to *T. gondii* have been recorded worldwide, being higher in adult ewes, suggesting a postnatal/horizontal infection as the main route of infection.

T. gondii is known to infect a large spectrum of avian species, ranging from passerine birds, like sparrows, to domesticated birds such as chickens (Dubey 2002). Adult backyard chickens can show infection rates up to 100%, while chickens raised indoors show very low prevalence rates of 0–5% (Dubey 2010a). Since parasite isolation from chicken tissues is relatively easier than from other animals, most parasite isolates worldwide have been obtained from this species. Moreover, due to the high susceptibility of infection observed in free-range chickens, they are an excellent environmental indicator of *T. gondii* presence in a given region (Moré et al. 2012; Pardini et al. 2016). In addition, migratory birds have been postulated to contribute to the dissemination of different *T. gondii* genotypes in their migratory routes.

The prevalence rates of *T. gondii* in pigs are remarkably variable according to raising conditions (indoor versus outdoor farms) and age (market age of fattening pigs versus breeder pigs) (Dubey 1986). Feral pigs can also be infected; however, the few studies conducted revealed lower prevalence than that observed in domestic pigs. Reported prevalence rates of *T. gondii* in pigs in the 1980s ranged between 22–78%, 19–58%, and 12–31% in Latin American countries, Argentina and Brazil; in European countries, Belgium and Italy; and in Southeast Asia, Indonesia and Japan, respectively (Dubey 2010b). More recent studies detected seroprevalence values of 59%, 37–58%, 51–72%, 4–39%, and 15.8% in Argentina, Central European countries, Latin American countries, Southeast Asia, and the USA, respectively (Jones et al. 2001a; Tenter et al. 2000).

Seroepidemiological studies in various geographical regions have detected positive bovines. However, the specificity of some serological tests for *T. gondii* is lowered by cross-reactivity with *Neospora caninum* and other related protozoa (Dubey 2010b). Many studies in the past probably misdiagnosed bovine abortions as due to *T. gondii,* while the protozoon involved might have been *N. caninum*. Seroprevalence rates ranging from 0 to 60% have been reported in different countries; however, care should be taken concerning the interpretation of these results (Dubey 2010b). In a national survey in the USA, antibodies to *T. gondii* or viable parasites were not detected in 2049 beef samples (Dubey et al. 2005). Parasite DNA could be detected

in cattle tissues; however, the importance of bovine tissues as source of infection for other hosts is uncertain (Moré et al. 2008).

Dogs are commonly infected by the ingestion of *T. gondii* oocysts from soil, vegetables, and water contaminated with infected feline feces, by the ingestion of raw or not well-cooked cyst-containing tissues from infected animals, and by transplacental infection to puppies (Dubey 2010b). Since dogs are usually living closely with humans and have a similar opportunity for *T. gondii* infection, prevalence of *T. gondii* in dogs might reflect human infection. Tenter et al. (2000) summarized prevalence rates to *T. gondii* in dogs of various countries and found strong regional differences. Recorded rates were of 60% by IFAT in Argentina, 46–84% by IFAT in Brazil, 36% by IFAT in Israel, 47% by IFAT in Spain, 5% by ELISA in China, and 8–25% by ELISA and LAT in Taiwan. In Trinidad and Tobago, rates measured by LAT were 60.5% in stray dogs, 30.5% in hunting dogs, and 25.5% in pet dogs (Ali et al. 2003).

Felids are important in the epidemiology of toxoplasmosis since they act as both definitive and intermediate hosts. Since *T. gondii* has been detected in most studied regions, it is assumed that felids (wild and domestic) could be infected all around the world. When cats are infected with tissue cysts, oocysts, or tachyzoites, prepatent periods of 3–10, 19–41, or 9–11 days, respectively, are followed by oocyst excretion in the feces (Dubey 2010b). As peak levels of specific IgG antibodies take place at 14 days post-infection, IgG antibodies might not be detected during oocyst excretion, especially after ingestion of tissue cysts. It is possible that a chronically infected animal, which has already shed oocysts, re-sheds after an immunosuppressive condition, which is commonly induced by feline immunodeficiency virus—FIV—infection, other chronic concomitant diseases, or age (Dubey and Carpenter 1993). Since sexual reproduction of the parasite is not associated with clinical signs, detection of oocysts in stool samples from symptomatic animals submitted to laboratory analysis is rare. Studies conducted using high number of stool samples submitted to copro-parasitological analysis revealed a prevalence of *T. gondii* oocysts in around 0.1 and 1% in domestic cats (Epe et al. 1993; Schares et al. 2008).

