CHAPTER 1

Transmission of Toxoplasmosis (*Toxoplasma gondii*) by Foods

Karen Signori Pereira,[*,1] **Regina M. B. Franco,**[†] and **Diego A. G. Leal**[†]

Contents

Abstract

Protozoan foodborne diseases are generally underrecognized. *Toxoplasma gondii* is the causative agent of toxoplasmosis, one of the most prevalent parasitic infections to humans and domestic animals. The most likely source of *T. gondii* occurring through food is the consumption of raw or undercooked meat contaminated with tissue cysts. Sporulated *T. gondii* oocysts, from the feces of infected cats, present in the environment are a potential source of infection. The ingestion of water contaminated with oocysts and

* Departamento de Engenharia Bioquímica, Escola de Química, Centro de Tecnologia Bloco E – Sala 203, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil
† Departamento de Parasitologia, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil
[1] Corresponding author: Karen Signori Pereira, *E-mail address:* karenspereira@gmail.com

Advances in Food and Nutrition Research, Volume 60
ISSN 1043-4526, DOI: 10.1016/S1043-4526(10)60001-0

the eating of unwashed raw vegetables or fruits were identified as an important risk factor in most epidemiological studies. This review presents information and data to show the importance of *T. gondii* transmission by foods.

I. TOXOPLASMOSIS

Toxoplasma gondii is the causative agent of toxoplasmosis, one of the most prevalent parasitic infections that afflict humans and other warm-blooded animals; *T. gondii* is the only known species associated with toxoplasmosis (Tenter *et al*., 2000). It is estimated that approximately one-third of the human population worldwide have the parasite.

Congenital toxoplasmosis is a special concern related to *T. gondii* infection which can be especially serious for the fetus if the mother is seronegative, that is, if the mother acquires the primary infection during the pregnancy (Wong and Remington, 1994). The first case of congenital toxoplasmosis in humans was reported by Wolf and Cowen (1937), who identified this protozoan parasite in the brain of a 3-day-old infant with encephalomyelitis. Until the 1940s, there was a scarcity of data of human toxoplasmosis with only a few isolated occurrence reports in children or adults. Improved knowledge about the real prevalence of toxoplasmosis in different regions of the world was made possible with the advent of Sabin–Feldman serologic test, also known as the dye-test, in 1948 (Sabin and Feldman, 1948). Two years later, the protozoan *Toxoplasma* was associated with an inflammatory disease of the eye (Frenkel and Jacobs, 1958).

Over the past several decades, toxoplasmosis has been increasingly recognized as a significant disease with strong implications for public health due to the seriousness of the sequelae derived from the parasitism and associated especially with the congenital or ocular forms. However, the disease has acquired even more importance since 1980, relating to its emergence as an opportunistic pathogen (Wong and Remington, 1993), especially among patients with AIDS, where concomitant infection with the parasite does not often present a favorable prognosis. Moreover, the incidence of toxoplasmosis, either the acquired or congenital forms even in healthy individuals with no immunological impairments, is high in many countries, although most affected individuals do not experience clinical symptoms (Neves *et al*., 2009). Toxoplasmosis is frequently misdiagnosed or underdiagnosed. Toxoplasmosis is the third most common cause of hospitalization due to foodborne infection overall (Mead *et al*., 1999).

II. DISCOVERY

T. gondii is a coccidian protozoan parasite that belongs to the Phylum Apicomplexa and Family Sarcocystidae, with a worldwide distribution (Smith, 2007). This parasite was described in 1908 by Alfonso Splendore, who identified this organism in a rabbit that died from parasitic disease (Splendore, 1908). In that same year, Nicolle and Manceaux (1909) found the same parasite in an African rodent and denominated this new organism as *Leishmania gondii*. Subsequently, these same researchers verified that the parasite earlier described as *Leishmania* did not possess the marked characteristics of protozoan parasites of the Phylum Kinetoplastida and thus they proposed the name *T. gondii*, based on the shape of the tachyzoite form of this parasite (''*toxon: arc; plasma: life*'') (Nicolle and Manceaux, 1909).

III. *T. GONDII* LIFE CYCLE

The life cycle of *T. gondii* includes felines, the definitive hosts that shed oocysts into the environment (Frenkel *et al*., 1970). It is well established that oocysts can be stable in the environment for up to a year (Dubey, 1998a). This parasite is also capable of infecting a wide range of other warm-blooded animals (mammals and birds) that are the intermediate hosts. Upon infection, the sporozoites located in two sporocysts inside the oocysts are released and establish a new infection in the enterocytes of both intermediate and definitive hosts. Thus, *T. gondii* has a life cycle that is comprised of three infective stages: tachyzoites, bradyzoites (in tissue cysts), and sporozoites (in oocysts).

The asexual developmental cycle of this protozoan occurs within any nucleated cell of warm-blooded animals. This cycle consists of two infective stages: *tachyzoites*, which undergo fast multiplication in various host cell types and are typically present in the acute infection, and *bradyzoites*, which undergo slow multiplication in latent tissue cysts, have a high affinity for neural and muscular tissues and are located predominantly in the central nervous system, the eye, and skeletal and cardiac muscles.

The bradyzoites characterize the chronic phase of toxoplasmosis. According to Dubey *et al*. (1998a,b), cysts can also develop, but to a lesser extent, in any visceral organs, such as lungs, liver, and kidneys. While infected host cells are filled by tachyzoites and are destroyed by their release, the bradyzoites contained in tissue cysts continue the process of multiplication through endodyogeny (Tenter, 2009).

The interaction between *T. gondii* and host cells involves an early stage of adhesion and posterior invasion of the host cell; these steps are both

crucial for the establishment of parasite infection. The parasitophorous vacuole is an interface between the parasite and host cell functions and facilitates the replication and differentiation of the parasite. In addition, this vacuole provides protection against free radicals, pH, and osmolarity changes, and also assists the parasite in the mechanism of evasion and activation of the host immune system (Laliberté and Carruthers, 2008).

Intermediate hosts become infected with *T. gondii* after ingesting infective oocysts that were shed in the environment or tissue cysts from infected animals. Parasite replication in the small intestine eventually leads to the lysis of enterocytes and consequently, tachyzoites disseminate throughout the host (Petersen and Dubey, 2001). By using stage-specific markers such as SAG1 for tachyzoites and BAG1 for bradyzoites, it was possible to follow the life cycle transitions through the acute and chronic phases in live mice (Ferguson, 2009).

Briefly, during the early stages of infection, tachyzoites are observed initially in the lymph nodes, spleen, and lungs, and 10 days postinfection, dissemination may occur to all organs of the body, including the brain and heart. The conversion of tachyzoite to bradyzoite occurs between 12 and 15 days postinfection and cyst formation takes place in the brain of the mouse. More tissue cysts are produced in mice that become mildly ill from infection than in those that become highly symptomatic (Weiss and Kim, 2007). In mice, the protozoan showed marked tissue tropism, since no evidence of stage conversion is observed in any other organ (however, there is a variation between different host species; in cats, the majority of tissue cysts are found in the muscles), and individual parasites may express both tachyzoite and bradyzoite antigens simultaneously as revealed by dual labeling techniques (Ferguson, 2009). While some organisms continue to proliferate as tachyzoites before being recognized and destroyed by the host immune response, other parasites invade new cells and develop into bradyzoites contained in tissue cysts.

