CHAPTER 5

Toxoplasmosis

Ynes R. Ortega

5.1 PREFACE

Toxoplasma gondii is a coccidia that is the most widespread and prevalent parasite in the world. It can infect warm-blooded animals, including man. It is responsible for 20.7% of food-borne deaths due to known infectious agents. Waterborne outbreaks have also been associated with *Toxoplasma* in Canada and Brazil (Aramini *et al*., 1999; Bahia-Oliveira *et al*., 2003; Dubey, 2004). Toxoplasmosis can be asymptomatic or can cause abortion in humans if an acute infection develops during pregnancy. Healthy individuals may develop encephalitis. Serologically, *Toxoplasma* can be identified in as high as 85% of the population in some European countries, where meat is primarily eaten undercooked. In Paris, 84% of pregnant women have been exposed to *Toxoplasma*, as compared to 32% of pregnant women in New York.

Few cases of waterborne toxoplasmosis have been reported. It is estimated that most cases are transmitted via contaminated foods. In the United States, between 400 and 4000 cases of congenital toxoplasmosis occur annually. Of the 750 deaths attributed to toxoplasmosis each year, 50% are believed to be caused by eating contaminated meat, making toxoplasmosis the third leading cause of food-borne deaths in this country (Lopez *et al*., 2000). These cases were assumed to have originated from mishandling in food service establishments and homes, not from food processing establishments. Acquisition of the parasite may be ingestion of raw or inadequately cooked infected meat or exposure to cat feces. Contamination may also occur from contact with soil when gardening or ingestion of unwashed fruits or vegetables contaminated with oocysts.

5.2 PARASITE DESCRIPTION

Toxoplasma gondii was described in the early 1900s. It has been identified as being able to infect over 300 species of mammals and 30 species of birds as intermediate hosts. Infection is acquired when a host ingests water or food which is contaminated with cat feces containing *Toxoplasma* oocysts. The oocysts excyst and the sporozoites migrate, and preferentially localize in muscle and the brain. The parasite can cross the placenta to infect the fetal tissues (Dubey, 1991).

A large variety of animals can acquire toxoplasmosis, but only cats (domestic and wild) are the definitive hosts. Outdoor cats are more likely to be infected with *Toxoplasma*.

The oocysts are highly resistant, even to desiccation, and can survive on dry surfaces for weeks or even months. The role of shellfish in parasite transmission is being studied (Arkush *et al*., 2003) because shellfish can filter large volumes

of water and concentrate viable *Toxoplasma* oocysts. Most marine mammals feed on mollusks and these mammals have a high mortality with meningoencephalitis caused by *Toxoplasma* (Dubey *et al*., 2003e; Oksanen *et al*., 1998).

In humans, most infections are asymptomatic; however, it can be fatal for immunocompromised individuals and the fetuses of women who acquire the infection during the first 4 to 5 months of pregnancy. Three different genotypes I, II, and III have been described in *T. gondii* (Howe and Sibley, 1995). Other strains fall into two classes: recombinant, which is closely related to the dominant types (I and III), and exotic. Type I is highly virulent in laboratory animals, whereas types II and III are non-virulent. In humans, Type II predominates in AIDS and congenital infections (encephalitis, pneumonitis, or disseminated infections). Type II has been isolated in about 75–80% of AIDS and non-AIDS immunocompromised patients. In Spain, the genotype I is more prevalent in congenital infections (Fuentes *et al*., 2001). Ocular toxoplasmosis is a common sequelae of congenital toxoplasmosis, but can be dormant for years and emerge at adulthood, causing severe retinochoriditis. In these individuals type I, type IV, or novel types were frequently isolated.

Type I was implicated in outbreaks in Canada and Brazil and was characterized by severe ocular toxoplasmosis (Boothroyd and Grigg, 2002). Immunocompetent adults may also suffer from retinitis and enlarged lymph nodes.

5.3 LIFE CYCLE

Unsporulated oocysts are excreted in the feces of infected cats. Oocysts undergo sporulation for 24–48 h to become infectious. The oocysts are the environmentally resistant form and are excreted in the feces (Fig. 5.1). Other animals or humans can acquire the infection when oocysts are ingested via water, food, or soil.

Sporulated oocysts consist of two sporocysts, each containing four sporozoites. Once released from the sporocyst, the sporozoites penetrate the intestinal cells and lymph nodes, becoming tachyzoites. These multiply very fast and disperse throughout the body via blood or lymph where they multiply and can eventually encyst in the brain, liver, skeletal, and cardiac muscle. These cysts contain bradyzoites which

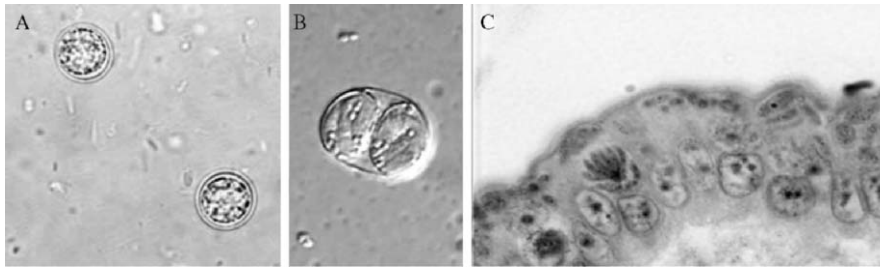

Figure 5.1. *Toxoplasma gondii* (a) unsporulated and (b) sporulated oocysts. (pictures obtained from http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary). (c) *Toxoplasma gondii* intracellular stages observed in cat intestinal tissue.

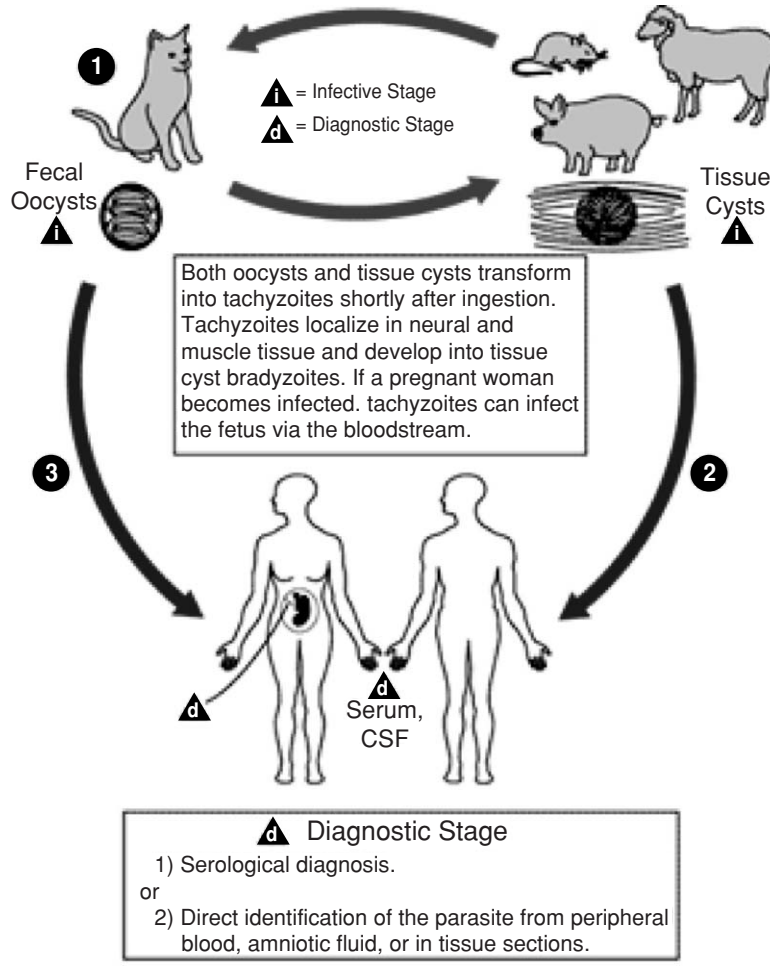

Figure 5.2. Life cycle of *Toxoplasma gondii*. Graph obtained from http:// www.dpd.cdc.gov/dpdx/HTML/ImageLibrary.

multiply slowly. Cysts may be viable for the duration of the hosts life. Tachyzoites are frequently present during the acute phase and bradyzoites in the chronic phase of the infection (Fig. 5.2).

When tissue cysts are ingested by a susceptible host, the cyst wall is digested by proteolytic enzymes. Bradyzoites are then released to infect the intestinal cells. Tachyzoites are dispersed and infect other tissues. If a cat ingests the tissues, tachyzoites infect the intestinal cells and begin asexual reproduction (schizogony) and sexual multiplication (gametogony). Macrogametocytes are fertilized by a male gamete (microgametocyte), forming the zygote which differentiates to become an oocyst, and is excreted in the feces.

A novel route of infection has been reported recently. *T. gondii* appeared in the bile and feces of interferon-gamma knockout (GKO) mice, but not wild mice after peroral infection with *T. gondii* cysts. The tachyzoite and bradyzoite specific mRNA were identified in bile and feces and was confirmed using the mouse infectivity assay (Piao *et al*., 2005).

5.4 TRANSMISSION

Toxoplasmosis can be acquired by ingestion of contaminated water, food, or soil (Choi *et al*., 1997; Coutinho *et al*., 1982; Dubey, 2004; Ruiz *et al*., 1973). Foodborne toxoplasmosis is most often acquired by consumption of raw or undercooked meats.

Congenital toxoplasmosis occurs when a pregnant female is exposed to *Toxoplasma* oocysts. *Toxoplasma* can cross the placenta and infect the fetus. This may result in diminished vision or blindness after birth. Symptoms include hydrocephaly, convulsion, and calcium deposits in the brain. Less frequently, toxoplasmosis is acquired by transfusion of blood or its components, or by organ transplantation. Latent toxoplasmosis can reactivate if the immune system of the host is compromised. Inhalation of aerosols containing oocysts from cat litters, farm animal feed, and bedding has also been suggested (Furuta *et al*., 2001).

The first documented toxoplasmosis outbreak associated with a municipal water supply was described in 1995 in British Columbia, Canada. It was hypothesized that domestic cat or cougar feces contaminated a surface water reservoir with *T. gondii* oocysts. These animals were observed around the watershed and could shed oocysts in their feces near the waters' edge (Aramini *et al*., 1999). During 1997–1999, a region of Brazil was surveyed for seropositivity to *Toxoplasma*. The survey population was selected randomly from schools, randomly chosen communities, and an army battalion. Out of 1436 persons tested, 84% of the population in the lower socioeconomic group was seropositive, compared with 62% and 23% of the middle and upper socioeconomic groups, respectively ($p < 0.001$). Multivariate analysis suggested that drinking unfiltered water increased the risk of seropositivity for the lower socioeconomic and middle socioeconomic populations (Bahia-Oliveira *et al*., 2003).

5.5 IDENTIFICATION

Toxoplasma infections can be diagnosed by serological assays examining the antibody response toward the infection. Commercial agglutination and Elisa assays are available (Dubey *et al*., 1995; Ryu *et al*., 1996). Western blot assays have also been reported in the literature (Bessieres *et al*., 1992; Saavedra and Ortega, 2004). *Toxoplasma* oocysts can be identified in the environment using conventional microscopy; however, the small number of parasites may be less sensitive. *Toxoplasma* oocysts have been isolated from mussels that could serve as paratenic hosts by concentrating oocysts (Arkush *et al*., 2003). *Toxoplasma* oocysts can be identified from water

samples using the current U.S. EPA method for concentration of *Cryptosporidium* (Isaac-Renton *et al*., 1998). Centrifugation and flocculation procedures using Aluminum sulfate and Ferric sulfate can also concentrate *Toxoplasma* oocysts. Sporulated oocysts were recovered more efficiently using aluminum sulfate while unsporulated oocysts could be better recovered using ferric sulfate (Kourenti and Karanis, 2004).

