Ocular Immunology & Inflammation, Early Online, 1–10, 2013

ISSN: 0927-3948 print / 1744-5078 online
DOI: 10.3109/09273948.2013.823214

informa
healthcare

Epidemiology, Pathophysiology, and the Future of Ocular Toxoplasmosis

Aize Kijlstra[1] and Eskild Petersen[2]

[1]*Livestock Research, Wageningen University and Research Centre, Lelystad, The Netherlands; and University Eye Clinic Maastricht, Maastricht, The Netherlands, Maastricht, The Netherlands and* [2]*Department of Infectious Diseases, Aarhus University Hospital Skejby, Aarhus, Denmark*

ABSTRACT

Despite large advances in the field of ocular toxoplasmosis, large gaps still exist in our knowledge concerning the epidemiology and pathophysiology of this potentially blinding infectious disease. Although ocular toxoplasmosis is considered to have a high health burden, still little is known about its exact prevalence and how it affects the quality of life. The epidemiology of toxoplasmosis depends on local habits throughout the globe, and changes are likely in view of increased meat consumption in developing countries and demands for higher animal welfare in the Western world. Water is increasingly seen as an important risk factor and more studies are needed to quantitate and control the role of water exposure (drinking, swimming). Tools are now becoming available to study both the human host as well as parasite genetic factors in the development of ocular toxoplasmosis. Further research on the role of *Toxoplasma* strains as well as basic studies on parasite virulence is needed to explain why *Toxoplasma* associated eye disease is so severe in some countries, such as Brazil. Although genetic analysis of the parasite represents the gold standard, further developments in serotyping using peptide arrays may offer practical solutions to study the role of parasite strains in the pathogenesis of *Toxoplasma* retinochoroiditis. More research is needed concerning the pathways whereby the parasite can infect the retina. Once in the retina further tissue damage may be due to parasite virulence factors or could be caused by an aberrant host immune response. Local intraocular immune responses are nowadays used for diagnostic procedures. Future developments may include the use of Raman technology or the direct visualization of a *Toxoplasma* cyst by optical coherence tomography (OCT). With the availability of ocular fluid specimens obtained for diagnostic purposes and the development of advanced proteomic techniques, a biomarker fingerprint that is unique for an eye with toxoplasmosis may become available. It is hoped that such a biomarker analysis may also be able to distinguish between acquired versus congenital disease. Recently developed mouse models of congenital ocular toxoplasmosis are extremely promising with regard to disease pathogenesis, diagnosis, and treatment.

Keywords: Drinking water, gene polymorphism, immunogenetics, meat, parasite strain, parasite virulence, *Toxoplasma gondii*, toxoplasmosis

Ocular toxoplasmosis is the most important cause of infectious posterior uveitis in the world and often leads to visual disability in the affected eye.[1] It has been estimated that approximately 2% of individuals experiencing toxoplasmosis will develop ocular manifestations, suggesting that 1 in 400 individuals across the world will have posterior uveitis due to *Toxoplasma gondii*.[2] Ocular toxoplasmosis poses a large burden on health care systems. A number of 250,000 visits of patients with ocular toxoplasmosis to ophthalmologists was recently estimated for a 2-year period in the United States.[3] The figures mentioned above are rough estimates and to date no exact data are available since ocular toxoplasmosis is not a notifiable disease. In addition, as a small peripheral retinal lesion may not cause a decrease in visual acuity, part of the ocular toxoplasmosis patients may not seek medical assistance. To be able to adequately address the health burden of the eye disease caused by *T. gondii* it is necessary to obtain reliable figures about its prevalence and how it affects the quality of life in those affected. Although there are multiple

Received 15 April 2013; revised 4 July 2013; accepted 4 July 2013; published online 16 October 2013

Correspondence: Aize Kijlstra, University Eye Clinic Maastricht, University Hospital Maastricht, PO Box 5800, 6262 AZ Maastricht, The Netherlands. E-mail: aize.kijlstra@wur.nl

routes of infection, ocular toxoplasmosis remains a preventable cause of blindness and in this review we would like to assess the steps that have already been made to control this disease and what is held in promise for the coming years. Important developments have been the improvement in the diagnostic laboratory methods, studies showing the importance of *T. gondii* genotype for ocular toxoplasmosis,[4] and the contribution of congenital versus acquired toxoplasmosis to ocular involvement.[5] Despite these developments many ophthalmologists still make the diagnosis on clinical grounds.

Earlier review papers published in this journal have addressed clinical features, transmission, therapy, and parasitology and will not be covered again in detail.[6–9] In this review we will outline the future of ocular toxoplasmosis epidemiology and pathogenesis.

FUTURE CHALLENGES OF OCULAR TOXOPLASMOSIS—EPIDEMIOLOGICAL CHALLENGES

The ubiquitous parasite *T. gondii* exists in three forms: (1) the tachyzoite, which infects almost any nucleated cell; (2) the tissue cyst, which is formed during the chronic phase of infection, resides in the brain or skeletal muscle, and contains many numbers of the so-called bradyzoite stage of the parasite, and (3) the oocyst (eggs of the parasite), which is formed in massive numbers in the intestinal tract of infected felines. Humans can become infected with *T. gondii* by eating undercooked meat containing tissue cysts, by the uptake of dirt, food, or water contaminated with oocysts, by transplacental transmission of tachyzoites (congenital toxoplasmosis), or following transplantation of an organ containing tissue cysts. Once a woman has been infected with *T. gondii*, transfer of the parasite to the fetus during a following pregnancy is considered unlikely, although exceptions can occur.[10]

T. gondii occurs worldwide and infects mammals and birds.[11] There is thus a huge animal reservoir from which humans can be infected. This reservoir can only be controlled with regards to domesticated animals, such as pigs and poultry, but even here there are huge challenges and obstacles. Apart from the animal reservoir there is also an enormous environmental source of *Toxoplasma* oocysts, which are maintained by all felines—domestic and wild. The relative contribution of these two reservoirs to *Toxoplasma* eye disease needs further clarification. Based on theoretical grounds it varies around the globe and depends on personal habits and local circumstances. Large changes are expected for the future in the light of increased meat consumption in developing countries[12] and increasing consumer demands for improved animal welfare in the Western world. Improving animal welfare may increase exposure to the environment and increase the presence of *T. gondii* in meat products from these animals[13] and unless appropriate measures are taken will put consumers at higher risk of contracting toxoplasmosis.[14] Changes in *T. gondii* prevalence in humans can occur in some regions in the world in the coming decades as a result of changing environmental conditions and may be predicted by using global climate change models.[15]