The mechanism that triggers the conversion of tachyzoites into bradyzoites is still not completely understood, but it appears that tissue cyst development is initiated at the time of parasite entry in the cell with the formation of a distinctive vacuole. After approximately 3 months, there is a reduction in the number of dividing organisms. The size of tissue cysts is variable (5–70 μm) and may harbor a few to several hundred bradyzoites (Dubey, 2004).

Generally, in most immunocompetent hosts, the immune response controls the replication, disease is limited, and the physiological stress suffered by the parasites causes the differentiation of the tachyzoites into bradyzoite cysts that may persist throughout the host's life (Tenter *et al.*, 2000). Tissue cysts undergo periodic reactivation, but these events are modulated by an intact immune system. In chronically infected hosts that lose T-cell function, reactivation may lead to disease (Petersen and Dubey,

2001). It is noteworthy that tissue cysts are the terminal life cycle stage in the intermediate hosts and are immediately infectious.

If ingested by a definitive host (members of Family Felidae), the bradyzoites initiate another asexual phase of proliferation in the epithelial cells of the small intestine. Then, the sexual phase of the life cycle is initiated with gamogony culminating in the formation of oocysts. Unsporulated oocysts are released into the intestinal lumen and are passed into the environment with the feces of felids. In the case of this apicomplexan protozoan, sporogony occurs outside the host and leads to the development of infectious oocysts (10 μm × 12 μm in diameter) which contain two sporocysts, each containing four sporozoites (Dubey *et al*., 1998a). The sporulation of the oocysts is directly dependent on environmental factors such as temperature and aeration, and after 1–5 days, the oocysts may become infectious (Dubey, 2004). Cats can shed upward of 360 million oocysts in their feces in a single day and oocysts were shed for 4–6 days (Dubey, 2002). These felines are essential in the life cycle of *T. gondii* (Dubey and Su, 2009).

IV. TRANSMISSION OF TOXOPLASMOSIS

Toxoplasma infection can be transmitted by the ingestion of oocysts—shed into the environment from cat feces—which may contaminate water, soil, and vegetables, or also by viable tissue cysts found in raw or undercooked meat of intermediate hosts. Oocysts are highly infectious to herbivores and bradyzoites to cats. Infections caused through the ingestion of oocysts are considered more severe clinically in intermediate hosts than those related through the ingestion of tissue cysts (Hill and Dubey, 2002).

Thus, one factor that contributes to the widespread distribution of *T. gondii* on all continents consists of the successful adaptability and development of different transmission modes exhibited by this protozoa (Dubey and Su, 2009) and that carnivorism and cannibalism contribute to and aggravate the persistence of the protozoan in nature, even in the absence of the felid sexual cycle (Su *et al*., 2003).

As tachyzoites are sensitive to environmental conditions (and they usually die very rapidly outside the host), foodborne transmission of *T. gondii* via tachyzoite is probably not significant epidemiologically and occurs only infrequently (Tenter, 2009). Tachyzoites of *T. gondii* have already been detected in body fluids such as saliva, sputum, urine, tears, semen, and milk of several intermediate hosts, including sheep, goats, cows, and camels (Tenter *et al*., 2000).

From literature data, there is great evidence that tachyzoites present in colostrum or maternal milk can infect infants via breast-feeding when mothers have a primary *T. gondii* infection (Bonametti *et al*., 1997b;

Tavares *et al.*, 2006). The lower concentration of proteolytic enzymes in the gastrointestinal tract of a child may explain these cases. In addition, Dubey *et al.* (1998a,b), reported that tachyzoites may occasionally survive for a short period of time (about 2 h) in acid pepsin solutions.

Moreover, oral infections by tachyzoite penetration in the host oral mucosal tissue were hypothesized by Johnson (1997), Riemann *et al.* (1975), and Sacks *et al.* (1982). On the other hand, bradyzoites of *T. gondii* are more resistant to digestive enzymes such as pepsin and trypsin (Dubey, 1998b; Jacobs *et al.*, 1960). It was also demonstrated that *T. gondii* cysts maintain their infectivity at temperatures of 4 °C over a period of 30 days (Tenter, 2009), while heating is the most efficient way to kill *T. gondii* tissue cysts (Kijlstra and Jongert, 2008a).

V. PATHOGENESIS AND HUMAN INFECTION SPECTRA

Infection with *T. gondii* is an important cause of diseases of the central nervous system and the eye in immunocompromised as well as immunocompetent individuals. When first acquired by the mother, this infection can be transmitted to the fetus. Infants with the most severe clinical signs in the brain and eye are those infected early in pregnancy when fetal immunity is low (Jamieson *et al.*, 2009). At birth, infants infected *in utero* may have intracranial calcification, hydrocephalus, convulsions, and ocular diseases such as retinochoroiditis or inflammation of the retina and choroid, with associated vitritis. The severity of disease is influenced by the trimester in which the infection is acquired by the mother (Dunn *et al.*, 1999; Remington *et al.*, 2006). A positive correlation exists between the rate of transmission and infection during the second or third trimesters of pregnancy (Desmonts and Couvreur, 1984; Dunn *et al.*, 1999).

In mothers previously exposed to *T. gondii*, the fetus is very rarely infected (Remington *et al.*, 2006), which suggests that natural maternal immunity against *T. gondii* is sufficient to protect the fetus from vertical transmission.

Untreated acute toxoplasmosis among pregnant women can lead to infection of the fetus via transplacental transmission (Varella *et al.*, 2009.). At first examination, newborns affected by congenital infection may seem normal; however, serious sequelae, such as neurological impairment and blindness, can develop within a few years later (Dunn *et al.*, 1999; Remington *et al.*, 2006; Safadi *et al.*, 2003.).

Some reports have suggested that pregnant women diagnosed with acute toxoplasmosis should be treated as soon as possible to reduce the risk and severity of congenital infection (Gilbert *et al.*, 2001; Gras *et al.*, 2005.). Moreover, the gestational age at primary infection is a critical factor that determines the clinical management of pregnant women, since the severity

of toxoplasmosis for the fetus decreases and the transmission rate increases with enhanced gestational age (Beguetto *et al.*, 2003).

When the mother is infected in the first trimester of pregnancy, abortion or stillbirth can occur. When mothers acquired their first infection in the second or third trimester, only 15% and 5% of children presented with a subclinical infection form at birth (Gras *et al.*, 2005).

Lopes *et al.* (2009) conducted a study with 492 pregnant women in Londrina, Paraná State, Brazil, in order to determine the risk factors associated with anti-*T. gondii* seropositivity, using a multivariate regression analysis approach. Age group, a low level of education, a low per capita income (< US$ 88.23), presence of a cat in the house, and the habit of eating green vegetables were considered important risk factors that might be related to the acquisition of the disease, while Spalding *et al.* (2005) showed that pregnant women who had contact with soil had the greatest risk of acquiring toxoplasmosis.

It is well established that the strain of *Toxoplasma* can have an effect on the pathology of the infections (Dubey, 1998a). The manifestations of the disease can vary significantly from one host to another and different components contribute to the severity of the disease, including (i) host species, (ii) immune status of host, and (iii) biological and genetic variation within the parasite (Innes, 1997).