5.5.1 Molecular Assays

Toxoplasma oocysts are usually present in low numbers in contaminated water and foods. Rapid and sensitive detection methods are necessary. Tissue culture and animal models available for *Toxoplasma* are time-consuming, expensive, and labor-intensive. Therefore, Polymerase chain reaction (PCR) amplification has become the preferred method. Most PCR assays used for *Toxoplasma* identification use primers targeting the B1 gene. It is a 35-fold-repetitive gene that is highly specific and conserved among strains of *Toxoplasma* (Buchbinder *et al*., 2003). It also has a PCR amplification and detection method for *T. gondii* oocyst nucleic acid that incorporates uracil-*N*-glycosylase to prevent false-positive results, an internal standard control to identify false-negative results, and uses PCR product oligoprobe confirmation using a nonradioactive DNA hybridization immunoassay. This method can provide positive, confirmed results in less than 1 day and can detect less than 50 oocysts (Schwab and McDevitt, 2003).

Other assays have focused on the sensitivity of the assay. DNA was extracted with a modified Qiagen DNA Mini Kit method and was amplified by PCR using specific primers for the *T. gondii* B1 gene. *T. gondii* was detected correctly in 90% of the clinical specimens examined in less than 5 h, with a detection limit of two parasites/sample (Jalal *et al*., 2004).

Toxoplasma oocyst detection can be included as part of the waterborne parasite detection protocol. Water samples are filtered followed by a sucrose density gradient. Oocyst detection is done using PCR and bioassay. In an experimental seeding assay with 100 L of deionized water, a parasite density of one oocyst/L was successfully detected by PCR in 60% of cases and 10 oocysts/L was detected in 100% of cases. The sensitivity of the PCR assay varied from less than 10 to more than 1000 oocysts/L, depending on the sample source. PCR was always more sensitive than mouse inoculation. Out of 139 environmental water samples, 125 could be analyzed. *Toxoplasma* DNA was identified in 8% of the cases; however none were positive by mouse inoculation (Villena *et al*., 2004).

DNA amplification using the18S-rRNA gene (MacPherson and Gajadhar, 1993) had a theoretical detection limit of 0.1 oocyst only when the samples were concentrated by aluminum sulfate flocculation (Kourenti and Karanis, 2004). TaqMan PCR assays using the B1 and ssrRNA genes have been successfully used in experimentally inoculated mussels.

The role of *Toxoplasma* on the morbidity and mortality of marine mammals has been studied extensively. Fatal meningoencephalitis has been reported in these animals. The sources of *T. gondii* oocysts in marine environments are unknown. However, bivalve shellfish have been demonstrated to serve as paratenic hosts by assimilation and concentration of infective cysts and oocysts. Therefore, *T. gondii*

oocysts can be concentrated by shellfish, and sea otters could acquire the infection by eating the shellfish. A TaqMan PCR assay for detection of *T. gondii* SSrRNA was evaluated using experimentally spiked mussels with *T. gondii* oocysts. *T. gondii*-specific SSrRNA was detected in mussels as long as 21 days postinoculation. Detection was found more frequently in the digestive gland homogenate. Parasite infectivity was confirmed using a mouse bioassay (Arkush *et al*., 2003).

A real-time PCR was developed in order to detect and quantify *T. gondii* B1 and bradizoite specific genes (SAG-4 and MAG-1) in serum and peripheral blood mononuclear cells specimens. The results were compared with those obtained with a nested PCR. Real-time PCR proved to be more sensitive than nested PCR for detection and quantification of either the B1 gene ($P < 0.001$) or the SAG-4/MAG-1 gene ($P < 0.05$). Real-time PCR has been shown to be particularly useful to accurately determine the parasite DNA load in follow-up specimens (Contini *et al*., 2005).

Other targets used for *Toxoplasma* identification include a 529 bp sequence, which has 300 copies in the genome of *Toxoplasma.* This fragment was used for the development of a very sensitive and specific PCR for diagnostic purposes, and a quantitative competitive-PCR for the evaluation of cyst numbers in the brains of chronically infected mice. Polymerase chain reaction with the 529 bp fragment was more sensitive than with the 35-copy B1 gene. A highly significant correlation between visual counting of brain cysts and quantitative competitive-PCR was obtained in mice chronically infected with *Toxoplasma* (Homan *et al*., 2000). Mobile genetic elements (MGE) that have 100–500 copies per cell were also used for design of assays for *Toxoplasma* identification. Two PCR-based strategies using specific primers amplified *T. gondii* MGEs; revealing information on element size and positional variation. The first PCR strategy involved the use of a standard two-primer PCR while the second strategy used a single specific primer in a step-up PCR protocol. The use of a standard two-primer PCR reaction revealed the presence of a virulence related marker in which all avirulent strains possessed an additional 688 bp band. The single primer PCR strategy demonstrated that all virulent strains had identical banding patterns suggesting invariance within this group of strains. However, all avirulent strains had different banding patterns indicating the presence of a number of individual lineages within this group (Terry *et al*., 2001).

Single copy genes SAG1-4 and GRA4 genes have been used as targets for *Toxoplasma* characterization and identification. The genes SAG5A, SAG5B, and SAG5C were also examined to characterize strain virulence in the three major genotypes of *T. gondii*. Southern blot analysis using a SAG5-specific probe could differentiate between genotype I virulent strains from the avirulent strains of either genotype II or genotype III. A PCR-restriction fragment length polymorphism method based on the SAG5C gene can discriminate between strains of genotype I, II, and III using a single endonuclease digestion (Meisel *et al*., 1996; Tinti *et al*., 2003).

5.5.2 Riboprinting

Characterization of *Toxoplasma* isolates is achieved by using PCR amplified products digested with 13 enzymes. Discrimination between intracellular stages of coccidia in human tissues can be achieved using riboprints (through restriction enzyme

analysis of the PCR-amplified small subunit rRNA gene). Together, the variation in riboprints and surface antigen gene structure reflects the phylogenetic diversity among these coccidia, and in addition, confirms the value of riboprinting in the identification of apicomplexan parasites such as *T. gondii* (Brindley *et al*., 1993). RFLP-PCR, RAPD, sequence length polymorphism, and sequencing has allowed for genotyping analysis (Aspinall *et al*., 2003; Bartova and Literak, 2004; Carme *et al*., 2002).

The coding region of GRA6 was amplified, sequenced, and compared for 30 *Toxoplasma* strains from eight different zymodemes (Z1–Z8). Sequence alignment demonstrated nucleotide polymorphisms. Types I, II, and III could be distinguished from each other. The large variety of amino acid changes supports the view that the GRA6 protein plays an important role in the antigenicity and pathogenicity of *T. gondii.* A PCR-RFLP method using MseI could differentiate the three *Toxoplasma* groups (Fazaeli *et al*., 2000).

5.6 PATHOGENICITY

Cell adhesion is a prerequisite for cell invasion. Various surface molecules are required in this process, including the SAG3 and SAG5 molecules. Once attachment occurs, tachyzoites release micronemal content, the conoid protrudes and forms an indentation in the host cell. Rhoptries containing proteins and lipids are released. A tight junction is formed between host cell and parasite. Tachyzoites multiply within the cell by binary fission. Multiplication continues until the host cell lyse and tachyzoites are released and can reinfect other cells. In chronic infections, bradyzoites are present and disease reactivation occurs when there is an impairment of the immune function. In murine models, tumor necrosis factor-α, interferon γ, and T cells are required to prevent disease reactivation (Roberts and McLeod, 2004).

5.7 EPIDEMIOLOGY

T. gondii can infect a variety of warm-blooded animals. The relevant species associated with transmission of *Toxoplasma* to humans will be described.

5.7.1 Humans

It has been estimated that 30–60% of adults in the United States have been exposed to *Toxoplasma* at some point in their lifetime (180, 185–191). A high seroprevalence of *Toxoplasma* in Europe and South American countries has also been reported. This may be due to the frequent consumption of raw meats.

According to the Third National Health and Nutrition Examination Survey in the United States (1988–1994), of 17,658 sera tested, the overall age-adjusted seroprevalence was 22.5%. Among women aged 15–44 years, seroprevalence was 15.0%. The seroprevalence in the Northeast was 29.2%, 22.8% in the South, 20.5% in the Midwest, and 17.5% in the West. Risks for acquiring *Toxoplasma* infection increased with age. It was higher among persons who were foreign-born, persons with a lower

educational level, those who lived in crowded conditions, and those who worked in soil-related occupations. About 25% of adults and adolescents in the United States have been infected with *T. gondii* (Jones *et al*., 2001). Sera collected in the National Health and Examination Survey (NHANES) from 1999–2000 was examined for seropositivity to *Toxoplasma*. Of 4234 persons 12–49 years of age, 15.8% were antibody positive; among women, 14.9% were seropositive. Prevalence was higher among non-Hispanic black persons (19.2%) than among non-Hispanic white persons (12.1%) (Jones *et al*., 2003). A cross-sectional seroprevalence study in healthy adults in Maryland included Seventh Day Adventists who were vegetarians and control community volunteers who were not vegetarians. Overall, seroprevalence was 31% in the study group. People with *T. gondii* infection were less likely to be Seventh Day Adventists (24% versus 50%) than people without *T. gondii* infection (Roghmann *et al*., 1999). In another study, seroprevalence of *Toxoplasma* in Yakima Indians (ages 1 to 66 years) was 20% (23/114) (Sturchler *et al*., 1987).

The seroprevalence of *T. gondii* infection using an ELISA test was determined in primigravid women in India. Between August 1996 and September 1997, *Toxoplasma* seroprevalence was 41.75% in 503 women (Akoijam *et al*., 2002). In Bombay, the seroprevalence of healthy adult voluntary blood donors (ages 13–50 years) was 30.9%, 67.8% in HIV infected hosts, and 28% in patients treated for cerebral tuberculoma or neurocysticercosis. *Toxoplasma* infection appears to be subclinical and prevalent throughout life but emerges as an important opportunistic infection in HIV/AIDS patients (Meisheri *et al*., 1997). In Kashmir, 53.14% of 2371 women with recurrent abortions and 69.35% of 310 women with neonatal deaths tested positive for IgM antibody against *Toxoplasma*. Of the 177 women who received followed up visits, 94.26% of 122 women with recurrent abortions and 63.64% of 55 women with neonatal deaths delivered normal babies after they were treated with spiramycin during pregnancy (Zargar *et al*., 1999). In Bangladesh, of 286 women examined by ELISA, 38.5% were seropositive for *Toxoplasma* IgG antibody, and of 88 randomly selected patients, 1.1% was positive for *Toxoplasma* IgM. The seroprevalence gradually increased with age and parity. The seroprevalence of antibody was higher among the poor women (53.0%) than the upper socio-economic class (22.0%) and among the women with jobs (55.0%) than the housewife group (35.0%) (Ashrafunnessa *et al*., 1998). In Nepal, the seroprevalence of *T. gondii* infection in 404 apparently healthy subjects was 65.3% (Rai *et al*., 1999).