As mentioned previously, *T. gondii* can infect mammals and birds; therefore, any wild animal could be potentially infected. Additionally, it is assumed that the parasites have a *domestic* cycle and a *wild or sylvatic* cycle. Apparently, coevolution of parasites and host tends to the equilibrium, and the adaptation is expressed as an asymptomatic infection. However, when a host adapted to one type of cycle is infected with a parasite adapted to other hosts, the infection tends to be severe or symptomatic (Carme et al. 2009).

6.2.2.1 Population Structure

The global population structure of *T. gondii* is very diverse and varies between continents. However, its differential appearance in distinct parts of the world is only partially understood. Most *T. gondii* isolates from humans and animals from North America and Europe have been classified into one of three genetic lineages—identified as type I, II, or III—based on PCR-RFLP analysis (Howe et al. 1997; Howe and Sibley 1995). These lineages have different virulence

phenotypes in mouse infection models: type I strain is highly lethal in outbred mice, while type II and III strains are significantly less virulent (Sibley and Boothroyd 1992). However, it is not known if this virulence phenotype described in mice may also be observed in other animal species. In the last 10 years, *T. gondii* isolates from South America were characterized as atypical, considering the abovementioned classification, as well as sequencing and microsatellite typing (Ajzenberg et al. 2004; Beck et al. 2009; Pena et al. 2008). While two major clonal lineages—type II and III—dominate clinical and natural isolates in Europe and North America, other parts of the world, like Brazil and Argentina, are dominated by non-clonal or other clonal *T. gondii* lineages representing a greater genetic diversity of *T. gondii* (Ajzenberg 2015; Ajzenberg et al. 2004, Shwab et al. 2014). Until now, 15 haplogroups have been reported worldwide (Su et al. 2012), but the existence of as yet unknown ones in more remote areas of the globe is expected (Ajzenberg 2015). Molecular and genomic *T. gondii* information from different strains reported worldwide can be retrieved from the *Toxoplasma* genomic resource (http://toxodb.org/toxo/). It has been hypothesized that genetic diversity in *T. gondii* is driven by selective pressures due to its adaptation to different intermediate or definitive hosts, included in domestic and wildlife cycles in different parts of the world (VanWormer et al. 2014).

6.3 Clinical Effects, Prevention, and Treatment

6.3.1 Clinical Effects

One of the principal clinical signs is the loss of vision due to a toxoplasmic retinochoroiditis known as ocular toxoplasmosis (Labalette et al. 2002). Congenital toxoplasmosis—the primo-infection during pregnancy—can produce neurological and ocular disorders in fetuses (Jones et al. 2001b). Another clinical presentation is encephalitis in immunocompromised patients caused by reactivation of *T. gondii*.

Toxoplasmosis is an important cause of small ruminant abortions, and the ingestion of undercooked meat and unpasteurized milk from infected animals can be a source of infection for humans (Dubey 2010b). *T. gondii*-caused abortion in sheep and goats is frequently associated with necrosis and calcification of the fetal cotyledons and normal inter-cotyledonary areas (Dubey 2010b). However, since similar lesions can be also observed in *N. caninum*-associated abortion, discrimination between these infections out of histopathological lesions is difficult (McAllister et al. 1996; Ortega-Mora et al. 2007). Repeated abortions in *T. gondii*-infected goats are frequently observed, suggesting a potential reactivation of the disease during successive gestations (Unzaga et al. 2014). Infection during early pregnancy may lead to fetal death and reabsorption; therefore, the ewe appears barren. An infection that takes place between approximately 50 and 120 days of gestation leads to abortion, stillborns, mummified fetuses, or the birth of weak lambs. The infected ewes acquire a protective immunity which prevents future abortions. This fact has been considered for the development and application of a vaccine to prevent sheep

abortion due to *T. gondii* which is available in a few countries like New Zealand, Great Britain, Ireland, Portugal, and Spain.