Not everybody who gets infected with *T. gondii* develops eye disease. It is increasingly clear that host genetic factors are important determinants of whether an infection results in ocular symptoms[16,17] and this knowledge may lead to new treatments modulating the immune response of the host. *T. gondii* can be divided into at least 138 unique genotypes and these genotypes have been shown to be unevenly distributed throughout the world.[18] Different genotypes show different pathogenicity, whereby some originating from South America can be very pathogenic with a high mortality.[19]

RESERVOIRS OF INFECTIONS

Freshwater

Numerous epidemiological studies over the past 20 years concerning risk factors for becoming *T. gondii* seropositive have suggested that in Europe, undercooked meat and meat products are the main route of transmission, but still can only explain up to half to two-thirds of infections.[20] Increasingly, infections from freshwater, being either lakes, streams, and rivers or *T. gondii*-contaminated water reservoirs demonstrate that this is an important route of infection in societies where people use unprocessed surface water for consumption.[21] Water sources are infected by oocysts excreted by cats and wild felines. These oocysts can persist in the environment for long periods and are resistant to many disinfection methods used by the water industry.[22,23]

Treatment of water by filtration through soil and sand beds is considered a method to remove parasite cysts from surface water[24] and since most developed countries use deep water wells as a source of drinking water it is not a major source of infection in the western world. Major improvements have recently been made to detect *Toxoplasma* oocysts in water samples, which now allow monitoring of drinking water and provide tools to identify possible sources causing waterborne outbreaks of toxoplasmosis.[25] These methods may also allow the evaluation of water management needed to provide *Toxoplasma*-safe water in many parts of the world. Providing safe water to a community will not only reduce the risk of toxoplasmosis but at the same time will address many other waterborne infections currently threatening global health.[26] However,

infections by exposure to freshwater like swimming, bathing, or washing in lakes and rivers will remain an important risk factor, not only in underdeveloped nations but also in western countries where freshwater recreation is very popular. Future epidemiological studies should pay more attention to this risk factor.

Although vaccination of domestic cats has been suggested as an option to control environmental parasite loads,[27,28] this has not yet led to a commercially available cat vaccine, and addressing the vaccination of wild felines will also remain an extraordinary challenge. *Toxoplasma* DNA has been detected on fruits and vegetables,[29] but as yet it is not possible to exactly quantitate the significance of this possible route.[30]

Saltwater Transmission

Transmission to mammals like dolphins and sea otters from estuarine freshwater washouts is well documented.[31,32] Oysters and shellfish can act as a vehicle for *Toxoplasma*[33] and epidemiological studies have suggested that humans can acquire toxoplasmosis via this route.[34]

EXPLAINING DIFFERENCES IN AGE-SPECIFIC SEROPREVALENCE BETWEEN COUNTRIES AND CONTINENTS

Seroprevalence is low in North America and Asia compared to certain parts of South America and Africa, with data from Europe lying in between.[35] This might be explained by differences in the infection rate of the animal reservoirs throughout the world, the parasite load in the environment, local habits, the presence of highly virulent strains in certain areas, and host genetic factors. Exposure can occur when food is traditionally cooked or enjoyed semi-processed, thus allowing *T. gondii* to survive food processing thereby resulting in the infection of the host. The tissue cyst of the parasite is killed when meat reaches an internal temperature of $-12\,^{\circ}C$.[14] Minimal freezing does not guarantee inactivation of the tissue cysts and differences concerning meat handling among countries may thus play a role in the infection with *Toxoplasma* in certain areas of the world.

As mentioned above exposure to other reservoirs like freshwater may explain observed differences in global seroprevalence. Elucidating the parasite stage causing the infection by further refinement of serological methods to discriminate an oocyst from tissue cyst infection may shed more light on this issue in the near future.[36,37] Socio-economic conditions are clearly a risk factor for toxoplasmosis in studies throughout the world.[38]

OVERALL RISK OF INFECTION AND RISK OF OCULAR SYMPTOMS

Ocular toxoplasmosis can be due to congenital infection or acquired toxoplasmosis after birth. In the Netherlands and United Kingdom it was calculated that approximately 1/3 of cases of chorioretinitis are due to congenital infection and 2/3 are caused by a *Toxoplasma* infection later in life.[39,40] In Brazil there is a very high rate of *Toxoplasma* infection, with up to 50% of school children already being infected and up to 80% of women at the childbearing age being seropositive for *Toxoplasma*.[41] This means that many women are already protected from transferring the parasite to their fetus. The remaining seronegative women have a chance of contracting infection during pregnancy, leading to an estimated incidence of 1 case of congenital toxoplasmosis for every 1000 live births. In the Netherlands the rate of *Toxoplasma* infection has drastically declined and currently seroconversion starts at the childbearing age of the women, whereby the majority is unprotected. This leads to an incidence of 1 case out of 500 live births.[42] The chance of developing chorioretinitis following congenital infection in The Netherlands has been calculated and the incidence of congenital infections can be traced by analyzing "dried blood spot filter paper cards" obtained from newborns for a variety of congenital disorders.[43] Assessing the incidence of *Toxoplasma* chorioretinitis is very difficult since it is not a notifiable disease. In view of the disease burden associated with ocular toxoplasmosis it is recommended that uveitis specialists set up a simple web-based system to register these patients. It should be noted that this will only include a registry of patients visiting a uveitis specialist and will not include cases with peripheral chorioretinal lesions not affecting visual function and who have not been seen by an ophthalmologist.