While asymptomatic infection with *T. gondii* resulting in a latent infection with tissue cysts is common in humans, symptomatic infection is much less frequent. In immunocompetent individuals, symptoms only occur in 10–20% of the cases; infected people develop chorioretinitis, lymphadenitis, myocarditis, or polymyositis. Although any lymph node may be infected, the most common manifestation is asymptomatic cervical lymphadenopathy (Weiss and Dubey, 2009).

In acute phase, the clinical manifestations include a mononucleosis-like syndrome, fever, lymph node enlargement, asthenia, and headache (Remington *et al.*, 2006). Neves *et al.* (2009) related the most frequent signs and symptoms in a cohort of 37 patients that attended Evandro Chagas Clinic Research Institute, FIOCRUZ, Brazil, as follows: lymph node enlargement was the most commonly observed alteration (35/37; 94.6%), abdominal ultrasound reveals liver enlargement in six patients (6/37; 16.2%), and splenomegaly in two patients (2/37; 5.4%). Asthenia (32/37; 86.5%), headache (26/37; 70.3%), fever (25/37; 67.6%), and weight loss (23/27; 62.2%) were the most common symptoms.

Immunocompromised patients that develop clinical disease have impairments in T-cell function, thus highlighting the importance of lymphocytes in controlling this persistent infection. In addition, the presence of bradyzoites and their subsequent rupture can cause life-threatening recrudescence of acute infection in immunocompromised individuals (Sullivan *et al.*, 2009). The immune response effectively prevents

dissemination of this protozoan, but sometimes a spontaneous reactivation of the latent infectious occurs. Production of Interferon-gamma (IFN-γ) by T cells, natural killer cells, and various other cell types in the brain protects the host against toxoplasmic encephalitis. The production of IFN-γ by microglia during the early stages of tachyzoite proliferation in the brain may be a critical factor in limiting parasitic growth (Wang and Suzuki, 2007).

The most vulnerable risk groups to acquire the infection and develop severe toxoplasmosis include persons with primary or acquired immunodeficiency and several deficits in T cell, monocyte, cytokine, and B cell functions; cancer patients with immunosuppressive cancers such as leukemia and lymphoma; transplant patients receiving immunosuppressive drugs and patients with hyper-IgM syndrome as well as those receiving corticosteroids (Weiss and Dubey, 2009). HIV-infected patients have an increased incidence of encephalomyopathy and encephalitis is the most important clinical manifestation of toxoplasmosis in these patients (Dubey, 2004; Horowitz *et al.*, 1983.; Jones *et al.*, 1996.). Nowadays, although widespread use of highly active anti-retroviral therapy (HAART) has led to better control of viral replication promoting an increase in immune function, new immunosuppressed cohorts are continually emerging like patients with autoimmune disorders treated with immunomodulatory factors (Hemmer *et al.*, 2006.).

Ocular toxoplasmosis is the most frequent cause of infectious blindness and visual morbidity among young adults in developed countries (Hovakimyan and Cunningham, 2002). The severity of the disease varies greatly between patients. The activation of dormant organisms within the retina causes necrotizing retinopathy and the severity of the disease is probably correlated with the most common causes of retinochoroiditis worldwide, hypersensitivity and inflammation (Garweg and Candolfi, 2009).

Recent evidence has identified interleukin (IL)-17 as a marker for disease severity. Other mediators include IL-23 which induces the proliferation of IL-17 producing cells and IL-27 which may regulate TH1-cell-mediated responses. CD25(+) regulatory T cells may control the local inflammatory response and protect the host against collateral inflammatory tissue damage (Garweg and Candolfi, 2009).

VI. LABORATORY DIAGNOSIS AND TREATMENT

According to Mozzato and Procianoy (2003), laboratory diagnosis poses a challenge for health care professionals due to the complexity in the interpretation of the results. Moreover, we need to consider that the modern laboratory techniques which have arisen in last decades are not

always available in the national public health care system of the developing countries by comparison to the more developed countries.

Generally, the diagnosis of toxoplasmosis in man may be done by serologic tests, PCR (which involves the amplification of specific nucleic acid sequences), histologic demonstration of the parasite, or by isolation of the protozoan that might be done by an animal infectivity assay or inoculation in human tissue cell cultures (Montoya, 2002).

Serologic test is the primary method of diagnosis and utilize a specific antibody to *T. gondii*. Other immunological methods include complement fixation tests, direct agglutination tests, ELISA, indirect agglutination tests, an immunosorbent agglutination test, and a latex agglutination test.

Toxoplasmosis is usually diagnosed based on the detection of specific IgG and IgM antibodies; however, the inclusion of other tests is mandatory for a conclusive diagnosis of toxoplasmosis during pregnancy, in HIV/AIDS patients and in neonates (Jones *et al.*, 2003.). These tests must include measurement of IgG avidity, IgA, IgE, and direct detection by PCR in all cases, including the amniotic fluid. Since IgG can persist for decades, IgM which typically persists for 6–9 months is used as a marker of recent infection.

While prenatal diagnosis is based on the detection of *T. gondii* in the amniotic fluid, neonatal screening is based on the detection of parasites in the placenta and on the detection of IgM and IgA antibodies in newborns. PCR for the detection of parasite DNA in amniotic fluid has improved the sensitivity of prenatal diagnosis (Bessiéres *et al.*, 2009). The accurate diagnosis of congenital toxoplasmosis is essential, since if the mother is treated it would reduce the probability of fetal infection by 50% (Desmonts and Couvreur, 1974).

Treatment of toxoplasmosis may vary with the form or the case. Generally, in immunocompetent patients treatment is usually unnecessary since the infection is subclinical and the immune response is able to control it. However, in immunocompromised patients (including HIV and other risk groups), the patients need to be treated and monitored since toxoplasmosis is a major cause of death among AIDS patients (Dubey, 2004). In these patients, the recommended treatment is a combination of two drugs, pyrimethamine (25–100 mg daily) and trisulfapyrimidines (2–6 g daily), administered for 1 month where this combination acts by inhibiting the enzyme, dihydrofolate reductase, of *T. gondii* preventing the synthesis of DNA and proteins.

In some cases, as in cerebral toxoplasmosis, which is frequently seen in HIV patients but may occur with atypical manifestations, it is extremely important to do the differential diagnosis from several other neurological infections like lymphoma and other cancers (Montoya, 2002).

The diagnosis of cerebral toxoplasmosis might be done in association with complementary methods such as imaging and immunological

methods principally on cerebrospinal fluid and blood (Montoya, 2002; Schroeder *et al.*, 2006.). The cerebral biopsy is considered the best and most definitive method of diagnosis for cerebral toxoplasmosis because this method may demonstrate the presence of tachyzoites. However, this approach is used sparingly (Hornef *et al.*, 1999) because it is an invasive procedure. The finding of lesions in the brain demonstrated with imaging methods and a positive serological test for toxoplasmosis must guide the physician to a specific therapeutic approach against the protozoa.

In the case of ocular toxoplasmosis where the signs and symptoms may be misdiagnosed as some other disease, the patients may present with a photophobia and see floaters; then a differential diagnosis is also necessary. The ophthalmologic examination with a slit-lamp allows the physician to observe the presence of a granulomatous inflammation and the fundoscopy demonstrates the presence of a yellow focus of retinochoroiditis (Guex-Crosier, 2009). The treatment for ocular toxoplasmosis consists of the administration of sulfadiazine which interferes with the formation of folic acid from *para*-aminobenzoic acid, associated with pyrimethamine, which interferes with the conversion of folic acid to folinic acid through dihydropteroate synthase (Guex-Crosier, 2009).