In Korea, the seroprevalence of *Toxoplasma* in pregnant women was found to be low. Seropositivity for *Toxoplasma* was 0.79% in 5175 sera and 1.33% in 750 amniotic fluid samples (Song *et al*., 2005). In Taiwan, a seroepidemiological survey of *T. gondii* infection among Atayal and Paiwan mountain aborigines and Southeast Asian laborers found that the overall seroprevalence of *T. gondii* infection was 19.4% for Atayal, 26.7% for Paiwan, 42.9% for Indonesian, 14.7% for Thai, and 11.3% for Filipinos. Atayal and Paiwan Indians with a history of eating raw meat seemed more susceptible to *T. gondii* infection than those who had never consumed raw meat (Fan *et al*., 2002).

In Catania (Sicily), the seroprevalence of *T. gondii* in fertile women is 41.1% (Condorelli *et al*., 1993). In the general northern Greek population, the prevalence of IgG-specific antibodies to *Toxoplasma* was 37, 29.9, and 24.1% in 1984, 1994,

and 2004, respectively, and was 35.6, 25.6 and 20%, respectively, in women of reproductive age (15–39 years). The significant decline in prevalence, and the shift toward an older age group, observed during this period could be explained by the improved socio-economic situation (Diza *et al*., 2005).

Sera from 144 Ethiopian immigrants living in the Jezreel Valley were tested for antibodies against *T. gondii*. Of these, 34% of the immigrants were positive and the prevalence in the Ethiopians was higher than in Jewish kibbutz members (22.8%) and lower than in Arab villagers (55.8%). Prevalence increased from 0% in children less than 10-years old to 46% in individuals 40 years or older (Flatau *et al*., 1993). The incidence of toxoplasmosis in rural areas of Central African Republic on a healthy population was determined. About 40% of the adults had IgG antibodies against *T. gondii,* but in a pre-desert area 25% were positive (Dumas *et al*., 1990). In Rwanda, 50% of the adults of two communities had antibodies to *T. gondii*. Only 12% of the Ngenda population group of 14-years old was positive, whereas The Nyarutovu (NVU) population had a 31% positivity; suggesting that the Nyarutovu acquired the infection earlier in life (Gascon *et al*., 1989). In Dar es Salaam, Tanzania, the infection rate in normal pregnant women was 41.9%, in anemic women 52.5%, and 66.7% in individuals with hypertension (Gill and Mtimavalye, 1982). In four regions of Southern Africa (Natal, Eastern Cape, Western Cape, and South West Africa and Botswana) the overall seroprevalence was 20% (of 3379 sera tested), the highest prevalence occurring in Blacks (34%) and Indians (33%) of Natal, and the lowest in San (Bushmen) (9%) and Whites (12%) of South West Africa and Botswana (Jacobs and Mason, 1978). In Bamako, Mali, toxoplasmosis seroprevalence was 60% from AIDS patients, 22.6% from the HIV-seropositive blood donors, and 21% from the HIV-seronegative blood donors (Maiga *et al*., 2001).

The seroprevalence of *T. gondii* in 191 pregnant women was 25.7% in a Primary Care setting in Malaga, Spain. Significant associations using univariate and multivariate analysis was demonstrated in individuals with previous abortions and low economic status (Guerra and Fernandez, 1995). In another study, the seroprevalence in intravenous drug users was 47.6%, 12.2% in infants, and 30% in pregnant women (Gutierrez *et al*., 1996).

In Sweden, the seroprevalence of *Toxoplasma* for Swiss women was 46.0% and 45.8% for women of other nationalities at the time of delivery. The risk of seroconversion among seronegative women during their 9 months of pregnancy was 1.21% (Jacquier *et al*., 1995). Blood samples from more than 40,000 newborns, from two geographically different areas, were examined for the presence of IgG antibodies to *Toxoplasma* to determine the seroprevalence of *Toxoplasma* in their mothers. During a 16-month period between April 1997 and July 1998, the seroprevalence was 14.0% in Stockholm County and 25.7% in Skane County. The seroprevalence among women born in Stockholm was 11.1% and 24.9% in Skane. The corresponding figures for women born outside the Nordic countries were 24.3% and 29.4% (Petersson *et al*., 2000).

In the Netherlands, between 1995 and 1996, 7521 sera were tested and the national seroprevalence was found to be 40.5 %. Living in the Northwest, having professional contact with animals, living in a moderately urbanized area, being divorced or widowed, being born outside The Netherlands, frequent gardening and

owning a cat were independently associated with *Toxoplasma* seropositivity. The seroprevalence among women aged 15–49 years was 35.2% in the study of 1995–1996 and was lower than in the pregnant women in the Southwest of The Netherlands in 1987–1988 (45.8 %). The steepest rise in seroprevalence occurred among women aged 25–44 years (Kortbeek *et al*., 2004).

In Slovenia, during 1981 to 1994, a serological screening for toxoplasmosis was carried out on 20,953 pregnant women. Seropositivity decreased from 52% in the 1980s to 37% during 1991–1994, while during the same period, the incidence of suspected primary infections acquired in pregnancy rose from 0.33% to 0.75% (Logar *et al*., 1995). Over a 12-month period, the incidence of congenital toxoplasmosis in 3959 pregnant women in Slovenia was 3/1000 (Logar *et al*., 1992).

In South America, the prevalence of *Toxoplasma* has been studied in the past years. The prevalence of *T. gondii* in indigenous Brazilian tribes with different degrees of acculturation was studied. During 2000-2001, seroprevalence varied from 57.3 to 78.8%. Differential contact with soil-harboring oocysts from wild felines may be responsible for the variable seroprevalence in the different tribes (Sobral *et al*., 2005). Also in Brazil, the seroprevalence of the Enawene-Nawe Indians of Mato Grosso was 80.4% (out of 148 samples). This community is isolated from non-Indians. They do not keep domestic animals, including cats. Their diet is based on insects, cassava, corn, honey, mushrooms, and fish. They do not consume other meats. Seropositivity increased significantly with age from 50 to 95%. Wild felines are considered a source of *Toxoplasma* which would contaminate soil, insect, and mushrooms (Amendoeira *et al*., 2003). A serologic survey for *Toxoplasma* was done in Ticuna Indians from five villages in western Brazil and was compared with non-Indian inhabitants of the town of Codajas, Amazonas. Seroprevalence was 39% in the Ticuna population and 77% in the Codajas population (Lovelace *et al*., 1978).

In the Yucpa community in Venezuela, the seroprevalence was 63% in 94 individuals (ages 3 months to 100 years) (az-Suarez *et al*., 2003); whereas in Amerindians (aged 1–69 years) the overall prevalence of infection was 49.7% (of 447). A higher antibody rate was found in lowland settings compared to the mountain areas. No age-antibody association was detected in the mountain communities contrary to the lowland setting (83.3% in the oldest group). The results suggest that transmission by infective cat feces plays a predominant role in the spread of infection in this population (Chacin-Bonilla *et al*., 2001). Another study conducted on 121 Amerindians of the Guajibo ethnic group, 4 to 45 years of age, found the overall prevalence to be 88% (de la *et al*., 1999).

The wide variation in humans is thought to be a result of cultural habits, environmental conditions, socioeconomic status, and proximity to animals. A steady increase in prevalence with age was noted in all surveys.

5.7.2 Swine

An average of 29% of pigs worldwide is estimated to have *Toxoplasma*. The distribution of the parasite varies according to various regions and farm management.

The regional prevalence of *T. gondii* in pigs from 85 farms in five New England states was 47.4%. Herd prevalence rate was 90.6%. Within the herd, the seroprevalence ranged from 4 to 100%. All farms studied had one or more risk factors for

exposure to *T. gondii*, suggesting that education on farm management practices should be targeted to include small producers (Gamble *et al*., 1999). In Swedish pigs in 1999, 5.2% of 807 meat juice samples collected from 10 abattoirs in different parts of the country were positive. The seroprevalence was 3.3% in fattening pigs and 17.3% in adult swine (Lunden *et al*., 2002). In Spain, seroprevalence of hunter-killed wild pigs between 1993 and 2004 from five geographic regions in the north and seven regions in the south was 38.4%. Seroprevalence was higher in pigs from high stocking per hectare. Sex, age, or hunting conditions (open or fenced) were not associated with high seroprevalence of *Toxoplasma* (Gauss *et al*., 2005). In Portugal, antibodies to *T. gondii* were found in 15.6% of 333 pigs prior to slaughter. Viable *T. gondii* was isolated from 15 of 37 pigs. Using the SAG2 -RFLP and microsatellite analysis, 11 isolates were Type II and 4 were Type III (Sousa *et al*., 2005). In Austria, blood samples were obtained from 4697 pigs. During a period of 10 years, the infection rate was reduced from 13.7 to 0.9%. Prevalence in breeding sows decreased from 43.4 to 4.3% and in fattening pigs 12.2 to 0.8% (Edelhofer, 1994). Under the Dutch field trial "Integrated Quality Control (IQC) for finishing pigs," 120 farms and 3 slaughterhouses were studied. The *Toxoplasma* seroprevalence was 2.1% in 23,348 serum samples. Seropositive animals were found from the earliest days of the finishing period. Housing and farm management play an important role in the prevention of *Toxoplasma* (Berends *et al*., 1991).

In Serbia, during June 2002 to 2003, the seroprevalence was 76.3% in 611 cattle, 84.5% in 511 sheep, and 28.9% in 605 pigs. The risk factors for cattle were small herd size and farm location in Western Serbia, while housing in stables with access to outside pens was protective. In sheep, an increased risk of infection was found in ewes from state-owned flocks vs. private flocks. In pigs, the risk of infection was highly increased in adult animals and in those from finishing type farms (Klun *et al*., 2005).

Some studies were also done in Asia. In northwestern Taiwan, in 1998, the overall seroprevalence of *T. gondii* infection was 28.8% among slaughtered pigs. No significant difference in seroprevalence was observed between male and female pigs (Fan *et al*., 2004). In 1994, in Sumatra Indonesia, the seropositivity in two slaughter houses varied from 3.6 to 9.2% (Inoue *et al*., 2001).

In Africa, *Toxoplasma* was also studied in domestic pigs. In Zimbabwe, *T. gondii* antibodies were found in 9.3% of 97 domestic pigs, 36.8% of 19 elands, 11.9% of 67 sables, 0% of 3 warthogs, 0% of 3 bushpigs, 50% of 2 white rhinos, 5.6% of 18 buffalos, 14.5% of 69 wildebeest, and 10.5% of 19 elephants examined (Hove and Dubey, 1999). In Ghana, the overall seroprevalence in pigs was 39%, and at different geographical locations, varied from 30.5, 42.5, and 43.9%. The age of the animal, the breed, the environmental conditions, and the management practices appeared to be the major determinants of prevalence of antibodies against *T. gondii.* Seroprevalence was significantly higher in crossbreed pigs (46.8%) than the Large White breed (38.8%) (rko-Mensah *et al*., 2000).

Federally inspected abattoirs in Canada during 1991-1992 were sampled. Seroprevalence of the 2800 market-age pigs ranged from 3.5 to 13.2% in the different regions of the country. *T. gondii* ribosomal RNA was identified in 9 of 36 animals, but mouse bioassay testing was negative in all pig muscle samples. This suggests that serological evidence of *T. gondii* infection in pigs alone does not accurately

assess the public health risks associated with consuming improperly cooked pork products (Gajadhar *et al*., 1998).