PATHOPHYSIOLOGY

Developments in Retinal Infection in Vitro Models

How *T. gondii* invades the human retina is not yet clear and future studies are necessary to address this important issue. It is possible that free tachyzoites directly invade the retina but it is also possible that infection proceeds according to a "Trojan horse" principle.[44] In vitro studies have shown that free tachyzoites cross a monolayer of retinal endothelial cells[45] and ex vivo experiments revealed that these tachyzoites can move within retinal layers in cadaveric eyes, where they preferentially infect glial cells.[46] Although free tachyzoites have been observed by light microscopy in human blood samples,[47] it is unlikely

that these parasites are still alive and capable of egressing from the bloodstream. In the bloodstream free tachyzoites will immediately bind anti-*Toxoplasma* antibodies and be killed by the action of the complement system.[48] Localization inside cells ("Trojan horse") is an efficient manner to evade the action of the host humoral immune defense.

Recent studies have investigated the types of cells that may function as a shuttle between tissues, whereby the bloodstream functions as the road between them.[49] Models using the so-called transwell system have allowed insight into the types of cells that can cross the vessel walls and invade the adjacent tissues. Of interest is the observation that although *Toxoplasma* can virtually infect any cell type it is mainly the activated macrophage and dendritic cells that show a highly efficient crossing of these borders.[49,50] Following infection with *T. gondii* these cells show a profound phenotypical change enhancing their transmigratory behavior. Dendritic cells (DCs) show the highest change in motility following *Toxoplasma* infection, which was also found to depend on parasite strain. Highest motility changes in human DCs or macrophages were observed with the type II strain of the parasite.[49,51]

Of interest are recent findings showing that once infected, DCs show an increased GABA release, turning them into a highly migratory cell.[52] GABA is one of the main neurotransmitters in the brain and it is hypothesized that the parasite makes an intelligent use of this pathway for its dissemination.

The vascular endothelial lining also plays an important role in transmigration into tissues and recent studies suggest that both brain and retinal vascular endothelium are preferred sites for the attachment of parasite infected DCs.[53,54] In the retina common adhesion molecules may be used by retinal infected DCs to cross the vessel wall.[54] Whether the infection of the "Trojan" cells leads to an increased expression of adhesion molecules is not yet clear and it is possible that the parasite uses the existing mechanisms of migration into the retina. The human retina contains a rich network of DCs and microglia, which are continuously replenished from the bloodstream.[55,56] Ex vivo experiments with human cadaver eyes have shown that once in the retina the tachyzoites mainly localize to the nerve fiber layer, although some migrate to the outer retina.[46] Further experiments with isolated retinal cultures provided evidence that tachyzoites preferentially localize in glial cells.[46] Future knowledge about how the parasite changes the regulation of host cell motility and which factors are exactly involved in tissue dissemination may offer novel therapeutic tools to control infection of the retina.

It has been postulated that parasite-infected DCs show an altered pattern of neuroendocrine mediator secretion, which may affect the local microenvironment.[57] In the brain this altered secretion of mediators is considered to play a role in behavioral changes of the *Toxoplasma*-infected host.[58] Whether local *Toxoplasma*-infected cells may lead to subtle changes in retinal or visual function has not yet been tested since most work using visual function tests has addressed the effects of overt retinal inflammation and scar formation.

Developments in Diagnostic Techniques with Intraocular Fluids

The value of intraocular fluid analysis for the diagnosis of ocular toxoplasmosis has been addressed extensively (for review, see[8]). While polymerase chain reaction (PCR) techniques are often easier than an enzyme-linked immunosorbent assay (ELISA), the latter is often more useful in the diagnosis of ocular toxoplasmosis in immunocompetent individuals. Many ophthalmologists do not perform intraocular fluid analysis and rely on the clinical picture and the presence of *Toxoplasma* antibodies in blood.[6] From a practical point of view, uveitis specialists often encounter the problem that the tests they require to establish or rule out a diagnosis include a set of different PCR and/or antibody tests against bacteria, viruses, and parasites. This often requires sending aliquots of the ocular fluid sample to different laboratories (virology, bacteriology, and parasitology).

Whether other methods to identify an infectious cause of uveitis other than PCR or ELISA will become available is not yet certain. Raman technology claims to be able to identify infections directly by, for instance, *Toxoplasma* antibody analysis in body fluids.[59,60] Raman spectroscopy is a technique whereby monochromatic light from a laser is transmitted through an aqueous environment.[61] The incident light results in a change in the vibrational or rotational state of the molecules in this environment and produces a scattered light signal. The so-called Raman effect is the difference in photon energy between the scattered and incident light and each molecule or set of molecules emits a specific signal. Raman probes that could noninvasively analyze the aqueous humor and provide a spectrum that could identify an intraocular infection with a certain microbe would be a revolutionary development. This technology is currently being developed at the Eye Clinic of Maastricht University but it will still take several years before the first results become available.[62]

Developments Concerning Ocular Immune/ Inflammatory Response (Genomics; Proteomics)

Uveitis can in some cases be seen as an aberrant intraocular reaction against a self or foreign antigen in

individuals with a certain permissive genetic background. A number of uveitis entities show a very strong association with genes encoding antigens of the HLA-system. Examples include birdshot choroidopathy, HLA-B27-associated uveitis, and ocular Behçet disease.[63] Many weaker associations are now being uncovered with a variety of genes involved in the immune response. Many of these genes show polymorphisms with an important biological consequence for the pathway in which the respective factor is involved.

Analysis of gene associations with the risk for certain uveitis entities can only be done if a sufficiently large sample size is available. Most studies to date have therefore concentrated on relatively frequent uveitis entities such as Behcet disease and are carried out in large uveitis referral centers.[64] For a similar approach toward identifying genes associated with ocular toxoplasmosis, centers would either have to cooperate or try to retrieve the *Toxoplasma* patients from their records to obtain DNA samples of these patients. Initial small-sample studies did not find an association between ocular toxoplasmosis and the HLA system.[65] Earlier studies from our group did identify an association between the severity of ocular toxoplasmosis and HLA-Bw62,[66] but this association may reflect a general genetic association with the ocular immune response, since a similar association was also found with patients having acute retinal necrosis.[67]

To date these studies have not been confirmed and there is a paucity of research related to the host genes involved in the development of ocular toxoplasmosis.[68] The fact that resistance to *Toxoplasma* encephalitis maps to genes within the HLA complex[69,70] should stimulate ocular immunologists to perform a thorough analysis of this topic. Despite the scarcity of research in this area, the human genetic predisposition to ocular symptoms is slowly becoming clear. It was first demonstrated in 2008 where it was shown that ocular and brain disease in congenital toxoplasmosis was associated with polymorphisms in ABCA4 encoding ATP-binding cassette transporter and with polymorphisms of the gene encoding COL2A1.[71,72]