For many years, ocular toxoplasmosis was traditionally considered a manifestation of congenital and postnatal infections. However, in the last decade, several studies have shown that most of the ocular disease caused by *T. gondii* is attributed to acquired disease and this fact was also evidenced in the epidemiological study of some waterborne outbreaks (Gilbert and Stanford, 2000; Holland, 1999). Ocular disease may also occur by the reactivation of the parasite when the cysts are present within the retina (Guex-Crosier, 2009). Summarizing, it is reasonable to assume that the investigation of ocular toxoplasmosis does not have to be focused only in pregnant women or in immunocompromised individuals but also must include the immunocompetent people.

VII. TOXOPLASMOSIS TRANSMISSION BY FOODS

One-third of the human world population is infected with the protozoan parasite *T. gondii*. Recent calculations of the disease burden of toxoplasmosis rank this foodborne disease at the same level as salmonellosis or campylobacteriosis (Kijlstra and Jongert, 2008a).

T. gondii does not grow in foods or in other environments outside of a suitable host; however, oocysts in environmental can survive for long periods at temperatures ranging from 4 to 37 °C. According to Waldeland (1977a,b,c), an oocyst can survive in soil for up to 2 years; so, any fecal material from infected cats will represent a hazard (Nesbakken, 2009).

As noted earlier, toxoplasmosis is acquired by ingesting food and water contaminated with oocysts from feces of infected cats or by the ingestion of raw or undercooked meat, containing tissue cysts (bradyzoites), of an infected intermediary host. Transmission of human toxoplasmosis occurs mainly through the ingestion of food containing cysts of *T. gondii*, found in sheep, pigs, cows, chickens, and goats. Uncooked pork and its derivatives have been the main foods implicated in outbreaks of toxoplasmosis. However, while consumption of raw or undercooked meat was consistently identified as a risk factor, the relative importance of the risk factor and the type of meat associated with it varied among different countries (Tenter *et al*., 2000).

Belford-Neto *et al*. (2007)., in Erechim (Rio Grande do Sul state, Brazil), collected samples from porcine tongue and diaphragm obtained in both large and small abattoirs and used molecular biological techniques to determine the prevalence of *T. gondii*. Seventeen out of 50 (34%) samples from the diaphragm and 33 out of 50 (66%) samples from the tongue demonstrated a positive PCR reaction for *T. gondii.* In Londrina, Paraná state of Brazil, 149 samples of sausage were collected from eight factories. Using a mouse bioassay, 13 (8.7%) sausage samples were positive; in one of them *T. gondii* was isolated and in the other 12, the mice seroconverted (Dias *et al*., 2005). In the United States, the survey of 698 retail outlets determined the prevalence of viable *T. gondii* tissue cysts in commercially available fresh pork products to be 0.38% (Dubey *et al*., 2005). In a study by Warnekulasuriya *et al*. (1998), in London, *T. gondii* was detected in ready-to-eat cured meat samples by amplification of the parasite's P30 gene using PCR. Viable *T. gondii* was detected in 1 (cured ham) out of 67 ready-to-eat cured meat samples. In 2002, Aspinall *et al.* (2002) analyzed, using primers specific for the *T. gondii SAG2* locus, 71 meat samples obtained from UK retail outlets and found that 27 of the meat samples showed the presence of this parasite.

While toxoplasmosis outbreaks have been mainly related to the ingestion of undercook meat, a few outbreaks could be attributed to milk ingestion; *T. gondii* was isolated in the milk of naturally infected cows (Hiramoto *et al*., 2001). Unpasteurized goat milk was implicated as a source of infection of *T. gondii* in several reports (Chiari and Neves, 1984; Riemann *et al*., 1975; Skinner *et al*., 1990). So, unpasteurized milk and dairy products could be an important source of human infection with *T. gondii.*

A study from Hiramoto *et al*. (2001) assessed the infectivity of cysts of the ME-49 strain of *T. gondii* in artificially infected bovine milk and derived fresh homemade cheese. The infectivity of cysts of the ME-49 strain of *T. gondii* was maintained in the milk even after storage for 20 days at refrigerator temperatures. Cysts were also able to survive the production process of homemade fresh cheese and storage for a period of 10 days in the same conditions.

According to Kapperud *et al*. (1996) oocysts from cat feces can contaminate fields and therefore vegetable products. The eating of unwashed raw vegetables or fruits was identified as an important risk factor in most epidemiological studies (Dorny *et al*., 2009). Waterborne outbreaks of toxoplasmosis are possible if water is untreated, and consuming seafood from contaminated water basins may pose a theoretical risk. Experimentally, viable *T. gondii* oocysts were recovered from oysters (*Crassostrea virginica*; Lindsay *et al*., 2004) and mussels (*Mytilus galloprovincialis*; Arkush *et al*., 2003).

VIII. TOXOPLASMOSIS OUTBREAKS ASSOCIATED WITH WATER AND FOODS

Between 1999 and 2008, in Brazil, four toxoplasmosis outbreaks associated with the ingestion of contaminated water and/or foods were registered according to the Brazilian Ministry of Health (Secretaria de Vigilância em Saúde (SVS) of Brasil, 2009).

According to Mead *et al*. (1999), *T. gondii* was associated with the third-highest number of deaths caused by foodborne pathogens in the United States. Research from Hoffmann *et al*. (2007) found that toxoplasmosis is the third most common cause of fatal foodborne disease associated with pork ingestion. In the United States, *T. gondii* and *Listeria monocytogenes* are the most important foodborne pathogens in pregnancy, and these organisms can induce death or grave disease in the fetus and newborn (Smith, 1999).

Outbreaks of toxoplasmosis are rarely seen since most infected immunocompetent individuals show few or no symptoms. However, 20 toxoplasmosis outbreaks were well described and discussed by Smith in 1993. Of them, 5 were associated with the ingestion of raw goat milk, 11 were associated with the ingestion of raw meat, and 1 was associated with the ingestion of creek water (Smith, 1993).

Some outbreaks of toxoplasmosis associated with the ingestion of oocyst-contaminated water have been documented. Benenson *et al*. (1982) described an outbreak that occurred among British troops in Panama in the jungle associated with the consumption of creek water probably contaminated with oocysts excreted by jungle cats.

Another outbreak occurred in British Columbia, Canada in 1995, and 110 acute toxoplasmosis cases were identified. The epidemiological studies demonstrated that the outbreak was consistent with a waterborne source and implicated contaminated municipal drinking water (Bowie *et al*., 1997).

The largest toxoplasmosis outbreak was registered in Santa Isabel do Ivai, Paraná state, Brazil, and unfiltered, municipally treated water was

the epidemiologically implicated source of *T. gondii* dissemination. The outbreak peaked between November 2001 and January 2002, and involved at least 426 people (Moura *et al*., 2006).

An outbreak of acquired ocular toxoplasmosis involving 248 people in India was described by Balasundaram *et al*. (2010). The suspected source of the infection was municipal drinking water.