In the United States, the prevalence of *Toxoplasma* during 1983-1984 was 23.9% in 11,842 commercial pigs. Seroprevalence was 42% in breeder pigs, whereas in market pigs it was 23% (Dubey *et al*., 1991). In Oahu, Hawaii, sera from 509 pigs from 31 farms were examined. *T. gondii* antibodies were found in 48.5% of pigs. The prevalence of *T. gondii* antibodies in garbage-fed pigs was 67.3% (of 199 pigs) and 33.8% in grain-fed pigs (of 180 pigs) (Dubey *et al*., 1992). In Iowa, using the SAG2 loci, 83.7% of the isolates from pigs were Type II genotype. The type III genotype was identified in only 16.3% of the isolates. The distribution of these genotypes was similar to those observed in humans, but was different from those previously reported in animals. The type I genotype was not identified in the isolates from pigs, although these strains have previously been shown to account for approximately 10–25% of toxoplasmosis cases in humans (Mondragon *et al*., 1998). In Montana, seropositive animals at 1:16 or higher were 13.2% of sheep, 5.0% of pigs, and 22.7% of goats. Using the MAT, 3.2% of cattle, 3.1% of bison were positive, and none of the elk were positive (Dubey, 1985). Viable *T. gondii* were isolated from hearts and tongues of 51 out of 55 pigs from a farm in Massachusetts (Dubey *et al*., 2002). In Ossabaw Island, Georgia; a remote, barrier island, antibodies to *T. gondii* were found in 0.9% of 1264 pigs from the island. Of 170 feral pigs from mainland Georgia, 18.2% were seropositive. The markedly low prevalence of *T. gondii* on Ossabaw Island was attributed to the virtual absence of cats; only 1 domestic cat was known to be present (Dubey *et al*., 1997).

In certain regions of South America, the prevalence of toxoplasmosis in pigs was estimated. In Brazil, antibodies to *T. gondii* were found in 17% of 286 pigs prior to slaughter. Viable *T. gondii* was isolated from seven out of 28 pigs. RFLP analysis using products of the SAG2 locus identified two isolates of Type I and five of Type III (de *et al*., 2005). In Sao Paulo Brazil, in 5-month-old pigs obtained at abattoirs, 9.6% were seropositive, which was lower than the same age animals in Lima, Peru (32.3%) (Suarez-Aranda *et al*., 2000). Another study showed a seroprevalence of 27.7% in 137 pigs at a slaughter house in Peru (Saavedra and Ortega, 2004). In Argentina, antibodies to *T. gondii* were detected in 37.8% of 230 slaughter sows belonging to 83 farms distributed in 5 provinces. Distribution among provinces varied from 3.3 to 62.8%. Monthly evaluation of pigs from an intensive management indoor farm demonstrated 4.5% seropositivity. A cross-sectional study in an outdoor farm demonstrated 40.2% seropositivity. This prevalence was related to the facilities and management of the farm (Venturini *et al*., 2004). Another study in Argentina from September 1991 to May 1992 demonstrated 11% seropositivity in 109 pigs at 1:1024 or higher serum dilutions, and 36.7% at 1:16 or less. *Toxoplasma* was isolated in 14 pigs using the mouse bioassay. The authors suggest that antibody production in infected pigs is apparently dependent on the pathogenicity of the parasite strain (Omata *et al*., 1994).

5.7.3 Poultry

Toxoplasma was isolated in 0.4% of 716 Croatian chicken brain tissues using the mouse bioassay (Kuticic and Wikerhauser, 2000). In Egypt, the seroprevalence of

Toxoplasma was 18.7% in 150 chickens. Of these, 10% in house-bred chickens and 11.1% of farm-bred chickens were positive. Tissue cysts of *T. gondii* were demonstrated in 78.6% of the positive chickens (Deyab and Hassanein, 2005). Further studies included not only in determining the seroprevalence, but also in determining the genotypes using the SAG2 locus, and their differences on virulence in various areas in the world. In the United States, the prevalence of *T. gondii* was determined in 118 free-range chickens from 14 counties in Ohio and in 11 chickens from a pig farm in Massachusetts. *T. gondii* antibodies were found in 20 of 118 chickens from Ohio. Viable parasites were isolated in 19 chickens and isolates were avirulent for mice. Five isolates were type II and 14 were type III (Dubey *et al*., 2003b). In Granada, West Indias, 52% of 102 free-range chickens were seropositive for *Toxoplasma* and parasites were isolated from 36 chickens. All were avirulent for mice. Of these chicken, 29 were Type III, 5 were Type I, 1 was Type II, and 1 had both Type I and III (Dubey *et al*., 2005). In Brazil, 16 of 40 free range chickens were seropositive from a rural area. Parasites were isolated in 81% of 16 seropositive chickens. Of these seven isolates were type I and six were type III (Dubey *et al*., 2003c). In Argentina, 65% of 29 free-range chickens were seropositive for *Toxoplasma* and parasites were isolated from 9 of 19 seropositive chickens. One was type I, 1 was type II, and 7 were type III (Dubey *et al*., 2003d). In Mexico, seroprevalence was 6.2% in 208 free-range chickens. *T. gondii* was isolated from 6 of 13 seropositive chickens. All were avirulent for mice, 5 were type III, and 1 was type I (Dubey *et al*., 2004b). In a commercial farm in Israel, antibodies to *Toxoplasma* were found in 45 of 96 free-range chickens. *T. gondii* was isolated in 42.2% of seropositive chickens and of these, 17 were type II, and 2 were type III (Dubey *et al*., 2004d). In a rural area surrounding Giza in Egypt, seroprevalence of *T. gondii* was 40.4% in 121 free range chickens and 15.8% of 19 ducks. Of 20 chicken isolates, 17 were type III and three were type II. The duck isolate of *T. gondii* was type III. None of the isolates were lethal for mice (Dubey *et al*., 2003a). *Toxoplasma* has also been found in other bird and animal species. In the United States, *T. gondii* type III has been isolated from skunks, lories, and goose, and Type II has been isolated in cats. All Type III isolates were mouse virulent (Dubey *et al*., 2004c). In Poland, the prevalence of *T. gondii* in chicks of wild birds and captive individuals was detected in 5.8% of 205 white stork chicks and 13.6% of 44 adult storks (Andrzejewska *et al*., 2004). The high prevalence in chicken may be associated with chicken feed from the ground.

5.7.4 Sheep and Goats

Congenital transmission in pedigree Charollais and outbred sheep flocks has been reported. Overall rates of transmission per pregnancy, as determined by PCR based diagnosis, were consistent over time in a commercial sheep flock (69%) and in sympatric (60%) and allopatric (41%) populations of Charollais sheep. The result of this was that 53.7 % of lambs were acquiring an infection prior to birth: 46.4% of live lambs and 90.0% of dead lambs (Williams *et al*., 2005). In Worcestershire, UK, sheep flocks were examined. Significant differences in the frequency of abortion between sheep families ranged between 0% and 48%, and infection frequencies with *T. gondii* for different families varied between 0% and 100% (Morley *et al*., 2005). In Morocco, 27.6% of 261 sheep intended for consumption in Marrakech

were seropositive for IgG specific anti-*Toxoplasma* (Sawadogo *et al*., 2005). In the southeastern region of Brazil 34.7% of the samples were seropositive (Figliuolo *et al*., 2004). In Italy, during the period 1999-2002, specific IgG antibodies were detected in 2048 (28.4%) sheep and 302 (12.3%) goats, and specific IgM antibodies were found in 652 (9%) sheep and 139 (5.6%) goats. From a total of 2471 ovine and 362 caprine fetal samples, 271 (11.1%) ovine and 23 (6.4%) caprine samples were positive by PCR (Masala *et al*., 2003). The seroprevalence of antibodies to *T. gondii* in goats of Satun Province in Thailand was 27.9% in 631 goats. Female goats were 1.73 times more likely than male to be seropositive and dairy goats were more seropositive than meat goats (Jittapalapong *et al*., 2005).

Serum samples from 4339 wild cervids collected in Norway were tested for antibodies against *T. gondii* using the direct agglutination test. Positive titers were found in 33.9% of 760 roe deer, 12.6% of 2142 moose, 7.7% of 571 red deer, and 1.0% of 866 reindeer. Significant factors such as age were relevant in roe deer, moose, and red deer. Sex was significant in moose, but not for roe deer or red deer, and geographic regions were significant in only roe deer and male moose (Vikoren *et al*., 2004). In the United States, *T. gondii* was isolated from white-tailed deer from Mississippi, raccoons, bobcats, gray fox, red fox, coyote from Georgia, and black bears from Pennsylvania. All three genotypes of *T. gondii* based on the SAG2 locus were circulating among wildlife (Dubey *et al*., 2004a). Llamas in the Peruvian Andean region also have been seropositive to *Toxoplasma.* Using the IFAT assay, 55.8% of 43 llamas and 5.5% of 200 vicunas tested positive (Chavez-Velasquez *et al*., 2005).

5.7.5 Other Animal Species

Animals other than pigs have also been studied to determine their role in parasite transmission to humans. Serologic surveys indicate that *T. gondii* infections are common worldwide from Alaska to Australia in wild carnivores, including pigs, bears, felids, fox, raccoons, and skunks. Clinical and subclinical toxoplasmosis has been reported from wild cervids, ungulates, marsupials, monkeys, and marine mammals (Hill *et al*., 2005).

Overall, *Toxoplasma* in cattle is suspected to be 25% worldwide. *Toxoplasma* parasites present in milk does not seem to be a high risk factor in parasite transmission. Chickens can get infected with *Toxoplasma*, but chickens are not usually eaten raw; therefore the risk of acquiring the infection is reduced. Although seroprevalence in animals is high, infectious parasites have been demonstrated in a few animal species; including swine and chickens. Food-borne outbreaks following ingestion of raw meats were described in France (Choi *et al*., 1997; Vaillant *et al*., 2005). Few cases associated with drinking unpasteurized goat milk (Sacks *et al*., 1982), and eating raw meats and organs from wild boars, seal, caribou, and lamb have been described.

Sera was obtained from 12,628 clinically ill, client-owned cats in the United States. Overall, 31.6% of the cats were seropositive for *T. gondii*-specific IgM, IgG, or both. Seroprevalence increases as cats age and is higher in male and domestic shorthair cats, compared with females and other breeds (Vollaire *et al*., 2005). In Brazil, antibodies to *T. gondii* were found in 40% of stray cats, and 50.5% in stray dogs (Meireles *et al*., 2004). In Austria, using the IFAT, 35% of foxes and 26% of the

dogs examined were positive (Wanha *et al*., 2005). In the UK, lung fluid from over 549 foxes was examined using IFAT. Of these, 20% were seropositive to *T. gondii* (Hamilton *et al*., 2005). In Southern Argentina, 20% of 84 free-ranging foxes had antibodies to *Toxoplasma* (Martino *et al*., 2004).

Marine mammals are also susceptible to *Toxoplasma* infection. Using an IFA, 36% of 80 California sea otters and 38% of 21 Washington sea otters examined were seropositive for *T. gondii*. None of 65 Alaskan sea otters examined had antibodies to *Toxoplasma* (Miller *et al*., 2002). Another study reported *T. gondii* in 77% of 115 dead, and in 60% of 30 apparently healthy sea otters, in 16% of 311 Pacific harbor seals, 42% of 45 sea lions, 16% of 32 ringed seals, and 50% of 8 bearded seals, 11.1% of 9 spotted seals, 98% of 141 Atlantic bottlenose dolphins, and 6% of 53 walruses (Dubey *et al*., 2003e).