Various studies have addressed the association of interleukin gene polymorphisms with ocular toxoplasmosis. A small study analyzing interleukin 1 gene polymorphisms did not reveal a difference between patients with ocular toxoplasmosis ($n = 100$) and controls ($n = 100$).[73] In reporting on this study the authors mentioned a significant difference in genotype and allele distributions of IL1A −889 C/T between ocular toxoplasmosis patients with ($n = 45$) and without ($n = 14$) recurrent episodes, but the data were not corrected for multiple comparisons and the significance of these findings therefore remains questionable. The same group also reported an association between ocular toxoplasmosis with genotypes related to a lower interleukin 6 and interleukin-10 production.[74,75] A small study from Brazil was not able to find a significant association between ocular toxoplasmosis and the interferon γ 874T/A genotype (rs2430561), but a larger sample size is needed to definitely rule out a possible relation.[76]

An association between gene polymorphisms of TLR2, TLR5, and TLR9 and congenital *Toxoplasma* eye disease in Brazil showed a significant association with the C allele of TLR9 rs352140.[16] These findings open new avenues in the study concerning the interaction between *T. gondii* and TLR9 and how this triggers inflammation in the eye. In the same patient group it was found that a gene polymorphism encoding the purinergic receptor P2X(7) (P2RX7) was strongly protective.[77] The P2X(7) receptor has been shown to play an important role in the inflammatory response following microbial infection.[78]

Recent studies in congenital toxoplasmosis also showed an association between the NALP1 rs8081261 and rs11652907 tag SNPs.[79] Since these SNPs could be in linkage disequilibrium with a presumptive causative gene, the authors performed various additional experiments to prove the direct involvement of NALP1 in the pathogenesis of *Toxoplasma*-related inflammation. They found convincing evidence indicating an important role for the NALP1 inflammasome in *Toxoplasma* infection. Lately an association was observed between the NOD2 tag-SNP rs3135499 and retinochoroiditis.[17] The studies mentioned above show the involvement of various pathways, each having an abundance of polymorphisms, which could either lead to enhanced risk or protection from disease caused by this parasite.

The exact role of gene polymorphisms of proteins involved in the immune response to the parasite will hopefully be uncovered in the next decade and it is hoped that studies will not be confined to congenital disease, but that the genes involved in, for instance, the development of acquired ocular toxoplasmosis will also be investigated. Apart from a genomics approach that is currently already applied to ocular toxoplasmosis, it can be envisaged that in the near future we will be hearing more about a proteomics approach.

Only few studies have recently been published on circulating biomarkers in relation to ocular toxoplasmosis and further developments in this field would be welcome. Although slightly increased levels of the TNF alpha receptor-2 were observed in the sera of patients with ocular toxoplasmosis, this was not related to the severity of the intraocular symptoms.[80] Ocular fluid is a unique medium in which to perform proteomic studies and has already been applied to the eye.[81] Since remnants of ocular fluids used for diagnostic purposes are readily available it can be expected that a proteomics analysis of these fluids will soon be performed, whereby various uveitis entities,

including toxoplasmosis, can be compared. Whether fluids from toxoplasmosis patients will show a specific proteomics signal apart from a general proinflammatory set of mediators that can also be expected from other uveitis entities is an open question.

Experimental Models

An important development in experimental models of ocular toxoplasmosis has been the availability of transgenic *Toxoplasma* strains. A β-galacosidase (lacZ) transgenic *Toxoplasma* has been used to detect tissue dissemination of the parasite into various organs using histochemical techniques.[82] The flat retinal mounting technique[83] has been used to successfully detect isolated retinal cysts of a lacZ transgenic *Toxoplasma* strain.[84] These studies showed that analysis of 1800 retinal cryostat sections from 3 infected mice didn't reveal any cysts, whereas a cyst was readily seen in a retinal flat mount section in 2 out of 4 infected mice.

Future questions that need an answer include the question what causes reactivation of a retinal cyst. It is also still a question whether a recurrence might be due to an influx of tachyzoites from another site of the body into the retina. Little work is currently being done on congenital animal models of ocular toxoplasmosis. The earlier work from McMenamin et al. still represents a hallmark study.[85] At that time the authors were able to detect tissue cysts in the eye but observed no parasites at locations with retinal inflammation, which led them to believe that autoimmunity might be playing a role in the pathogenesis. Currently, the idea is that the parasite has already been removed due to the inflammatory reaction and no further hard evidence has been presented concerning the autoimmune theory.[86]

One of the major drawbacks of current animal models is that mice don't have a macula. In humans it has been hypothesized that the macula is not often involved during acquired disease since this area of the retina lacks blood vessels.[87] Later reactivation, on the other hand, may lead to spread of parasites within the retina to the macular area. During congenital infection there might be a higher chance of initial macular localization due to the local microenvironment of the developing macula. Some have suggested that if *Toxoplasma* first manifests itself in the macula that this could be seen as evidence of congenital infection.[1] Others, however, do not believe that congenital origin of the retinal infection is associated with unique features.[87]

Humans and mice also differ concerning receptor expression. For example, TLR11 is an important receptor in the pathogenesis of toxoplasmosis in mice, but it is not expressed in humans.[88]

Despite the drawbacks of murine models of ocular toxoplasmosis, they do have advantages in that the complete genome of the mouse is known and various knockout mouse strains are available. A new model of congenital toxoplasmosis in mice was established by infecting Swiss-Webster mice subcutaneously immediately after birth.[89] The fact that the development of the retina in a mouse is completed during the first week of life makes this model similar to the in utero infection in humans at the end of pregnancy, the time point where transmission of the parasite to the fetus results in a high incidence of ocular involvement.[90] The advantage of this approach is that all infected murine pups survive and that all eyes are infected with the parasite at 4 weeks of life. Furthermore, all eyes become inflamed, whereby dormant cysts without evidence of adjacent inflammation are observed in the ganglion cell layer, the inner plexiform layer, and the inner nuclear layer of the retina, similar to what was observed earlier in the classical model of murine congenital toxoplasmosis.[85] This new neonatal congenital model has promising prospects for future studies into the pathogenesis and treatment of human ocular toxoplasmosis.