Clinical effects in avian species vary considerably. Severe toxoplasmosis has been reported in canaries, which can develop ophthalmitis (including cataracts and blindness). Chickens are considered highly susceptible to become infected; however, they are resistant to the development of clinical signs. Nevertheless, a recent case report showed symptomatic chicken, and the highest rates of affected animals were adults with central nervous signs. Further histological examination revealed encephalitis, neuritis, myocarditis, and retina lesions (Dubey et al. 2007). Recently, a study that involved the experimental infection with *T. gondii* of 1-day old chicken was conducted. A significantly higher mortality was observed in white layer lines, as compared to brown layer lines, suggesting that differences in the genetic background may influence the susceptibility of chicken to toxoplasmosis (Schares et al. 2016). Many other avian species have been reported as carrying *T. gondii* infections, although only few cases were reported as suffering a severe disease, most of which were birds raised in captivity.

Pigs infected with *T. gondii* are the most important source of human infection in several countries, especially in the USA (Dubey 2010b). Humans are commonly infected by the ingestion of tissue cysts through the consumption of raw or not well-cooked pork. Clinical toxoplasmosis in pigs is considered rare; however, a few reports from Taiwan, Japan, Switzerland, and the USA have indicated increases in abortions and neonatal mortality and/or febrile and neurological symptoms (Basso et al. 2015; Dubey 2010b). In addition, experimental infection with *T. gondii* in pigs demonstrated that some neonatal infected pigs died after birth, although abortion and congenital infections were rarely observed (Dubey 2010b; Wingstrand et al. 1997).

Cattle are considered one of the least susceptible animal species to develop clinical toxoplasmosis. Moreover, although animals can be infected experimentally, the parasite is eliminated or reduced to undetectable levels in a few weeks post-infection (Dubey 1983). On the other hand, some researchers have detected parasite DNA in bovine fetuses (Gottstein et al. 1998), and the parasite was successfully isolated from two aborted bovine fetuses (Canada et al. 2002). From the above mentioned, it is possible to assume that *T. gondii* can be transmitted vertically in cattle; however, this appears to be a rare occurrence.

Domestic cats and other felids acting as intermediate hosts can develop neurological or ocular signs due to the extraintestinal multiplication of the protozoon. Susceptibility for developing clinical toxoplasmosis increases with feline immunodeficiency virus (FIV) infection, indicating cooperative interaction between *T. gondii* and FIV, as well as in older cats, suggesting a potential reduction of the immunological control of the infection (Dubey and Carpenter 1993). Pallas' cats (*Felis manul manul*) and sand cats (*Felis margarita*) are highly susceptible to develop generalized toxoplasmosis (Basso et al. 2005; Dubey et al. 2010). Intestinal cycle regularly occurs without clinical signs, where an infected feline could be eliminating millions of oocysts.

In dogs, *T. gondii* infections lead to respiratory or neuromuscular clinical signs. However, the latter signs are difficult to differentiate from those produced by *N. caninum* (Dubey et al. 1988a). Therefore, it is important to remark the need of differential diagnosis between these two parasitic infections. Concurrent infections with canine distemper virus could potentiate or reactivate a *T. gondii* infection, and animals can suffer a severe associated disease (Dubey 2010b).

Some species like New World monkeys (Dietz et al. 1997), lemurs (Spencer et al. 2004), Pallas' cats (Basso et al. 2005), slender-tailed meerkats, and some Australian marsupials (Basso et al. 2007; Dubey et al. 1988b) are considered highly susceptible to clinical toxoplasmosis. Most of these animal species suffer a multi-organic or generalized fatal toxoplasmosis. Apparently, animals with short evolutionary contact with *T. gondii* (desert- or tree-living animals) can suffer a severe disease, even if a low-virulence parasite strain is involved (Basso et al. 2009). As example, a colony of slender-tailed meerkats in a zoo was reported to suffer severe and disseminated toxoplasmosis due to a genotype III strain of *T. gondii*, which shows low virulence in mice (Basso et al. 2009). Wallabies, especially females, are considered more susceptible to acute toxoplasmosis than kangaroos (Basso et al. 2007; Dubey et al. 1988b). However, sudden death and similar lesions to those reported previously in the wallaby *Macropus rufogriseus* have been reported in male kangaroos (Adkesson et al. 2007; Basso et al. 2007; Bermudez et al. 2009; Dubey and Crutchley 2008). In addition, a large amount of *T. gondii* cysts was detected in muscles of a *Macropus rufus* kangaroo, which could be related to species or individual susceptibility and/or related to poor immune control of parasite multiplication (Moré et al. 2010). Additionally, the *T. gondii* strain affecting this animal was identified as genotype III, and the case occurred in the same zoo where the meerkats abovementioned died, suggesting that this genotype may be frequent in the zoo environment. Fatal toxoplasmosis has been described in most genera of New World primates in several parts of the world (Gyimesi et al. 2006; Pardini et al. 2015). The infection generally takes the form of acute disease, and animals die suddenly, sometimes without preceding signs. If signs are present, they are nonspecific and include lethargy, malaise, depression, anorexia, diarrhea, hypothermia, serosanguineous nasal discharge, and respiratory distress (Gyimesi et al. 2006; Pardini et al. 2015).