Toxoplasma gondii antibodies were present in 13.9% of 961 Polish farmed mink. On large farms, the seropositivity was lower (2.9%), than on small farms (26.33%). On farms feeding fish, percentage of seropositivity was lower (2.2%), than on farms based on non-frozen slaughter offal (43.4%) (Smielewska-Los and Turniak, 2004).

Rodents have also been reported as having *Toxoplasma* antibodies. In 456 wild rabbits, the prevalence was 14.2%. Prevalence of infection was significantly higher in wild rabbits from northeast Spain (53.8%), where rabbits lived in a forest. In other areas with drier conditions, prevalence ranged from 6.1 to 14.6% (Almeria *et al*., 2004). Capybaras, the largest rodent used for meat in South and Central America were seropositive by IFAT (69.8%) and with the MAT 63 (42.3%) (Canon-Franco *et al*., 2003). Raccoons from Fairfax County, Virginia were also surveyed. Out of 256 racoons, 84.4% had been exposed to *T. gondii* (Hancock *et al*., 2005). In Sao Paulo Brazil, antibodies to *T. gondii* were found in 82 (20.4%) of the 396 opossums using the MAT assay, and using the IFAT 148 of 396 were positive (Yai *et al*., 2003).

In Zimbabwe, wild animals have also been examined for the presence of *Toxoplasma* antibodies. Significantly high seroprevalence were found in the felidae (92% of 26), bovidae (55.9% of 34), and farm-reared struthionidae (48% of 50). The nyala had the highest seroprevalence at 90% (9/10). Low anti-*Toxoplasma* antibody prevalence was found in greater kudu (20% of 10), giraffe (10% of 10), and elephant (10% of 20). No antibodies were detected in the wild African suidae and bushpig (Hove and Mukaratirwa, 2005). In Thailand, 45.5% of 156 captive elephants were positive by MAT. In the same region, 14.09% of 447 dairy cattle on 14 dairy farms were also positive for *Toxoplasma.* Coinfections of *Neospora* and *Toxoplasma* were identified in 4.76% of the cattle (Gondim *et al*., 1999).

5.8 TREATMENT

Individuals with acute toxoplasmosis and congenital infections can be treated with pyrimethamine and sulfadiazine. These drugs are effective with tachyzoites, but not with the bradizoites in mature cysts. Spiramycin and clindamicin are effective, but have serious side effects (Alves and Vitor, 2005; Chakraborty *et al*., 1997; Djurkovic-Djakovic *et al*., 2005; guirre-Cruz *et al*., 1998; Lescano *et al*., 2004; Schmidt *et al*., 2005; Sordet *et al*., 1998; Tabbara *et al*., 2005).

Five hundred forty women of child bearing age with still births and spontaneous abortions in their obstetrical history were tested serologically for anti-*Toxoplasma* antibody using microlatex agglutination test. Maximum prevalence (10.2%) and highest titer of anti-*Toxoplasma* antibodies were observed in women of 35–42 years age group. The overall prevalence of toxoplasmosis in these women was 7.7%. Seropositive pregnant women were treated using a combined regimen of sulfadiazine and pyrimethamine. Incidence of toxoplasmosis in women is low because of infrequent and uncommon practices, such as a substantial number of the population surveyed ingested undercooked or uncooked food stuff, especially meat (Chakraborty *et al*., 1997).

Mice intraperitonealy inoculated with *Toxoplasma* tachizoites were treated with nifurtimox alone or in combination with pyrimethamine. Nifurtimox alone was not significantly effective against murine toxoplasmosis. However, when combined with pyrimethamine, a strong anti-*Toxoplasma* effect was obtained in comparison with survival rates associated with pyrimethamine or nifurtimox alone (guirre-Cruz *et al*., 1998).

The *in vitro* activity of atovaquone-loaded nanocapsules against tachyzoites of *T. gondii* was comparable to atovaquone suspension form. The sensibility of *T. gondii* to atovaquone varies according to the strains, and the activity of atovaquone in the treatment of toxoplasmosis is enhanced when administered in nanoparticular form (Sordet *et al*., 1998).

The efficacy of prolonged administration of azithromycin and pyrimethamine was evaluated in mice experimentally infected with a cystogenic strain of *T. gondii.* Mice started an oral treatment of 120 days, 20 days post infection. The association of both drugs provided the best results by diminishing the cyst count in the brain of the animals (Lescano *et al*., 2004).

The tolerability and efficacy of pyrimethamine and sulfadiazine in children with congenital toxoplasmosis was evaluated. Anemia or thrombocytopenia was not observed in treated children; however, progression of eye lesions was observed during the follow-up period. Although treatment was well tolerated in 86% (25/29) of the children and did not affect their weight gain, drug effectiveness at recommended concentrations was limited (Schmidt *et al*., 2005).

5.9 INACTIVATION

Cats, mice, and chickens have been used to determine infectivity and viability of *Toxoplasma* (Hellesnes and Mohn, 1977; Hiramoto *et al*., 2001; Lindsay *et al*., 2005; Piao *et al*., 2005). Whether this infectivity is selective to certain genotypes is still under study. *Toxoplasma* tachyzoites can be propagated using the MRC-5 cell line and most other fibroblast cell lines.

Cysts produced in mouse brains were used to experimentally spike milk and prepare homemade cheese. Cysts were infectious for 20 days at refrigeration temperature and survived the production process of homemade fresh cheese and storage for a period of 10 days. These findings support the importance of milk pasteurization before any processing or ingestion (Hiramoto *et al*., 2001).

High pressure processing (HPP) is an effective non-thermal method of eliminating non-spore forming bacteria. *Toxoplasma* oocysts were exposed from 100 to 550 MPa for 1 min in the HPP unit. Oocysts treated with 550 to 340 MPa were rendered noninfectious for mice. These results suggest that HPP technology may be useful in the removal of *T. gondii* oocysts from food products (Lindsay *et al*., 2005).

Oocysts remain viable when stored at 10–25°C for 200 days. At 35°C, oocysts were infective for 32 days, but not for 62 days; at 40°C they were still infective for 9 days, but not for 28 days. At 45°C, oocysts were non-infectious for a 2 day incubation. At 60°C, oocysts were rendered non infectious after 1 min. At 4°C, oocysts can be infectious for up to 54 months. At freezing temperatures, oocysts were still infectious at −5°C and −10°C after 106 days of storage. Sporulated oocysts are highly resistant and can survive freezing at −20°C. Freezing to −12°C and cooking to an internal temperature of 67°C can kill *Toxoplasma* cysts in meats (Dubey, 1996).

Unsporulated oocysts irradiated at 0.4 to 0.8 kGy sporulated, but were not infective to mice. Sporulated oocysts irradiated at ≥ 0.4 kGy were able to excyst, and sporozoites were infective, but not capable of inducing a viable infection in mice. *T. gondii* was detected in histologic sections of mice up to 5 days, but not at 7 days after feeding oocysts irradiated at 0.5 kGy. Raspberries inoculated with sporulated *T. gondii* oocysts were rendered innocuous after irradiation at 0.4 kGy (Dubey *et al*., 1998).

Toxoplasma gondii cysts were stored at −21°C for various periods of time and then inoculated into mice. Parasite cysts were rendered inactive only after freezing for 5 h or longer (Hellesnes and Mohn, 1977).

Pigs acquire *Toxoplasma* infection more commonly after birth than via transplacental infection. In most pigs, toxoplasmosis presents subclinically, whereas in young pigs clinical toxoplasmosis is observed. Tissue cysts persist in brain, heart, and tongue for several months (Dubey, 1986).

REFERENCES

Akoijam, B. S., Shashikant, Singh, S., and Kapoor, S. K., 2002, Seroprevalence of *Toxoplasma* infection among primigravid women attending antenatal clinic at a secondary level hospital in North India, *J. Indian Med. Assoc.* **100**:591–596, 602.

Almeria, S., Calvete, C., Pages, A., Gauss, C., and Dubey, J. P., 2004, Factors affecting the seroprevalence of *Toxoplasma gondii* infection in wild rabbits (Oryctolagus cuniculus) from Spain, *Vet. Parasitol.* **123**:265–270.

Alves, C. F., and Vitor, R. W., 2005, Efficacy of atovaquone and sulfadiazine in the treatment of mice infected with *Toxoplasma gondii* strains isolated in Brazil, *Parasite* **12**:171–177.

Amendoeira, M. R., Sobral, C. A., Teva, A., de Lima, J. N., and Klein, C. H., 2003, Serological survey of *Toxoplasma gondii* infection in isolated Amerindians, Mato Grosso, *Rev. Soc. Bras. Med. Trop.* **36**:671–676.

Andrzejewska, I., Tryjanowski, P., Zduniak, P., Dolata, P. T., Ptaszyk, J., and Cwiertnia, P., 2004, *Toxoplasma gondii* antibodies in the white stork Ciconia ciconia, *Berl. Munch. Tierarztl. Wschr.* **117**:274–275.

Aramini, J. J., Stephen, C., Dubey, J. P., Engelstoft, C., Schwantje, H., and Ribble, C. S., 1999, Potential contamination of drinking water with *Toxoplasma gondii* oocysts, *Epidemiol. Infect.* **122**:305–315.

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 galloprovincialis), *Int. J. Parasitol.* **33**:1087–1097.

Ashrafunnessa, Khatun, S., Islam, M. N., and Huq, T., 1998, Seroprevalence of *Toxoplasma* antibodies among the antenatal population in Bangladesh, *J. Obstet. Gynaecol. Res.* **24**:115–119.

Aspinall, T. V., Guy, E. C., Roberts, K. E., Joynson, D. H., Hyde, J. E., and Sims, P. F., 2003, Molecular evidence for multiple *Toxoplasma gondii* infections in individual patients in England and Wales: Public health implications, *Int. J. Parasitol.* **33**:97–103.

az-Suarez, O., Estevez, J., Garcia, M., Cheng-Ng, R., Araujo, J., and Garcia, M., 2003, Seroepidemiology of toxoplasmosis in a Yucpa Amerindian community of Sierra de Perija, Zulia State, Venezuela, *Rev. Med. Chil.* **131**:1003–1010.

Bahia-Oliveira, L. M., Jones, J. L., zevedo-Silva, J., Alves, C. C., Orefice, F., and Addiss, D. G., 2003, Highly endemic, waterborne toxoplasmosis in north Rio de Janeiro state, Brazil, *Emerg. Infect. Dis.* **9**:55–62.

Bartova, E., and Literak, I., 2004, K24 *T. gondii* isolate is a hybrid and has the virulence of lineage I isolates, *Parasite* **11**:183–188.

Berends, B. R., Smeets, J. F., Harbers, A. H., van, K. F., and Snijders, J. M., 1991, Investigations with enzyme-linked immunosorbent assays for Trichinella spiralis and *Toxoplasma gondii* in the Dutch "Integrated Quality Control for finishing pigs" research project, *Vet. Q.* **13**:190–198.

Bessieres, M. H., Le, B. S., and Seguela, J. P., 1992, Analysis by immunoblotting of *Toxoplasma gondii* exo-antigens and comparison with somatic antigens, *Parasitol. Res.* **78**:222–228.