Advances have been made using experimental models of ocular toxoplasmosis. In the mouse model, using intravitreal parasite injections, a significant increase of annexin1-expressing neutrophils was observed and the authors proposed that this might be used as a target to control the intraocular inflammatory response.[91]

Parasite Imaging in the Retina

Parasite imaging in the retina has long been the dream of uveitis specialists. Experimental models have shown that by using genetically engineered parasites one can show parasites in the brain or retina when these have genes incorporated that can be visualized with histological[84] or bioluminescence techniques.[92] Nanogold-conjugated anti-*Toxoplasma* antibodies have been used to target *Toxoplasma* tachyzoites, but the use of these particles to image native *Toxoplasma* cysts in the human retina has not yet been achieved.[93] This is probably due to the fact that the *Toxoplasma* cyst wall is impermeable to such reagents. A retinal cyst may have a diameter between 5 and 100 μm and novel imaging techniques, such as optical coherence tomography (OCT), with a micrometer resolution capability[94] may possibly be used in the future to visualize the parasite either directly or after the uptake of enhancing dyes by the cysts. Direct visualization may allow noninvasive eradication of the retinal cysts by laser technology.[95] This idea has been around for some time and was abandoned due to the fact that it was not successful, possibly because photocoagulation was performed around foci,

without knowing the exact localization of the parasite. Even if the parasite location is known exactly, care should be taken not to aggravate or even induce a marked inflammatory reaction by the sudden local release of parasite antigens following laser treatment.

OCT has recently been applied to assess the morphological changes associated with ocular toxoplasmosis and thickening of all layers of the retina was described using spectral optical coherence tomography (SOCT).[96] Future developments with this technique can be envisaged to follow the course of ocular toxoplasmosis and its response to treatment.

Role of Parasite Strains

Why most individuals experiencing a *T. gondii* infection do not get sick while others develop complications such as retinal disease has puzzled scientists for quite some time. It is now emerging that some parasites are more virulent, whereas some humans may be more susceptible than others. Until recently, three strains of *T. gondii* were recognized, types I, II, and III. With the availability of modern techniques and following the analysis of over 950 isolates from around the world a complex picture has now arisen with 138 different genotypes.[18] These genotypes are now organized in 6 ancestral populations or clades. It has become clear that the frequency of ocular toxoplasmosis as well as the disease severity is strain associated.[97,98] In Europe and the United States, the frequency of ocular involvement in *T. gondii*-infected individuals is approximately 1–2%, whereas in certain areas of Brazil this may be as high as 18%.[2] South American strains may have a higher tropism for the eye than European strains, but it might also be explained by the fact that the so-called atypical South American strains cause more tissue damage due to a higher expression of certain virulence factors, such as ROP18.[99] These explanations are theoretical and further study is needed to explain the more frequent involvement of the eye following *Toxoplasma* infection in certain parts of Brazil.

The retina is particularly vulnerable to *T. gondii* infection because retinal endothelial cells express more molecules that interact with the parasite than other cell types[100] and retinal inflammation following *Toxoplasma* infection may have more serious consequences for a small and delicate organ such as the eye as compared to large organs such as the brain and heart. Whether specific virulence factors affect tropism and ensuing inflammation in different organs remains to be clarified.[101]

Identification of strains in clinical specimens from patients with ocular toxoplasmosis is hampered by the fact that parasite DNA is often not detected. To overcome this problem ELISA methods have been developed that are based on the antibody repertoire of the patient directed against certain allelic peptide motifs.[102] At present this technique only recognizes groups of genotypes and at present a distinction is made between type II and non-type II strains.[103]

T. gondii is one of the most successful parasites on this planet. This is due to the fact that it has a unique balance between parasite virulence (host mortality) and host resistance (host survival). To ensure completion of its life cycle the parasite has developed strategies to end up in its definite host, the cat (or other felines). Due to the fact that the parasite can infect a wide variety of vertebrate hosts it is quite reasonable to hypothesize that some strains are better adapted to certain hosts than others. Infection of humans does not have a direct advantage to the parasite and complications in humans can be regarded as a bystander effect.

CONCLUSIONS

Changes in the epidemiology of ocular toxoplasmosis can be expected in coming years in view of increased meat consumption in certain areas of the world and changing patterns of animal husbandry leading to increased risk of exposure to meat that is contaminated with *Toxoplasma.* Waterborne infections with *Toxoplasma* oocysts are particularly a problem in poor tropical areas where untreated surface water is used for consumption. Improved monitoring and control of municipal or local drinking water may, on the other hand, lead to a decrease of toxoplasmosis.

The role of parasite virulence and the role of human host genetic factors may offer explanations concerning the observation why the severity of *Toxoplasma*-associated eye lesions can be so different among patients around the globe. Future developments in parasite serotyping are expected, although DNA typing is still the gold standard.

New developments are expected concerning knowledge about mechanisms whereby the parasite invades the retina and how it uses certain cell types and their intracellular machinery for its migration within tissues. Further developments are also expected in the field of bioimaging of the parasite in the retina. Whether this may lead to a local directed noninvasive destruction of the parasite is an option that requires careful evaluation. Ocular fluid analysis is a tool that plays an important role in the diagnosis of atypical cases of ocular toxoplasmosis and opens the door for the development of further advanced proteomic techniques, whereby a future goal would be a biomarker fingerprint that is unique for an eye with toxoplasmosis. Such techniques may also be applied to distinguish between acquired versus congenital ocular toxoplasmosis. The development of new mouse models of congenital ocular toxoplasmosis may hopefully parallel the research in human