Chiari and Neves (1984) described an acute toxoplasmosis outbreak involving three members of the same family in Minas Gerais State, Brazil. The source of the infection was unpasteurized goat's milk. Another outbreak, involving 10 people, associated with ingestion of unpasteurized goat's milk was registered by Sacks *et al*. (1982) and occurred in California, USA.

Despite these episodes, the most likely source of *T. gondii* infection is the consumption of raw or undercooked meat contaminated with tissue cysts.

In 1969, an outbreak of acute toxoplasmosis involved five medical students from Cornell Medical College. The students all ate rare hamburger at the same place, on the same night, and evidence indicates that this was the vehicle for transmission of the infection (Kean *et al*., 1969).

Fertig *et al*. (1977) related a toxoplasmosis outbreak involving three people in London; there was no direct contact with cats, and the relevant communal meal taken included inadequately grilled lamb. In Brazil, Bandeirantes city (Paraná State), one registered outbreak of acute toxoplasmosis involved 17 people. The illness was acquired by the ingestion of raw mutton offered during a party in September 1993 (Bonametti *et al*., 1997a). In 2005, 10 people from the same family acquired toxoplasmosis in Santa Vitória do Palmar (Rio Grande do Sul State, Brazil). Epidemiological studies pointed to the source of the outbreak as an industrialized cured meat (*copa*) (Tavares *et al*., 2006).

Two outbreaks of acute toxoplasmosis in Korea were linked to eating undercooked pork. In the first, three people were infected after eating a meal consisting of raw spleen and liver from a wild pig. In the second outbreak, five soldiers were infected after eating a meal of raw liver from a domestic pig (Choi *et al*., 1997.; Dawson, 2005). In Australia, a family outbreak of toxoplasmosis involving five members of a Lebanese family was described by De Silva *et al*. (1984). Kibbi, a traditional Lebanese dish, prepared with raw meat may have been the source of the infection. Another outbreak occurred in Australia and was associated with ingestion of undercooked kangaroo and lamb meat (Robson *et al*., 1995).

IX. *T. GONDII* CONTROL (IN FOODS)

T. gondii control in foods is primarily associated with adequate cooking and/or prevention of cross-contamination. According to Hillers *et al*. (2003), the main consumer food-handling behaviors associated with

prevention are, respectively: (i) use a thermometer to make sure that meat and poultry (including ground) are cooked to safe temperatures, (ii) keep pets out of food preparation areas, (iii) wash hands with warm soapy water before and after handling raw foods, and (vi) knives, cutting boards, and food preparation surfaces should be washed with hot water and soap after contact with raw poultry, meat, and seafood. Washing is effective because the stages of *T. gondii* in meat are killed by contact with soap and water (Dubey and Beattie, 1988).

Commercial procedures of curing with salt or low temperature smoke and the treatment of meat with enhancing solutions such as potassium or sodium lactate, can also kill *T. gondii* tissue cysts, although the inactivation of these cysts depends of the synergistic interaction between salt concentration, maturation time, and temperature of storage (Kijlstra and Jongert, 2008b). With contaminated pork and pork products, Hill *et al.* (2004) found that the injection, within 8 h, of 2.0% NaCl or 1.4% or higher lactate-based salt solutions into pork loins containing infective tissue cysts prevented transmission of *T. gondii*. Storage at meat case temperatures at or below 0 °C (32 F) for 7 days also killed *T. gondii* tissue cysts in pork loins (Hill *et al.*, 2004.). Lundén and Uggla (1992) investigated the effects of curing with sodium chloride and sucrose, low-temperature smoking, freezing at −20 °C, and cooking in a microwave oven, respectively, on the infectivity of *T. gondii* encysted in mutton. *T. gondii* was not isolated from cured, smoked, or frozen meat. However, in two of four steaks processed in a microwave oven, according to a standard household recipe, the parasite remained infective, possibly due to uneven heating of the meat. The tissue cysts of *T. gondii* are relatively resistant to changes in temperature and remain infectious in refrigerated (1–4 °C) carcasses or minced meat for up to 3 weeks (Tenter, 2009). According to Dubey (2004), tissue cysts in meat are killed by heating to an internal temperature of 67 °C or by cooling to −13 °C. However, occasionally some tissue cysts may survive deep-freezing and it has even been suggested that some strains of *T. gondii* may be resistant to freezing (Tenter, 2009).

More recently, modern food processing technologies using irradiation or high pressure treatment were analyzed for the inactivation of the parasite with promising results (Kijlstra and Jongert, 2008a).

High pressure processing (HPP) at 340, 400, 480, and 550 MPa has been shown to inactivate *T. gondii* oocysts under experimental conditions (Lindsay *et al.*, 2005). *T. gondii* in tissue cysts and oocysts are also killed by exposure to 0.5 krad of γ-irradiation (Dubey, 1998a,b.; Dubey and Thayer, 1994).

REFERENCES

Arkush, K. D., Miller, M. A., Leutenegger, C. M., Gardner, I. A., Packham, A. E., Heckeroth, A. R., Tenter, A. M., Barr, B. C., and Conrad, P. A. (2003). Molecular and bioassay-based detection of *Toxoplasma gondii* oocyst uptake by mussels (*Mytilus gallo-provincialis*). *Int. J. Parasitol.* **33**(10), 1087–1097.

Aspinall, T. V., Marlee, D., Hyde, J. E., and Sims, P. F. G. (2002). Prevalence of *Toxoplasma gondii* in commercial meat products as monitored by polymerase chain reaction – food for thought? *Int. J. Parasitol.* **32**(9), 1193–1199.

Balasundaram, M. B., Andavar, R., Palaniswamy, M., and Venkatapathy, N. (2010). Outbreak of acquired ocular toxoplasmosis involving 248 patients. *Arch. Ophthal.* **128**(1), 28–32.

Beguetto, E., Buffolano, W., Spadoni, A., Del Pezzo, M., Di Cristina, M., Minenkova, O., Petersen, E., Felici, F., and Gargano, N. (2003). Use of an Immunoglobulin G avidity assay based on recombinant antigens for diagnosis of primary *T. gondii* infection during pregnancy. *J. Clin. Microbiol.* **41**, 5414–5418.

Belford-Neto, R., Nussenblatt, V., Rizzo, L., Muccioli, C., Silveira, C., Nussenblatt, R., Khan, A., Sibley, D., and Belford, R., Jr. (2007). High prevalence of unusual genotypes of *Toxoplasma gondii* infection in pork meat samples from Erechim, Southern Brazil. *An. Acad. Bras. Ciênc.* **79**(1), 111–114.

Benenson, M. W., Takafuji, E. T., Lemon, S. M., Greenup, R. L., and Suler, A. J. (1982). Oocyst-transmitted toxoplasmosis associated with ingestion of contaminated water. *N. Engl. J. Med.* **307**(11), 666–669.

Bessiéres, M. H., Berrebi, A., Cassaing, S., Fillaux, J., Cambus, J. P., Berry, A., Assouline, C., Ayoubi, J. M., and Magnaval, J. F. (2009). Diagnosis of congenital toxoplasmosis: prenatal and neonatal evaluation of methods used in Toulouse University Hospital and incidence of congenital toxoplasmosis. *Mem. Inst. Oswaldo Cruz* 389–392.

Bonametti, A. M., Passos, J. N., da Silva, E. M. K., and Bortoliero, A. L. (1997a). Surto de toxoplasmose aguda transmitida através da ingestão de carne crua de gado ovino. *Rev. Soc. Bras. Med. Trop.* **30**(1), 21–25 [online].