Boothroyd, J. C., and Grigg, M. E., 2002, Population biology of *Toxoplasma gondii* and its relevance to human infection: Do different strains cause different disease? *Curr. Opin. Microbiol.* **5**:438–442.

Brindley, P. J., Gazzinelli, R. T., Denkers, E. Y., Davis, S. W., Dubey, J. P., Belfort, R., Jr., Martins, M. C., Silveira, C., Jamra, L., and Waters, A. P., 1993, Differentiation of *Toxoplasma gondii* from closely related coccidia by riboprint analysis and a surface antigen gene polymerase chain reaction, *Am. J. Trop. Med. Hyg.* **48**:447–456.

Buchbinder, S., Blatz, R., and Rodloff, A. C., 2003, Comparison of real-time PCR detection methods for B1 and P30 genes of *Toxoplasma gondii*, *Diagn. Microbiol. Infect. Dis.* **45**:269–271.

Canon-Franco, W. A., Yai, L. E., Joppert, A. M., Souza, C. E., D'Auria, S. R., Dubey, J. P., and Gennari, S. M., 2003, Seroprevalence of *Toxoplasma gondii* antibodies in the rodent capybara (Hidrochoeris hidrochoeris) from Brazil, *J. Parasitol.* **89**:850.

Carme, B., Bissuel, F., Ajzenberg, D., Bouyne, R., Aznar, C., Demar, M., Bichat, S., Louvel, D., Bourbigot, A. M., Peneau, C., Neron, P., and Darde, M. L., 2002, Severe acquired toxoplasmosis in immunocompetent adult patients in French Guiana, *J. Clin. Microbiol.* **40**:4037–4044.

Chacin-Bonilla, L., Sanchez-Chavez, Y., Monsalve, F., and Estevez, J., 2001, Seroepidemiology of toxoplasmosis in amerindians from western Venezuela, *Am. J. Trop. Med. Hyg.* **65**:131–135.

Chakraborty, P., Sinha, S., Adhya, S., Chakraborty, G., and Bhattacharya, P., 1997, Toxoplasmosis in women of child bearing age and infant follow up after in-utero treatment, *Indian J. Pediatr.* **64**:879–882.

Chavez-Velasquez, A., varez-Garcia, G., Gomez-Bautista, M., Casas-Astos, E., Serrano-Martinez, E., and Ortega-Mora, L. M., 2005, *Toxoplasma gondii* infection in adult llamas (Lama glama) and vicunas (Vicugnavicugna) in the Peruvian Andean region, *Vet. Parasitol.* **130**:93–97.

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, Food-borne outbreaks of human toxoplasmosis, *J. Infect. Dis.* **175**:1280–1282.

Condorelli, F., Scalia, G., Stivala, A., Costanzo, M. C., Adragna, A. D., Franceschino, C., Santagati, M. G., Furneri, P. M., Marino, A., and Castro, A., 1993, Seroprevalence to some TORCH agents in a Sicilian female population of fertile age, *Eur. J. Epidemiol.* **9**:341–343.

Contini, C., Seraceni, S., Cultrera, R., Incorvaia, C., Sebastiani, A., and Picot, S., 2005, Evaluation of a Real-time PCR-based assay using the lightcycler system for detection of *Toxoplasma gondii* bradyzoite genes in blood specimens from patients with toxoplasmic retinochoroiditis, *Int. J. Parasitol.* **35**:275–283.

Coutinho, S. G., Lobo, R., and Dutra, G., 1982, Isolation of *Toxoplasma* from the soil during an outbreak of toxoplasmosis in a rural area in Brazil, *J. Parasitol.* **68**:866–868.

de la, R. M., Bolivar, J., and Perez, H. A., 1999, *Toxoplasma gondii* infection in Amerindians of Venezuelan Amazon, *Medicina* (*B Aires*) **59**:759–762.

de, A. D. S. C., de Carvalho, A. C., Ragozo, A. M., Soares, R. M., Amaku, M., Yai, L. E., Dubey, J. P., and Gennari, S. M., 2005, First isolation and molecular characterization of *Toxoplasma gondii* from finishing pigs from Sao Paulo State, Brazil, *Vet. Parasitol.* **131**:207–211.

Deyab, A. K., and Hassanein, R., 2005, Zoonotic toxoplasmosis in chicken, *J. Egypt. Soc. Parasitol.* **35**:341–350.

Diza, E., Frantzidou, F., Souliou, E., Arvanitidou, M., Gioula, G., and Antoniadis, A., 2005, Seroprevalence of *Toxoplasma gondii* in northern Greece during the last 20 years, *Clin. Microbiol. Infect.* **11**:719–723.

Djurkovic-Djakovic, O., Nikolic, A., Bobic, B., Klun, I., and Aleksic, A., 2005, Stage conversion of *Toxoplasma gondii* RH parasites in mice by treatment with atovaquone and pyrrolidine dithiocarbamate, *Microbes. Infect.* **7**:49–54.

Dubey, J. P., 1985, Serologic prevalence of toxoplasmosis in cattle, sheep, goats, pigs, bison, and elk in Montana, *J. Am. Vet. Med. Assoc.* **186**:969–970.

Dubey, J. P., 1986, A review of toxoplasmosis in pigs, *Vet. Parasitol.* **19**:181–223.

Dubey, J. P., 1991, Toxoplasmosis–an overview, *Southeast Asian J. Trop. Med. Public Health*, **22**(Suppl):88–92.

Dubey, J. P., 1996, Strategies to reduce transmission of *Toxoplasma gondii* to animals and humans, *Vet. Parasitol.* **64**:65–70.

Dubey, J. P., 2004, Toxoplasmosis–a waterborne zoonosis, *Vet. Parasitol.* **126**:57–72.

Dubey, J. P., Leighty, J. C., Beal, V. C., Anderson, W. R., Andrews, C. D., and Thulliez, P., 1991, National seroprevalence of *Toxoplasma gondii* in pigs, *J. Parasitol.* **77**:517–521.

Dubey, J. P., Gamble, H. R., Rodrigues, A. O., and Thulliez, P., 1992, Prevalence of antibodies to *Toxoplasma gondii* and Trichinella spiralis in 509 pigs from 31 farms in Oahu, Hawaii, *Vet. Parasitol.* **43**:57–63.

Dubey, J. P., Weigel, R. M., Siegel, A. M., Thulliez, P., Kitron, U. D., Mitchell, M. A., Mannelli, A., Mateus-Pinilla, N. E., Shen, S. K., and Kwok, O. C., 1995, Sources and reservoirs of *Toxoplasma gondii* infection on 47 swine farms in Illinois, *J. Parasitol.* **81**:723–729.

Dubey, J. P., Rollor, E. A., Smith, K., Kwok, O. C., and Thulliez, P., 1997, Low seroprevalence of *Toxoplasma gondii* in feral pigs from a remote island lacking cats, *J. Parasitol.* **83**:839–841.

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

Dubey, J. P., Gamble, H. R., Hill, D., Sreekumar, C., Romand, S., and Thuilliez, P., 2002, High prevalence of viable *Toxoplasma gondii* infection in market weight pigs from a farm in Massachusetts, *J. Parasitol.* **88**:1234–1238.

Dubey, J. P., Graham, D. H., Dahl, E., Hilali, M., El-Ghaysh, A., Sreekumar, C., Kwok, O. C., Shen, S. K., and Lehmann, T., 2003a, Isolation and molecular characterization of *Toxoplasma gondii* from chickens and ducks from Egypt, *Vet. Parasitol.* **114**: 89–95.

Dubey, J. P., Graham, D. H., Dahl, E., Sreekumar, C., Lehmann, T., Davis, M. F., and Morishita, T. Y., 2003b, *Toxoplasma gondii* isolates from free-ranging chickens from the United States, *J. Parasitol.* **89**:1060–1062.

Dubey, J. P., Navarro, I. T., Graham, D. H., Dahl, E., Freire, R. L., Prudencio, L. B., Sreekumar, C., Vianna, M. C., and Lehmann, T., 2003c, Characterization of *Toxoplasma gondii* isolates from free range chickens from Parana, Brazil, *Vet. Parasitol.* **117**:229–234.

Dubey, J. P., Venturini, M. C., Venturini, L., Piscopo, M., Graham, D. H., Dahl, E., Sreekumar, C., Vianna, M. C., and Lehmann, T., 2003d, Isolation and genotyping of *Toxoplasma gondii* from free-ranging chickens from Argentina, *J. Parasitol.* **89**:1063–1064.

Dubey, J. P., Zarnke, R., Thomas, N. J., Wong, S. K., Van, B. W., Briggs, M., Davis, J. W., Ewing, R., Mense, M., Kwok, O. C., Romand, S., and Thulliez, P., 2003e, *Toxoplasma gondii*, Neospora caninum, Sarcocystis neurona, and Sarcocystis canis-like infections in marine mammals, *Vet. Parasitol.* **116**:275–296.

Dubey, J. P., Graham, D. H., De Young, R. W., Dahl, E., Eberhard, M. L., Nace, E. K., Won, K., Bishop, H., Punkosdy, G., Sreekumar, C., Vianna, M. C., Shen, S. K., Kwok, O. C., Sumners, J. A., Demarais, S., Humphreys, J. G., and Lehmann, T., 2004a, Molecular and biologic characteristics of *Toxoplasma gondii* isolates from wildlife in the United States, *J. Parasitol.* **90**:67–71.

Dubey, J. P., Morales, E. S., and Lehmann, T., 2004b, Isolation and genotyping of *Toxoplasma gondii* from free-ranging chickens from Mexico, *J. Parasitol.* **90**:411–413.

Dubey, J. P., Parnell, P. G., Sreekumar, C., Vianna, M. C., De Young, R. W., Dahl, E., and Lehmann, T., 2004c, Biologic and molecular characteristics of *Toxoplasma gondii* isolates from striped skunk (Mephitis mephitis), Canada goose (Branta canadensis), black-winged lory (Eos cyanogenia), and cats (Felis catus), *J. Parasitol.* **90**:1171–1174.

Dubey, J. P., Salant, H., Sreekumar, C., Dahl, E., Vianna, M. C., Shen, S. K., Kwok, O. C., Spira, D., Hamburger, J., and Lehmann, T. V., 2004d, High prevalence of *Toxoplasma gondii* in a commercial flock of chickens in Israel, and public health implications of free-range farming, *Vet. Parasitol.* **121**:317–322.

Dubey, J. P., Bhaiyat, M. I., de, A. C., Macpherson, C. N., Sharma, R. N., Sreekumar, C., Vianna, M. C., Shen, S. K., Kwok, O. C., Miska, K. B., Hill, D. E., and Lehmann, T., 2005, Isolation, tissue distribution, and molecular characterization of *Toxoplasma gondii* from chickens in Grenada, West Indies, *J. Parasitol.* **91**:557–560.

Dumas, N., Cazaux, M., Meunier, D. M., Seguela, J. P., and Charlet, J. P., 1990, Toxoplasmosis in the Central African Republic. Complementary study in a rural area, *Bull. Soc. Pathol. Exot.* **83**:342–348.

Edelhofer, R., 1994, Prevalence of antibodies against *Toxoplasma gondii* in pigs in Austria—an evaluation of data from 1982 and 1992, *Parasitol. Res.* **80**:642–644.