ocular toxoplasmosis and be able to increase our understanding with regard to disease pathogenesis, diagnosis, and treatment.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1. Bosch-Driessen LEH, Berendschot T, Ongkosuwito JV, et al. Ocular toxoplasmosis: clinical features and prognosis of 154 patients. *Ophthalmology* 2002;109:869–878.
2. Holland GN. LX Edward Jackson memorial lecture: ocular toxoplasmosis—a global reassessment, part 1: epidemiology and course of disease. *Am J Ophthalmol.* 2003;136: 973–988.
3. Kirby T. Calls for more detailed studies on toxoplasmosis. *Lancet Infect Dis.* 2012;12:912–913.
4. Vaudaux JD, Muccioli C, James ER, et al. Identification of an atypical strain of *Toxoplasma gondii* as the cause of a waterborne outbreak of toxoplasmosis in Santa Isabel do Ivai, Brazil. *J Infect Dis.* 2010;202:1226–1233.
5. Vasconcelos-Santos DV. Ocular manifestations of systemic disease: toxoplasmosis. *Curr Opin Ophthalmol.* 2012;23: 543–550.
6. Wakefield D, Cunningham ET, Pavesio C, et al. Controversies in ocular toxoplasmosis. *Ocul Immunol Inflamm.* 2011;19:2–9.
7. Vasconcelos-Santos DV, Dodds EM, Orefice F. Review for disease of the year: differential diagnosis of ocular toxoplasmosis. *Ocul Immunol Inflamm.* 2011;19:171–179.
8. Garweg JG, de Groot-Mijnes JDF, Montoya JG. Diagnostic approach to ocular toxoplasmosis. *Ocul Immunol Inflamm.* 2011;19:255–261.
9. Petersen E, Kijlstra A, Stanford M Epidemiology of ocular toxoplasmosis. *Ocul Immunol Inflamm.* 2012;20:68–75.
10. Silveira C, Ferreira R, Muccioli C, et al. Toxoplamosis transmitted to a newborn from the mother infected 20 years earlier. *Am J Ophthalmol.* 2003;136:370–371.
11. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. *Int J Parasitol.* 2000;30:1217–1258.
12. Popkin BM. Nutrition in transition: the changing global nutrition challenge. *Asia Pacific J Clin Nutr.* 2001;10: S13–S18.
13. Kijlstra A, Meerburg BG, Mul MF. Animal-friendly production systems may cause re-emergence of *Toxoplasma gondii*. *Njas-Wageningen J Life Sci.* 2004;52:119–132.
14. Kijlstra A, Jongert E. Control of the risk of human toxoplasmosis transmitted by meat. *Int J Parasitol.* 2008; 38:1359–1370.
15. Meerburg BG, Kijlstra A. Changing climate-changing pathogens: *Toxoplasma gondii* in North-Western Europe. *Parasitol Res.* 2009;105:17–24.
16. Peixoto-Rangel AL, Miller EN, Castellucci L, et al. Candidate gene analysis of ocular toxoplasmosis in Brazil: evidence for a role for toll-like receptor 9 (TLR9). *Mem Instit Oswaldo Cruz.* 2009;104:1187–1190.
17. Dutra MS, Bela SR, Peixoto-Rangel AL, et al. Association of a NOD2 gene polymorphism and T-helper 17 cells with presumed ocular toxoplasmosis. *J Infect Dis.* 2013;207: 152–163.
18. Su CL, Khan A, Zhou P, et al. Globally diverse *Toxoplasma gondii* isolates comprise six major clades originating from a small number of distinct ancestral lineages. *Proc Natl Acad Sci U S A.* 2012;109:5844–5849.
19. Demar M, Ajzenberg D, Maubon D, et al. Fatal outbreak of human toxoplasmosis along the Maroni River: epidemiological, clinical, and parasitological aspects. *Clin Infect Dis.* 2007;45:E88–E95.
20. Cook AJC, Gilbert RE, Buffolano W, et al. Sources of toxoplasma infection in pregnant women: European multicentre case-control study. *Br Med J.* 2000;321:142–147.
21. Bahia-Oliveira LMG, Jones JL, Azevedo-Silva J, et al. Highly endemic, waterborne toxoplasmosis in north Rio de Janeiro state, Brazil. *Emerg Infect Dis.* 2003;9:55–62.
22. Frenkel JK, Ruiz A, Chinchilla M Soil survival of *Toxoplasma* oocysts in Kansas and Costa Rica. *Am J Trop Med Hyg.* 1975;24:439–443.
23. Dumetre A, Le Bras C, Baffet M, et al. Effects of ozone and ultraviolet radiation treatments on the infectivity of *Toxoplasma gondii* oocysts. *Vet Parasitol.* 2008;153:209–213.
24. Hijnen WAM, Brouwer-Hanzens AJ, Charles KJ, et al. Transport of MS2 phage, *Escherichia coli*, *Clostridium perfringens*, *Cryptosporidium parvum* and *Giardia intestinalis* in a gravel and a sandy soil. *Environ Sci Technol.* 2005;39: 7860–7868.
25. Karanis P, Aldeyarbi HM, Mirhashemi ME, et al. The impact of the waterborne transmission of *Toxoplasma gondii* and analysis efforts for water detection: an overview and update. *Environ Sci Pollut Res.* 2013;20:86–99.
26. Karanis P, Kourenti C, Smith H Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. *J Water Health* 2007;5:1–38.
27. Frenkel JK, Pfefferkorn ER, Smith DD, et al. Prospective vaccine prepared from a new mutant of *Toxoplasma gondii* for use in cats. *Am J Vet Res.* 1991;52:759–763.
28. Mateus-Pinilla NE, Dubey JP, Choromanski L, et al. A field trial of the effectiveness of a feline *Toxoplasma gondii* vaccine in reducing *T. gondii exposure for swine*. *J Parasitol.* 1999;85:855–860.
29. Lass A, Pietkiewicz H, Szostakowska B, et al. The first detection of DNA in environmental fruits and vegetables samples. *Eur J Clin Microbiol Infect Dis.* 2012;31:1101–1108.
30. Alvarado-Esquivel C, Estrada-Martinez S, Liesenfeld O *Toxoplasma gondii* infection in workers occupationally exposed to unwashed raw fruits and vegetables: a case control seroprevalence study. *Parasites Vectors.* 2011; 4:235.
31. Shapiro K, Miller M, Mazet J, et al. Effect of estuarine wetland degradation on transport of *Toxoplasma gondii* surrogates from land to sea. *Appl Environ Microbiol.* 2010; 76:6821–6828.
32. Shapiro K, Miller M, Mazet J Temporal association between land-based runoff events and California sea otter (*Enhydra lutris nereis*) protozoal mortalities. *J Wildlife Dis.* 2012;48: 394–404.
33. Robertson LJ. The potential for marine bivalve shellfish to act as transmission vehicles for outbreaks of protozoan infections in humans: a review. *Int J Food Microbiol.* 2007; 120:201–216.
34. Chiang TY, Hsieh HH, Kuo MC, et al. Seroepidemiology of *Toxoplasma gondii* infection among healthy blood donors in Taiwan. *Plos One* 2012;7:e48139.
35. Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. *Int J Parasitol.* 2009;39:1385–1394.
36. Hill D, Coss C, Dubey JP, et al. Identification of a sporozoite-specific antigen from *Toxoplasma gondii*. *J Parasitol.* 2011;97:328–337.