It is possible to assume that stressed animals in some environments suffer more severe toxoplasmosis due to a poor immunity control of parasite multiplication.

6.3.2 Prevention

Since *T. gondii* can persist in different animal species and uses different transmission routes, a complete control and eradication appears utopic. Most recommendations to prevent infection with this protozoon are related to the predominant route of infection in each animal species. Bradyzoites from tissues are inactivated by incubation at 65 °C, freezing at −20 °C during a week, or gamma irradiation at 0.5 kGy (Dubey 1996). Therefore, cooking or freezing potentially infected animal tissues

before ingestion by other species is important to reduce parasite viability and minimize infection. Consumption of raw meat or undercooked products is a frequent cultural behavior; therefore, slaughtered animals destined to human consumption should be analyzed for the presence of *T. gondii* and/or proceed as previously mentioned to minimize the risk of infection.

Once excreted by felids, oocysts are immature. Therefore, daily disposing of stools from domestic cats is useful to reduce potential oocyst dispersion in the environment. Mature sporulated oocysts are long-term resistant, but they are destroyed by boiling water. This can be used for disinfection of surfaces in contact with cat feces. To minimize the chances of a cat being infected and lately contaminate the environment with oocysts, the use of commercial dry or canned feed is recommended. Neutering of cats is recommended to reduce their behavior of hunting birds and rodents. Washing vegetables with safe water is a main recommendation to eliminate or decrease the amounts of potentially present oocysts, as well as preventing felids to take contact with farms or markets that produce or sell vegetables. Humans in contact with soil (potentially contaminated with oocysts) should wash their hands as frequently as possible and/or protect them with garden gloves.

Gamble et al. (1999) reported regional differences in prevalence of *T. gondii* in pigs and suggested that education on farm management practices should be targeted to reduce toxoplasmosis.

Keeping animals in zoo environments is a controversial issue. Several wild felid species can act as definitive hosts, contaminating the environment with oocysts. Unfortunately, the presence of stray domestic cats within zoos around the world is frequently observed, a fact which can have a negative impact on environment contamination. Moreover, several animal species are extremely susceptible to developing fatal toxoplasmosis. In spite of this, a main recommendation to zoo authorities is to build blackout-type enclosures to maintain susceptible species protected from accidental ingestion of oocysts, as well as to minimize stress to avoid potential reactivation of chronic infections.

A live vaccine (Toxovax), using a nonpersistent modified strain of *T. gondii* (S48), available in New Zealand, the UK, and Europe, prevents *T. gondii* abortion in sheep (Buxton et al. 1991). Also, a live vaccine using a mutant strain of *T. gondii* (T-263) is being developed in the USA to reduce oocyst shedding by cats (Verma and Khanna 2013).

6.3.3 Treatment

As yet, there are no drugs able to kill *T. gondii* tissue cysts in humans or animals. Treatment is recommended to reduce tachyzoite multiplication. Drugs such as clindamycin, sulfadiazine, pyrimethamine, toltrazuril, or ponazuril are effective both in vitro and in vivo on *T. gondii* multiplication. These drugs need to be applied for periods of about 30 days to be effective in clinical cases. Corticosteroids are indicated to reduce the extension of inflammatory ocular lesions. A prophylactic treatment of ewes with monensin has been proposed; however, its application is

controversial considering current international protocols to avoid antimicrobial resistance (Dubey 2010b).

It is now apparent that many atypical genotypes exist besides the typical three genotypes first described. These genotypes can differ in virulence and transmissibility from the typical genotypes that have been used in the majority of scientific research over the past 70 years. Recommendations to prevent congenital toxoplasmosis (CT) have been determined based on the information produced by researching with these typical strains (Lindsay and Dubey 2011). It is important to identify the biological behavior of atypical strains and to develop new recommendations for the prevention and the monitoring of women at risk for developing CT, especially in South American countries where a higher diversity of *T. gondii* genotypes has been reported.

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