Fan, C. K., Su, K. E., Wu, G. H., and Chiou, H. Y., 2002, Seroepidemiology of *Toxoplasma gondii* infection among two mountain aboriginal populations and Southeast Asian laborers in Taiwan, *J. Parasitol.* **88**:411–414.

Fan, C. K., Su, K. E., and Tsai, Y. J., 2004, Serological survey of *Toxoplasma gondii* infection among slaughtered pigs in northwestern Taiwan, *J. Parasitol.* **90**:653–654.

Fazaeli, A., Carter, P. E., Darde, M. L., and Pennington, T. H., 2000, Molecular typing of *Toxoplasma gondii* strains by GRA6 gene sequence analysis, *Int. J. Parasitol.* **30**:637–642.

Figliuolo, L. P., Kasai, N., Ragozo, A. M., de, P., V, Dias, R. A., Souza, S. L., and Gennari, S. M., 2004, Prevalence of anti-*Toxoplasma gondii* and anti-Neospora caninum antibodies in ovine from Sao Paulo State, Brazil, *Vet. Parasitol.* **123**:161–166.

Flatau, E., Nishri, Z., Mates, A., Qupty, G., Reichman, N., and Raz, R., 1993, Seroprevalence of antibodies against *Toxoplasma gondii* among recently immigrating Ethiopian Jews, *Isr. J. Med. Sci.* **29**:395–397.

Fuentes, I., Rubio, J. M., Ramirez, C., and Alvar, J., 2001, Genotypic characterization of *Toxoplasma gondii* strains associated with human toxoplasmosis in Spain: Direct analysis from clinical samples, *J. Clin. Microbiol.* **39**:1566–1570.

Furuta, T., Une, Y., Omura, M., Matsutani, N., Nomura, Y., Kikuchi, T., Hattori, S., and Yoshikawa, Y., 2001, Horizontal transmission of *Toxoplasma gondii* in squirrel monkeys (Saimiri sciureus), *Exp. Anim.* **50**:299–306.

Gajadhar, A. A., Aramini, J. J., Tiffin, G., and Bisaillon, J. R., 1998, Prevalence of *Toxoplasma gondii* in Canadian market-age pigs, *J. Parasitol.* **84**:759–763.

Gamble, H. R., Brady, R. C., and Dubey, J. P., 1999, Prevalence of *Toxoplasma gondii* infection in domestic pigs in the New England states, *Vet. Parasitol.* **82**:129–136.

Gascon, J., Torres-Rodriguez, J. M., Soldevila, M., and Merlos, A. M., 1989, Seroepidemiology of toxoplasmosis in 2 communities of Rwanda (Central Africa), *Rev. Inst. Med. Trop. Sao Paulo*, **31**:399–402.

Gauss, C. B., Dubey, J. P., Vidal, D., Ruiz, F., Vicente, J., Marco, I., Lavin, S., Gortazar, C., and Almeria, S., 2005, Seroprevalence of *Toxoplasma gondii* in wild pigs (Sus scrofa) from Spain, *Vet. Parasitol.* **131**:151–156.

Gill, H. S., and Mtimavalye, L. A., 1982, Prevalence of *Toxoplasma* antibodies in pregnant African women in Tanzania, *Afr. J. Med. Med. Sci.* **11**:167–170.

Gondim, L. F., Sartor, I. F., Hasegawa, M., and Yamane, I., 1999, Seroprevalence of Neospora caninum in dairy cattle in Bahia, Brazil, *Vet. Parasitol.* **86**:71–75.

Guerra, G. C., and Fernandez, S. J., 1995, Seroprevalence of *Toxoplasma gondii* in pregnant women, *Aten. Primaria* **16**:151–153.

guirre-Cruz, L., Velasco, O., and Sotelo, J., 1998, Nifurtimox plus pyrimethamine for treatment of murine toxoplasmosis, *J. Parasitol.* **84**:1032–1033.

Gutierrez, J., Roldan, C., and Maroto, M. C., 1996, Seroprevalence of human toxoplasmosis, *Microbios* **85**:73–75.

Hamilton, C. M., Gray, R., Wright, S. E., Gangadharan, B., Laurenson, K., and Innes, E. A., 2005, Prevalence of antibodies to *Toxoplasmagondii* and Neosporacaninum in red foxes (Vulpesvulpes) from around the UK, *Vet. Parasitol.* **130**:169–173.

Hancock, K., Thiele, L. A., Zajac, A. M., Elvingert, F., and Lindsay, D. S., 2005, Prevalence of antibodies to *Toxoplasma gondii* in raccoons (Procyon lotor) from an urban area of Northern Virginia, *J. Parasitol.* **91**:694–695.

Hellesnes, I., and Mohn, S. F., 1977, Effects of freezing on the infectivity of *Toxoplasma gondii* cysts for white mice, *Zentralbl. Bakteriol. [Orig. A]* **238**:143–148.

Hill, D. E., Chirukandoth, S., and Dubey, J. P., 2005, Biology and epidemiology of *Toxoplasma gondii* in man and animals, *Anim. Health Res. Rev.* **6**:41–61.

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. Saude Publica* **35**:113–118.

Homan, W. L., Vercammen, M., De, B. J., and Verschueren, H., 2000, Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR, *Int. J. Parasitol.* **30**:69–75.

Hove, T., and Dubey, J. P., 1999, Prevalence of *Toxoplasma gondii* antibodies in sera of domestic pigs and some wild game species from Zimbabwe, *J. Parasitol.* **85**:372–373.

Hove, T., and Mukaratirwa, S., 2005, Seroprevalence of *Toxoplasma gondii* in farm-reared ostriches and wild game species from Zimbabwe, *Acta Trop.* **94**:49–53.

Howe, D. K., and Sibley, L. D., 1995, *Toxoplasma gondii* comprises three clonal lineages: Correlation of parasite genotype with human disease, *J. Infect. Dis.* **172**:1561–1566.

Inoue, I., Leow, C. S., Husin, D., Matsuo, K., and Darmani, P., 2001, A survey of *Toxoplasma gondii* antibodies in pigs in Indonesia, *Southeast Asian J. Trop. Med. Public Health* **32**:38–40.

Isaac-Renton, J., Bowie, W. R., King, A., Irwin, G. S., Ong, C. S., Fung, C. P., Shokeir, M. O., and Dubey, J. P., 1998, Detection of *Toxoplasma gondii* oocysts in drinking water, *Appl. Environ. Microbiol.* **64**:2278–2280.

Jacobs, M. R., and Mason, P. R., 1978, Prevalence of *Toxoplasma* antibodies in Southern Africa, *S. Afr. Med. J.* **53**:619–621.

Jacquier, P., Hohlfeld, P., Vorkauf, H., and Zuber, P., 1995, Epidemiology of toxoplasmosis in Switzerland: National study of seroprevalence monitored in pregnant women 1990–1991, *Schweiz. Med. Wochenschr. Suppl.* **65**:29S–38S.

Jalal, S., Nord, C. E., Lappalainen, M., and Evengard, B., 2004, Rapid and sensitive diagnosis of *Toxoplasma gondii* infections by PCR, *Clin. Microbiol. Infect.* **10**:937–939.

Jittapalapong, S., Sangvaranond, A., Pinyopanuwat, N., Chimnoi, W., Khachaeram, W., Koizumi, S., and Maruyama, S., 2005, Seroprevalence of *Toxoplasma gondii* infection in domestic goats in Satun Province, Thailand, *Vet. Parasitol.* **127**:17–22.

Jones, J. L., Kruszon-Moran, D., Wilson, M., McQuillan, G., Navin, T., and McAuley, J. B., 2001, *Toxoplasma gondii* infection in the United States: Seroprevalence and risk factors, *Am. J. Epidemiol.* **154**:357–365.

Jones, J. L., Kruszon-Moran, D., and Wilson, M., 2003, *Toxoplasma gondii* infection in the United States, 1999-2000, *Emerg. Infect. Dis.* **9**:1371–1374.

Klun, I., Djurkovic-Djakovic, O., Katic-Radivojevic, S., and Nikolic, A., 2005, Cross-sectional survey on *Toxoplasma gondii* infection in cattle, sheep, and pigs in Serbia: Seroprevalence and risk factors, *Vet. Parasitol.* **135**:121–131.

Kortbeek, L. M., De Melker, H. E., Veldhuijzen, I. K., and Conyn-Van Spaendonck, M. A., 2004, Population-based *Toxoplasma* seroprevalence study in The Netherlands, *Epidemiol. Infect.* **132**:839–845.

Kourenti, C., and Karanis, P., 2004, Development of a sensitive polymerase chain reaction method for the detection of *Toxoplasma gondii* in water, *Water Sci. Technol.* **50**:287–291.

Kuticic, V., and Wikerhauser, T., 2000, A survey of chickens for viable toxoplasms in Croatia, *Acta Vet. Hung.* **48**:183–185.

Lescano, S. A., Amato, N., V, Chieffi, P. P., Bezerra, R. C., Gakiya, E., Ferreira, C. S., and Braz, L. M., 2004, Evaluation of the efficacy of azithromycin and pyrimethamine, for treatment of experimental infection of mice with *Toxoplasma gondii* cystogenic strain, *Rev. Soc. Bras. Med. Trop.* **37**:460–462.

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**:699–701.

Logar, J., Novak-Antolic, Z., Zore, A., Cerar, V., and Likar, M., 1992, Incidence of congenital toxoplasmosis in the Republic of Slovenia, *Scand. J. Infect. Dis.* **24**:105–108.

Logar, J., Novak-Antolic, Z., and Zore, A., 1995, Serological screening for toxoplasmosis in pregnancy in Slovenia, *Scand. J. Infect. Dis.* **27**:163–164.

Lopez, A., Dietz, V. J., Wilson, M., Navin, T. R., and Jones, J. L., 2000, Preventing congenital toxoplasmosis, *MMWR Recomm. Rep.* **49**:59–68.

Lovelace, J. K., Moraes, M. A., and Hagerby, E., 1978, Toxoplasmosis among the Ticuna Indians in the state of Amazonas, Brazil, *Trop. Geogr. Med.* **30**:295–300.

Lunden, A., Lind, P., Engvall, E. O., Gustavsson, K., Uggla, A., and Vagsholm, I., 2002, Serological survey of *Toxoplasma gondii* infection in pigs slaughtered in Sweden, *Scand. J. Infect. Dis.* **34**:362–365.

MacPherson, J. M., and Gajadhar, A. A., 1993, Sensitive and specific polymerase chain reaction detection of *Toxoplasma gondii* for veterinary and medical diagnosis, *Can. J. Vet. Res.* **57**:45–48.

Maiga, I., Kiemtore, P., and Tounkara, A., 2001, Prevalence of anti *Toxoplasma* antibodies in patients with acquired immunodeficiency syndrome and blood donors in Bamako, *Bull. Soc. Pathol. Exot.* **94**:268–270.

Martino, P. E., Montenegro, J. L., Preziosi, J. A., Venturini, C., Bacigalupe, D., Stanchi, N. O., and Bautista, E. L., 2004, Serological survey of selected pathogens of free-ranging foxes in southern Argentina, 1998–2001, *Rev. Sci. Tech.* **23**:801–806.

Masala, G., Porcu, R., Madau, L., Tanda, A., Ibba, B., Satta, G., and Tola, S., 2003, Survey of ovine and caprine toxoplasmosis by IFAT and PCR assays in Sardinia, Italy, *Vet. Parasitol.* **117**:15–21.