37. Boyer K, Hill D, Mui E, et al. Unrecognized ingestion of *Toxoplasma gondii* oocysts leads to congenital toxoplasmosis and causes epidemics in North America. *Clin Infect Dis.* 2011;53:1081–1089.
38. Fan CK, Lee LW, Liao CW, et al. Toxoplasma gondii infection: relationship between seroprevalence and risk factors among primary schoolchildren in the capital areas of Democratic Republic of Sao Tome and Principe, West Africa. *Parasite Vectors.* 2012;5:141.
39. Havelaar AH, Haagsma JA, Mangen MJJ, et al. Disease burden of foodborne pathogens in the Netherlands, 2009. *Int J Food Microbiol.* 2012;156:231–238.
40. Gilbert RE, Stanford MR. Is ocular toxoplasmosis caused by prenatal or postnatal infection?. *Br J Ophthalmol.* 2000; 84:224–226.
41. Dubey JP, Lago EG, Gennari SM, et al. Toxoplasmosis in humans and animals in Brazil: high prevalence, high burden of disease, and epidemiology. *Parasitology.* 2012; 139:1375–1424.
42. Hofhuis A, Van Pelt W, Van Duynhoven Y, et al. Decreased prevalence and age-specific risk factors for *Toxoplasma gondii* IgG antibodies in The Netherlands between 1995/ 1996 and 2006/2007. *Epidemiol Infect.* 2011;139:530–538.
43. Kortbeek LM, Hofhuis A, Nijhuis CDM, et al. Congenital toxoplasmosis and DALYs in the Netherlands. *Mem Instit Oswaldo Cruz.* 2009;104:370–373.
44. Lachenmaier SM, Deli MA, Meissner M, et al. Intracellular transport of *Toxoplasma gondii* through the blood–brain barrier. *J Neuroimmunol.* 2011;232:119–130.
45. Furtado JM, Bharadwaj AS, Chipps TJ, et al. *Toxoplasma gondii* tachyzoites cross retinal endothelium assisted by intercellular adhesion molecule-1 in vitro. *Immunol Cell Biol.* 2012;90:912–915.
46. Furtado JM, Ashander LM, Mohs K, et al. *Toxoplasma gondii* migration within and infection of human retina. *Plos One.* 2013;8:e54358.
47. Silveira C, Vallochi AL, da Silva UR, et al. *Toxoplasma gondii* in the peripheral blood of patients with acute and chronic toxoplasmosis. *Br J Ophthalmol.* 2011;95:396–400.
48. Dubey JP. The history of *Toxoplasma gondii*: the first 100 years. *J Eukaryotic Microbiol.* 2008;55:467–475.
49. Lambert H, Dellacasa-Lindberg I, Barragan A. Migratory responses of leukocytes infected with *Toxoplasma gondii*. *Microbes Infect.* 2011;13:96–102.
50. Lambert H, Hitziger N, Dellacasa I, et al. Induction of dendritic cell migration upon *Toxoplasma gondii* infection potentiates parasite dissemination. *Cell Microbiol.* 2006;8: 1611–1623.
51. Lambert H, Vutova PP, Adams WC, et al. The *Toxoplasma gondii*-shuttling function of dendritic cells is linked to the parasite genotype. *Infect Immun.* 2009;77:1679–1688.
52. Fuks JM, Arrighi RBG, Weidner JM, et al. GABAergic signaling is linked to a hypermigratory phenotype in dendritic cells infected by *Toxoplasma gondii*. *Plos Pathogens.* 2012;8:e1003051.
53. Courret N, Darche S, Sonigo P, et al. CD11c- and CD11b-expressing mouse leukocytes transport single *Toxoplasma gondii* tachyzoites to the brain. *Blood.* 2006;107:309–316.
54. Furtado JM, Bharadwaj AS, Ashander LM, et al. Migration of *Toxoplasma gondii*-infected dendritic cells across human retinal vascular endothelium. *Invest Ophthalmol Vis Sci.* 2012;53:6856–6862.
55. Yang PZ, Das PK, Kijlstra A. Localization and characterization of immunocompetent cells in the human retina. *Ocul Immunol Inflamm.* 2000;8:149–157.
56. Chen L, Yang PZ, Kijlstra A. Distribution, markers, and functions of retinal microglia. *Ocul Immunol Inflamm.* 2002; 10:27–39.
57. Weidner JM, Kanatani S, Hernandez-Castaneda MA, et al. Rapid cytoskeleton remodelling in dendritic cells following invasion by *Toxoplasma gondii* coincides with the onset of a hypermigratory phenotype. *Cell Microbiol.* 2013;in press.
58. Flegr J. How and why *Toxoplasma* makes us crazy. *Trends Parasitol.* 2013;29:156–163.
59. Duarte J, Pacheco MTT, Villaverde AB, et al. Near-infrared Raman spectroscopy to detect anti-*Toxoplasma gondii* antibody in blood sera of domestic cats: quantitative analysis based on partial least-squares multivariate statistics. *J Biomed Optics.* 2010;15:047002.
60. Araujo-Andrade C, Pichardo-Molina JL, Barbosa-Sabanero G, et al. Detection of the presence of antibodies against *Toxoplasma gondii* in human colostrum by Raman spectroscopy and principal component analysis. *J Biomed Optics.* 2007;12:034006.
61. Erckens RJ, Jongsma FHM, Wicksted JP, et al. Raman spectroscopy in ophthalmology: from experimental tool to applications in vivo. *Lasers Med Sci.* 2001;16:236–252.
62. Elshout M, Erckens RJ, Webers CA, et al. Detection of Raman spectra in ocular drugs for potential in vivo application of Raman spectroscopy. *J Ocul Pharmacol Therapeut.* 2011;27:445–451.
63. Du LP, Kijlstra A, Yang PZ. Immune response genes in uveitis. *Ocul Immunol Inflamm.* 2009;17:249–256.
64. Hou S, Kijlstra A, Yang P. The genetics of Behcet's disease in a Chinese population. *Front Med.* 2012;6:354–359.
65. Nussenblatt RB, Mittal KK, Fuhrman S, et al. Lymphocyte proliferative responses of patients with ocular toxoplasmosis to parasite and retinal antigens. *Am J Ophthalmol.* 1989;107:632–641.
66. Meenken C, Rothova A, Dewaal LP, et al. HLA typing in congenital toxoplasmosis. *Br J Ophthalmol.* 1995;79:494–497.
67. Holland GN, Cornell PJ, Park MS, et al. An association between acute retinal necrosis syndrome and HLA-DQW7 and phenotype-BW62,DR4. *Am J Ophthalmol.* 1989;108: 370–374.
68. Subauste CS, Ajzenberg D, Kijlstra A. Review of the series "disease of the year 2011: toxoplasmosis" pathophysiology of toxoplasmosis. *Ocul Immunol Inflamm.* 2011;19:297–306.
69. Henriquez FL, Woods S, Cong H, et al. Immunogenetics of *Toxoplasma gondii* informs vaccine design. *Trends Parasitol.* 2010;26:550–555.
70. Suzuki Y. Host resistance in the brain against *Toxoplasma gondii*. *J Infect Dis.* 2002;185:S58–S65.
71. Jamieson SE, de Roubaix LA, Cortina-Borja M, et al. Genetic and epigenetic factors at COL2A1 and ABCA4 influence clinical outcome in congenital toxoplasmosis. *Plos One.* 2008;3:e2285.
72. Jamieson SE, Cordell H, Petersen E, et al. Host genetic and epigenetic factors in toxoplasmosis. *Mem Instit Oswaldo Cruz.* 2009;104:162–169.
73. Cordeiro CA, Moreira PR, Costa GC, et al. Interleukin-1 gene polymorphisms and toxoplasmic retinochoroiditis. *Mol Vision.* 2008;14:1845–1849.
74. Cordeiro CA, Moreira PR, Bessa TF, et al. Interleukin-6 gene polymorphism (-174 G/C) is associated with toxoplasmic retinochoroiditis. *Acta Ophthalmol.* 2013;91: e311–314.
75. Cordeiro CA, Moreira PR, Andrade MS, et al. Interleukin-10 gene polymorphism (-1082G/A) is associated with toxoplasmic retinochoroiditis. *Invest Ophthalmol Vis Sci.* 2008;49:1979–1982.
76. Neves ED, Curi ALL, de Albuquerque MC, et al. Genetic polymorphism for IFN gamma+874T/A in patients with acute toxoplasmosis. *Rev Soc Brasileira Med Trop.* 2012;45: 757–760.