Bonametti, A. M., Passos, J. N., da Silva, E. M. K., and Macedo, Z. S. (1997b). Probable transmission of acute toxoplasmosis through breast feeding. *J. Trop. Pediatr.* **43**, 116.

Bowie, W. R., King, A. E., Werker, D. H., Isaac-Renton, J. L., Bell, A., Eng, S. B., and Marion, S. A. (1997). Outbreak of toxoplasmosis associated with municipal drinking water. The BC Toxoplasma investigation team. *Lancet* **350**(9072), 173–177.

Chiari, C. A. and Neves, D. P. (1984). Toxoplasmose humana adquirida através da ingestão de leite de cabra. *Mem. Inst. Oswaldo Cruz* **79**, 337–340.

Choi, W. Y., Nam, H. W., Kwak, N. H., Huh, W., Kim, Y. R., Kang, M. W., Cho, S. Y., and Dubey, J. P. (1997). Foodborne outbreaks of human toxoplasmosis. *J. Infect. Dis.* **175**(5), 1280–1282.

Dawson, D. (2005). Foodborne protozoan parasites. *Int. J. Food Microbiol.* **103**(2), 207–227.

De Silva, L. M., Mulcahy, D. L., and Kamath, K. R. A. (1984). Family outbreak of toxoplasmosis: a serendipitous finding. *J. Infect.* **08**, 163–167.

Desmonts, G. and Couvreur, J. (1974). Toxoplasmosis in pregnancy and its transmission to the fetus. *Bull. N. Y. Acad. Med.* **50**, 146–159.

Desmonts, G. and Couvreur, J. (1984). Toxoplasmose congénitale. *Ann. de Pediatr. (Paris)* **31**, 805–809.

Dias, R. A. F., Navarro, I. T., Ruffolo, B. B., Bugni, F. M., Castro, M. V., and Freire, R. I. (2005). *Toxoplasma gondii* in fresh pork sausage and seroprevalence in butchers from factories in Londrina, Paraná state, Brazil. *Rev. Inst. Med. Trop. Sao Paulo* **47**(4), 185–189.

Dorny, P., Praet, N., Deckers, N., and Gabriel, S. (2009). Emerging food-borne parasites. *Vet. Parasitol.* **163**(3), 196–206.

Dubey, J. P. (1998a). *Toxoplasma gondii* oocysts survival under defined temperatures. *J. Parasitol.* **84**, 862–865.

Dubey, J. P. (1998b). Re-examination of resistance of *Toxoplasma gondii* tachyzoites and bradyzoites to pepsin and trypsin digestion. *Parasitology* **116**, 43–50.

Dubey, J. P. (2002). Tachyzoite-induced life cycle of *Toxoplasma gondii* in cats. *J. Parasitol.* **88**, 713–717.

Dubey, J. P. (2004). Toxoplasmosis – a waterborne review. *Vet. Parasitol.* **126**, 57–72.

Dubey, J. P. and Beattie, C. P. (1988). Toxoplasmosis of Animals and Man. CRC Press, Boca Raton, FL, 220 pp.

Dubey, J. P. and Su, C. (2009). Population biology of *Toxoplasma gondii:* What's out and where did they come from? *Mem. Inst. Oswaldo Cruz* **104**, 190–195.

Dubey, J. P. and Thayer, D. W. (1994). Killing of different strains of *Toxoplasma gondii* tissue cysts by irradiation under defined conditions. *J. Parasitol.* **80**, 764–767.

Dubey, J. P., Lindsay, D. S., and Speer, C. A. (1998a). Structures of *Toxoplasma gondii* tachyzoites, bradyzoites and sporozoites and biology and development of tissue cysts. *Clin. Microbiol. Rev.* **11**, 267–299.

Dubey, J. P., Thayer, D. W., Speer, C. A., and Shen, S. K. (1998b). Effect of gamma irradiation on unsporulated and sporulated *Toxoplasma gondii* oocysts. *Int. J. Parasitol.* **28**(3), 369–375.

Dubey, J. P., Hill, D. E., Jones, J. L., Hightower, A. W., Kirkland, E., Roberts, J. M., Marcet, P. L., Lehmann, T., Vianna, M. C. B., Miska, K., Sreekumar, C., Kwok, O. C. H., Shen, S. K., and Gamble, H. R. (2005). Prevalence of viable *Toxoplasma gondii* in beef, chicken, and pork from retail meat stores in the United States; risk assessment to consumers. *J. Parasitol.* **91**, 1082–1093.

Dunn, D., Wallon, M., Peyron, F., Petersen, E., Peckham, C., and Gilbert, R. (1999). Mother-to-child transmission of toxoplasmosis: Risk estimates for clinical counseling. *Lancet* **353**, 1829–1833.

Ferguson, D. J. P. (2009). *Toxoplasma gondii*: 1908–2008, homage to Nicolle, Manceaux and Splendore. *Mem. Inst. Oswaldo Cruz* **104**, 133–148.

Fertig, A., Selwyn, S., and Tibble, M. J. (1977). Tetracycline treatment in a food-borne outbreak of toxoplasmosis. *Br. Med. J.* **1**, 1064.

Frenkel, J. K. and Jacobs, L. (1958). Ocular toxoplasmosis. *Arch. Ophthal.* **48**, 260–279.

Frenkel, J. K., Dubey, J. P., and Miller, N. L. (1970). *Toxoplasma gondii* in cats: Fecal stages identified as coccidian oocysts. *Science* **167**, 893–896.

Garweg, J. G. and Candolfi, E. (2009). Immunopathology in ocular toxoplasmosis: Facts and clues. *Mem. Inst. Oswaldo Cruz* **104**, 211–220.

Gilbert, R. E. and Stanford, M. R. (2000). Is ocular toxoplasmosis caused by prenatal or postnatal infection? *Br. J. Ophtalmol.* **84**, 224–226.

Gilbert, R., Dunn, D., Wallon, M., Hayde, M., Prusa, A., Lebech, M., Kortbeer, T., Peyron, F., Pollak, A., and Petersen, E. (2001). Ecological comparison of the risks of mother-to-child transmission and clinical manifestations of congenital toxoplasmosis according to prenatal treatment protocol. *Epidemiol. Infect.* **127**, 113–120.

Gras, L., Wallon, M., Pollak, A., Cortina-Borja, M., Evengard, B., Hayde, M., Petersen, E., and Gilbert, R. (2005). Association between prenatal treatment and clinical manifestations of congenital toxoplasmosis in infancy: A cohort study in 13 European centres. *Acta Paediatr.* **94**, 1721–1731.

Guex-Crosier, Y. (2009). Update on the treatment of ocular toxoplasmosis. *Int. J. Med. Sci.* **6**, 140–142.

Hemmer, B., Frohman, E., Hartung, H. P., and Stiive, O. (2006). Central nervous system infections – a potential complication of systemic immunotherapy. *Curr. Opin. Neurol.* **19**, 271–276.

Hill, D. and Dubey, J. P. (2002). *Toxoplasma gondii*: Transmission, diagnosis and prevention. *Clin. Microbiol. Infect.* **8**, 634–640.