Meireles, L. R., Galisteo, A. J., Jr., Pompeu, E., and Andrade, H. F., Jr., 2004, *Toxoplasma gondii* spreading in an urban area evaluated by seroprevalence in free-living cats and dogs, *Trop. Med. Int. Health* **9**:876–881.

Meisel, R., Stachelhaus, S., Mevelec, M. N., Reichmann, G., Dubremetz, J. F., and Fischer, H. G., 1996, Identification of two alleles in the GRA4 locus of *Toxoplasma gondii* determining a differential epitope which allows discrimination of type I versus type II and III strains, *Mol. Biochem. Parasitol.* **81**:259–263.

Meisheri, Y. V., Mehta, S., and Patel, U., 1997, A prospective study of seroprevalence of Toxoplasmosis in general population, and in HIV/AIDS patients in Bombay, India, *J. Postgrad. Med.* **43**:93–97.

Miller, M. A., Gardner, I. A., Packham, A., Mazet, J. K., Hanni, K. D., Jessup, D., Estes, J., Jameson, R., Dodd, E., Barr, B. C., Lowenstine, L. J., Gulland, F. M., and Conrad, P. A., 2002, Evaluation of an indirect fluorescent antibody test (IFAT) for demonstration of antibodies to *Toxoplasma gondii* in the sea otter (*Enhydra lutris*), *J. Parasitol.* **88**:594–599.

Mondragon, R., Howe, D. K., Dubey, J. P., and Sibley, L. D., 1998, Genotypic analysis of *Toxoplasma gondii* isolates from pigs, *J. Parasitol.* **84**:639–641.

Morley, E. K., Williams, R. H., Hughes, J. M., Terry, R. S., Duncanson, P., Smith, J. E., and Hide, G., 2005, Significant familial differences in the frequency of abortion and *Toxoplasma gondii* infection within a flock of Charollais sheep, *Parasitology* **131**:181–185.

Oksanen, A., Tryland, M., Johnsen, K., and Dubey, J. P., 1998, Serosurvey of *Toxoplasma gondii* in North Atlantic marine mammals by the use of agglutination test employing whole tachyzoites and dithiothreitol, *Comp. Immunol. Microbiol. Infect. Dis.* **21**:107–114.

Omata, Y., Dilorenzo, C., Venturini, C., Venturini, L., Igarashi, I., Saito, A., and Suzuki, N., 1994, Correlation between antibody levels in *Toxoplasma gondii* infected pigs and pathogenicity of the isolated parasite, *Vet. Parasitol.* **51**:205–210.

Petersson, K., Stray-Pedersen, B., Malm, G., Forsgren, M., and Evengard, B., 2000, Seroprevalence of *Toxoplasma gondii* among pregnant women in Sweden, *Acta Obstet. Gynecol. Scand.* **79**:824–829.

Piao, L. X., Aosai, F., Mun, H. S., and Yano, A., 2005, Peroral infectivity of *Toxoplasma gondii* in bile and feces of interferon-gamma knockout mice, *Microbiol. Immunol.* **49**:239–243.

Rai, S. K., Matsumura, T., Ono, K., Abe, A., Hirai, K., Rai, G., Sumi, K., Kubota, K., Uga, S., and Shrestha, H. G., 1999, High *Toxoplasma* seroprevalence associated with meat eating habits of locals in Nepal, *Asia Pac. J. Public Health* **11**:89–93.

rko-Mensah, J., Bosompem, K. M., Canacoo, E. A., Wastling, J. M., and Akanmori, B. D., 2000, The seroprevalence of toxoplasmosis in pigs in Ghana, *Acta Trop.* **76**:27–31.

Roberts, C., and McLeod, R., 2004, *Toxoplasma gondii*. In Gorbach, S. L., Bartlett, J. G., and Blacklow, N. R. (eds), *Infections Diseases*, Vol. 282, Lippincott Williams & Wilkins, Philadelphia, PA, pp. 2334–2339.

Roghmann, M. C., Faulkner, C. T., Lefkowitz, A., Patton, S., Zimmerman, J., and Morris, J. G., Jr., 1999, Decreased seroprevalence for *Toxoplasma gondii* in Seventh Day Adventists in Maryland, *Am. J. Trop. Med. Hyg.* **60**:790–792.

Ruiz, A., Frenkel, J. K., and Cerdas, L., 1973, Isolation of *Toxoplasma* from soil, *J. Parasitol.* **59**:204–206.

Ryu, J. S., Min, D. Y., Ahn, M. H., Choi, H. G., Rho, S. C., Shin, Y. J., Choi, B., and Joo, H. D., 1996, *Toxoplasma* antibody titers by ELISA and indirect latex agglutination test in pregnant women, *Korean J. Parasitol.* **34**:233–238.

Saavedra, G. M., and Ortega, Y. R., 2004, Seroprevalence of *Toxoplasma gondii* in swine from slaughterhouses in Lima, Peru, and Georgia, U.S.A, *J. Parasitol.* **90**:902–904.

Sacks, J. J., Roberto, R. R., and Brooks, N. F., 1982, Toxoplasmosis infection associated with raw goat's milk, *JAMA* **248**:1728–1732.

Sawadogo, P., Hafid, J., Bellete, B., Sung, R. T., Chakdi, M., Flori, P., Raberin, H., Hamouni, I. B., Chait, A., and Dalal, A., 2005, Seroprevalence of T. *gondii* in sheep from Marrakech, Morocco, *Vet. Parasitol.* **130**:89–92.

Schmidt, D. R., Hogh, B., Andersen, O., Hansen, S. H., Dalhoff, K., and Petersen, E., 2005, Treatment of infants with congenital toxoplasmosis: Tolerability and plasma concentrations of sulfadiazine and pyrimethamine, *Eur. J. Pediatr.* **165**:19–25.

Schwab, K. J., and McDevitt, J. J., 2003, Development of a PCR-enzyme immunoassay oligoprobe detection method for *Toxoplasma gondii* oocysts, incorporating PCR controls, *Appl. Environ. Microbiol.* **69**:5819–5825.

Smielewska-Los, E., and Turniak, W., 2004, *Toxoplasma gondii* infection in Polish farmed mink, *Vet. Parasitol.* **122**:201–206.

Sobral, C. A., Amendoeira, M. R., Teva, A., Patel, B. N., and Klein, C. H., 2005, Seroprevalence of infection with *Toxoplasma gondii* in indigenous Brazilian populations, *Am. J. Trop. Med. Hyg.* **72**:37–41.

Song, K. J., Shin, J. C., Shin, H. J., and Nam, H. W., 2005, Seroprevalence of toxoplasmosis in Korean pregnant women, *Korean J. Parasitol.* **43**:69–71.

Sordet, F., Aumjaud, Y., Fessi, H., and Derouin, F., 1998, Assessment of the activity of atovaquone-loaded nanocapsules in the treatment of acute and chronic murine toxoplasmosis, *Parasite* **5**:223–229.

Sousa, S. D., Ajzenberg, D., Canada, N., Freire, L., Costa, J. M., Darde, M. L., Thulliez, P., and Dubey, J. P., 2005, Biologic and molecular characterization of *Toxoplasma gondii* isolates from pigs from Portugal, *Vet. Parasitol.* **135**:133–136.

Sturchler, D., DiGiacomo, R. F., and Rausch, L., 1987, Parasitic infections in Yakima Indians, *Ann. Trop. Med. Parasitol.* **81**:291–299.

Suarez-Aranda, F., Galisteo, A. J., Hiramoto, R. M., Cardoso, R. P., Meireles, L. R., Miguel, O., and Andrade, H. F., Jr., 2000, The prevalence and avidity of *Toxoplasma gondii* IgG antibodies in pigs from Brazil and Peru, *Vet. Parasitol.* **91**:23–32.

Tabbara, K. F., Hammouda, E., Tawfik, A., Al-Omar, O. M., and bu El-Asrar, A. M., 2005, Azithromycin prophylaxis and treatment of murine toxoplasmosis, *Saudi. Med. J.* **26**:393–397.

Terry, R. S., Smith, J. E., Duncanson, P., and Hide, G., 2001, MGE-PCR: A novel approach to the analysis of *Toxoplasma gondii* strain differentiation using mobile genetic elements, *Int. J. Parasitol.* **31**:155–161.

Tinti, M., Possenti, A., Cherchi, S., Barca, S., and Spano, F., 2003, Analysis of the SAG5 locus reveals a distinct genomic organisation in virulent and avirulent strains of *Toxoplasma gondii*, *Int. J. Parasitol.* **33**:1605–1616.

Vaillant, V., de, V. H., Baron, E., Ancelle, T., Colin, P., Delmas, M. C., Dufour, B., Pouillot, R., Le, S. Y., Weinbreck, P., Jougla, E., and Desenclos, J. C., 2005, Food-borne infections in France, *Foodborne Pathog. Dis.* **2**:221–232.

Venturini, M. C., Bacigalupe, D., Venturini, L., Rambeaud, M., Basso, W., Unzaga, J. M., and Perfumo, C. J., 2004, Seroprevalence of *Toxoplasma gondii* in sows from slaughterhouses and in pigs from an indoor and an outdoor farm in Argentina, *Vet. Parasitol.* **124**:161–165.

Vikoren, T., Tharaldsen, J., Fredriksen, B., and Handeland, K., 2004, Prevalence of *Toxoplasma gondii* antibodies in wild red deer, roe deer, moose, and reindeer from Norway, *Vet. Parasitol.* **120**:159–169.

Villena, I., Aubert, D., Gomis, P., Ferte, H., Inglard, J. C., is-Bisiaux, H., Dondon, J. M., Pisano, E., Ortis, N., and Pinon, J. M., 2004, Evaluation of a strategy for *Toxoplasma gondii* oocyst detection in water, *Appl. Environ. Microbiol.* **70**:4035–4039.

Vollaire, M. R., Radecki, S. V., and Lappin, M. R., 2005, Seroprevalence of *Toxoplasma gondii* antibodies in clinically ill cats in the United States, *Am. J. Vet. Res.* **66**:874–877.

Wanha, K., Edelhofer, R., Gabler-Eduardo, C., and Prosl, H., 2005, Prevalence of antibodies against Neospora caninum and *Toxoplasma gondii* in dogs and foxes in Austria, *Vet. Parasitol.* **128**:189–193.

Williams, R. H., Morley, E. K., Hughes, J. M., Duncanson, P., Terry, R. S., Smith, J. E., and Hide, G., 2005, High levels of congenital transmission of *Toxoplasma gondii* in longitudinal and cross-sectional studies on sheep farms provides evidence of vertical transmission in ovine hosts, *Parasitology* **130**:301–307.

Yai, L. E., Canon-Franco, W. A., Geraldi, V. C., Summa, M. E., Camargo, M. C., Dubey, J. P., and Gennari, S. M., 2003, Seroprevalence of Neospora caninum and *Toxoplasma gondii* antibodies in the South American opossum (Didelphis marsupialis) from the city of Sao Paulo, Brazil, *J. Parasitol.* **89**:870–871.

Zargar, A. H., Wani, A. I., Masoodi, S. R., Laway, B. A., Kakroo, D. K., Thokar, M. A., Sofi, B. A., and Bashir, M. I., 1999, Seroprevalence of toxoplasmosis in women with recurrent abortions/neonatal deaths and its treatment outcome, *Indian J. Pathol. Microbiol.* **42**:483–486.