Hill, D. E., Sreekumar, C., Gamble, H. R., and Dubey, J. P. (2004). Effect of commonly used enhancement solutions on the viability of *Toxoplasma gondii* tissue cysts in pork loin. *J. Food Prot.* **67**(10), 2230–2233.

Hillers, V. N., Medeiros, L., Kendall, P., Chen, G., and DiMascola, S. (2003). Consumer food-handling behaviors associated with prevention of 13 foodborne illnesses. *J. Food Prot.* **66** (10), 1893–1899.

Hiramoto, R. M., Mayrbaurl-Borges, M., Galisteo, A. J., Jr., Meireles, L. R., Macre, M. S., and Andrade, H. F., Jr. (2001). Infectivity of cysts of the ME-49 *Toxoplasma gondii* strain in bovine milk and homemade cheese. *Rev. Saúde Pública* **35**(2), 113–118.

Hoffmann, S., Fischbeck, P., Krupnick, A., and McWilliams, M. (2007). Using expert elicitation to link foodborne illnesses in the United States to foods. *J. Food Prot.* **70**(5), 1220–1229.

Holland, G. N. (1999). Reconsidering the pathogenesis of ocular toxoplasmosis. *Am. J. Ophthalmol.* **128**, 502–505.

Hornef, M. W., Iten, A., Maeder, P., Villemure, J. G., and Regli, L. (1999). Brain biopsy in patients with acquired immunodeficiency syndrome. *Arch. Intern. Med.* **159**, 2590–2596.

Horowitz, S. L., Bentson, J. R., Benson, F., Davos, I., Pressman, B., and Gotlieb, M. S. (1983). CNS toxoplasmosis in acquired immunodeficiency syndrome. *Arch. Neurol.* **40**, 649–652.

Hovakimyan, A. and Cunningham, E. T., Jr. (2002). Ocular toxoplasmosis. *Ophthalmol. Clin. North Am.* **15**, 327–332.

Innes, E. A. (1997). Toxoplasmosis: comparative species susceptibility and host immune response. *Comp. Immunol. Microbiol. Infect. Dis.* **20**, 131–138.

Jacobs, L., Remington, J. S., and Melton, M. L. (1960). The resistance of the encysted form of *Toxoplasma gondii*. *J. Parasitol.* **46**, 11–21.

Jamieson, S. E., Cordell, H., Petersen, E., Mcleod, R., Gilbert, R. E., and Blackwell, J. M. (2009). Host genetic and epigenetic factors in toxoplasmosis. *Mem. Inst. Oswaldo Cruz* **104**, 162–169.

Johnson, A. M. (1997). Speculation on possible life cycles for the clonal lineages in the genus *Toxoplasma*. *Parasitol. Today* **13**, 393–397.

Jones, J. L., Hanson, D. L., Chu, S. Y., Ciesielski, C. A., Kaplan, J. E., Ward, J. W., and Navin, T. R. (1996). Toxoplasmic encephalitis in HIV-infected persons: Risk factors and trends. The adult/adolescent spectrum of disease group. *AIDS* **10**, 1393–1399.

Jones, J. L., Lopez, A., Wilson, M., Schulkin, J., and Gibbs, R. (2003). Congenital toxoplasmosis: A review. *Obstet. Gynecol. Surv.* **56**, 296–305.

Kapperud, G., Jenum, P. A., Stray-Pedersen, B., Melby, K. K., Eskild, A., and Eng, J. (1996). Risk factors for *Toxoplasma gondii* infection in pregnancy. Results of a prospective case-control study in Norway. *Am. J. Epidemiol.* **144**, 405–412.

Kean, B. H., Kimball, A. C., and Christenson, W. N. (1969). An epidemic of acute toxoplasmosis. *JAMA* **208**(6), 1002–1004.

Kijlstra, A. and Jongert, E. (2008a). Control of the risk of human toxoplasmosis transmitted by meat. *Int. J. Parasitol.* **38**, 1359–1370.

Kijlstra, A. and Jongert, E. (2008b). *Toxoplasma*-safe meat: Close to reality? *Trends Parasitol.* **25**, 18–21.

Laliberté, J. and Carruthers, V. B. (2008). Host cell manipulation by the human pathogen *Toxoplasma gondii*. *Cell. Mol. Life Sci.* **65**, 1900–1915.

Lindsay, D. S., Collins, M. V., Mitchell, S. M., Wetch, C. N., Rosypal, A. C., Flick, G. J., Zajac, A. M., Lindquist, A., and Dubey, J. P. (2004). Survival of *Toxoplasma gondii* oocysts in Eastern oysters (*Crassostrea virginica*). *J. Parasitol.* **90**(5), 1054–1057.

Lindsay, D. S., Collins, M. V., Jordan, C. N., Flick, G. J., and Dubey, J. P. (2005). Effects of high pressure processing on infectivity of *Toxoplasma gondii* oocysts for mice. *J. Parasitol.* **91**(3), 699–701.

Lopes, F. M. R., Mitsuka-Breganó, R., Gonçalves, D. D., Freire, R. L., Karigyo, C. G. T., Wedy, G. F., Matsuo, T., Reiche, E. M. V., Morimoto, H. K., Capobiango, J. D., Inoue, I. T., Garcia, J. L., and Navarro, I. T. (2009). Factors associated with seropositivity

for anti-*Toxoplasma gondii* antibodies in pregnant women of Londrina, Paraná, Brazil. *Mem. Inst. Oswaldo Cruz* **104**, 378–382.
Lundén, A. and Uggla, A. (1992). Infectivity of *Toxoplasma gondii* in mutton following curing, smoking, freezing or microwave cooking. *Int. J. Food Microbiol.* **15**, 357–363.
Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M., and Tauxe, R. V. (1999). Food-related illness and death in the United States. *Emerging Infect. Dis.* **5**(5), 606–625.
Montoya, J. G. (2002). Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. *J. Infect. Dis.* **185**, S73–S82.
Moura, L., de Moura, Lenildo, Bahia-Oliveira, L. M. G., Wada, M. W., Jones, J. L., Tuboi, S. H., Carmo, E. H., Ramalho, W. M., Camargo, N. J., Trevisan, R., Graça, R. M. T., Silva, A. J., *et al*. (2006). Waterborne toxoplasmosis, Brazil, from field to Gene. *Emerging Infect. Dis.* **12** (2), 326–329.
Mozzato, L. and Procianoy, R. S. (2003). Incidence of congenital toxoplasmosis in Southern Brazil: A prospective study. *Rev. Inst. Med. Trop.* **45**, 147–151.
Nesbakken, T. (2009). Food safety in a global market – Do we need to worry? *Small Rumin. Res.* **86**(1), 63–66.
Neves, E. S., Bicudo, L. N., Al Curi, E., Carregal, E., Bueno, W. F., Ferreira, R. G., Amendoeira, M. R., Benchimol, E., and Fernandes, O. (2009). Acute toxoplasmosis: clinical-laboratorial aspects and ophthalmologic evaluation in a cohort of immunocompetent patients. *Mem. Inst. Oswaldo Cruz* **104**, 393–396.
Nicolle, C. and Manceaux, L. (1909). Sur un protozoaire nouveau du gondi. *C. R. Acad. Sci.* **148**, 369.
Petersen, E. and Dubey, J. P. (2001). Biology of Toxoplasmosis. *In* ''Toxoplasmosis'', (D. H. M. Johnson and T. G. Wreghitt, eds), pp. 1–42. Cambridge University Press, Cambridge.
Remington, J. S., McLeod, R., Thulliez, P., and Desmonts, G. (2006). Toxoplasmosis. *In* ''Infectious Diseases of the Fetus and Newborn Infant'', (J. S. Remington, J. O. Klein, C. B. Wilson, and C. J. Baker, eds), 6th edn, pp. 947–1091. Philadelphia: Elsevier Saunders.
Riemann, H. P., Meyer, M. E., Theis, J. H., Kelso, G., and Behymer, D. E. (1975). Toxoplasmosis in an infant fed unpasteurized goat milk. *J. Pediatr.* **87**, 573–576.
Robson, J. M. B., Wood, R. N., Sullivan, J. J., Nicolaides, N., and Lewis, B. R. (1995). A probable foodborne outbreak of toxoplasmosis. *Commun. Dis. Intell.* **19**, 517–522.
Sabin, A. A. B. and Feldman, H. A. (1948). Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite (*Toxoplasma*). *Science* **108**, 660–663.
Sacks, J. J., Roberto, R. R., and Brookes, N. F. (1982). Toxoplasmosis infection associated with raw goat's milk. *J. Am. Med. Assoc.* **248**, 1728–1732.
Safadi, M. A., Berezin, E. N., Farhat, C. K., and Carvalho, E. S. (2003). Clinical presentation and follow up of children with congenital toxoplasmosis in Brazil. *Braz. J. Infect. Dis.* **7**, 325–331.
Schroeder, P. C., Post, M. J. D., Oschatz, E., Stadler, A., Bruce-Gregorios, J., and Thurnher, M. M. (2006). Analysis of the utility of diffusion-weighted MRI and apparent diffusion coefficient values in distinguishing central nervous system toxoplasmosis from lymphoma. *Neuroradiology* **48**, 715–720.
Secretaria de Vigilância em Saúde (SVS) of Brasil (2009). Doenças Transmitidas por Alimentos. Análise Epidemiológica dos Surtos de Doenças Transmitidas por Alimentos no Brasil. http://portal.saude.gov.br/portal/arquivos/pdf/surtos_dta_15.pdf, (Accessed 12 September 2009).
Skinner, L. J., Timperley, A. C., Wightman, D., Chatterton, J. M., and Ho-Yen, D. O. (1990). Simultaneous diagnosis of toxoplasmosis in goats and goatowner's family. *Scand. J. Infect. Dis.* **22**, 359–361.

Smith, J. L. (1993). Documented outbreaks of toxoplasmosis transmission of *Toxoplasma gondii* to humans. *J. Food Prot.* **56**(7), 630–639.
Smith, J. L. (1999). Foodborne infections during pregnancy. *J. Food Prot.* **62**(7), 818–829.
Smith, D. D. (2007). The Sarcocystidae: *Sarcocystis*, *Frenkelia*, *Toxoplasma*, *Besnoitia*, *Hammondia*, and *Cystoisospora*. *J. Eukaryot. Microbiol.* **28**(2), 262–266.
Splendore, A. (1908). Un nuovo protozoa parassita deconigli d'une malattia che ricorda in molti punti il kala-azar dell'uoma. Nota preliminare. *Rev. Soc. Sci. São Paulo* **3**, 109–112.
Spalding, S. M., Amendoeira, M. R. R., Klein, C. H., and Ribeiro, L. C. (2005). Serological screening and toxoplasmosis exposure factors among pregnant women in South of Brazil. *Rev. Soc. Bras. Med. Trop.* **38**, 173–177.
Su, C., Evans, D., Cole, R. H., Kissinger, J. C., Ajioka, J. W., and Sibley, L. D. (2003). Recent expansion of *Toxoplasma* through enhanced oral transmission. *Science* **299**, 414–416.
Sullivan, W. J., Jr., Smith, A. T., and Joyce, B. R. (2009). Understanding mechanisms and the role of differentiation in pathogenesis of *Toxoplasma gondii* – A review. *Mem. Inst. Oswaldo Cruz* **104**, 155–161.
Tavares, A. L. C., Souza, A., Fabres, A. D., Almeida, M. A. B., Junior, L. R. A., Carmo, G. M. I., Araújo, W. N., Garcia, M. H. O., Reis, A. K. V., Figueiredo, D. M. S., Branco, N., Franco, R. M. B., and Hatch, D. L. (2006). Surto intra-familiar de toxoplasmose, Santa Vitória do Palmar, RS. *Bol. Eletrônico Epidemiol.* http://portal.saude.gov.br/portal/arquivos/pdf/boletim_03_06.pdf, (Accessed 12 December 2009).
Tenter, A. M. (2009). *Toxoplasma gondii* in animals used for human consumption. *Mem. Inst. Oswaldo Cruz* **104**, 364–369.
Tenter, A. M., Heckeroth, A. R., and Weiss, L. M. (2000). *Toxoplasma gondii*: From animals to humans. *Int. J. Parasitol.* **30**(12–13), 1217–1258.
Varella, I. S., Canti, I. C. T., Santos, B. R., Coppini, A. Z., Argondizzo, L. C., Tonin, C., and Wagner, M. B. (2009). Prevalence of acute toxoplasmosis infection among 41, 112 pregnant women and the mother-to-child transmission rate in a public hospital in South Brazil. *Mem. Inst. Oswaldo Cruz* **104**, 383–388.
Waldeland, H. (1977a). Toxoplasmosis in sheep I. Long-term epidemiological studies in four breeding flocks. *Acta Vet. Scand.* **18**(2), 227–236.
Waldeland, H. (1977b). Toxoplasmosis in sheep. II. Influence of various factors on the antibody contents. *Acta Vet. Scand.* **18**(2), 237–247.
Waldeland, H. (1977c). Toxoplasmosis in sheep. III. Hematological, serological and parasitological studies. *Acta Vet. Scand.* **18**(2), 248–256.
Wang, X. and Suzuki, Y. (2007). Microglia produces IFN-gamma independently from T-cells during acute toxoplasmosis in the brain. *J. Interferon Cytokine Res.* **27**, 599–695.
Warnekulasuriya, M. R., Johnson, J. D., and Holliman, R. E. (1998). Detection of *Toxoplasma gondii* in cured meats. *Int. J. Food Microbiol.* **45**(3), 211–215.
Weiss, L. M. and Dubey, J. P. (2009). Toxoplasmosis: A history of clinical observations. *Int. J. Parasitol.* **39**, 895–901.
Weiss, L. M. and Kim, K. (2007). Bradyzoite Development. *In ''Toxoplasma gondii.* The Model Apicomplexan – Perspectives and Methods'', (L. M. Weiss and K. Kim, eds), pp. 341–366. Elsevier.
Wolf, A. and Cowen, D. (1937). Granulomatous encephalomyelitis due to an encephalitozoon (encephalitozic ancephalomyelitis): A new protozoan disease of man. *Bull. Neurol. Inst. NY.* **6**, 306–335.
Wong, S. Y. and Remington, J. S. (1993). Biology of *Toxoplasma gondii*. *AIDS* **7**, 299–316.
Wong, S. Y. and Remington, J. S. (1994). Toxoplasmosis in pregnancy. *Clin. Infect. Dis.* **18**, 853–861.