77. Jamieson SE, Peixoto-Rangel AL, Hargrave AC, et al. Evidence for associations between the purinergic receptor P2X(7) (P2RX7) and toxoplasmosis. *Genes Immun.* 2010;11: 374–383.
78. Miller CM, Boulter NR, Fuller SJ, et al. The role of the P2X(7) receptor in infectious diseases. *Plos Pathogens.* 2011; 7:e1002212.
79. Witola WH, Mui E, Hargrave A, et al. NALP1 Influences susceptibility to human congenital toxoplasmosis, proinflammatory cytokine response, and fate of *Toxoplasma gondii*-infected monocytic cells. *Infect Immun.* 2011;79: 756–766.
80. Bessa TF, Cordeiro CA, Goncalves RM, et al. Increased serum levels of soluble tumor necrosis factor receptor-2 (sTNFR2) in patients with active toxoplasmic retinochoroiditis. *Brazilian J Infect Dis.* 2012;16:540–544.
81. Jay NL, Gillies M. Proteomic analysis of ophthalmic disease. *Clin Exp Ophthalmol.* 2012;40:755–763.
82. Seeber F, Boothroyd JC. *Escherichia coli* beta-galactosidase as an in vitro and in vivo reporter enzyme and stable transfection marker in the intracellular protozoan parasite *Toxoplasma gondii*. *Gene.* 1996;169:39–45.
83. McMenamin PG. Optimal methods for preparation and immunostaining of iris, ciliary body, and choroidal wholemounts. *Invest Ophthalmol Vis Sci.* 2000;41: 3043–3048.
84. Escoffier P, Jeanny JC, Marinach-Patrice C, et al. *Toxoplasma gondii*: flat-mounting of retina as a new tool for the observation of ocular infection in mice. *Exp Parasitol.* 2010;126:259–262.
85. McMenamin PG, Dutton GN, Hay J, et al. The ultrastructural pathology of congenital murine toxoplasmic retinochoroiditis, 1: the localization and morphology of *Toxoplasma* cysts in the retina. *Exp Eye Res.* 1986;43: 529–543.
86. Garweg JG, de Kozak Y, Goldenberg B, et al. Anti-retinal autoantibodies in experimental ocular and systemic toxoplasmosis. *Graefes Arch Clin Exp Ophthalmol.* 2010;248: 573–584.
87. Holland GN. LX Edward Jackson Memorial Lecture: ocular toxoplasmosis—a global reassessment, part II: disease manifestations and management. *Am J Ophthalmol.* 2004; 137:1–17.
88. Pifer R, Yarovinsky F Innate responses to *Toxoplasma gondii* in mice and humans. *Trends Parasitol.* 2011;27:388–393.
89. Lahmar I, Guinard M, Sauer A, et al. Murine neonatal infection provides an efficient model for congenital ocular toxoplasmosis. *Exp Parasitol.* 2010;124:190–196.
90. Wallon M, Peyron F, Cornu C, et al. Congenital *Toxoplasma* infection: monthly prenatal screening decreases transmission rate and improves clinical outcome at age 3 years. *Clin Infect Dis.* 2013;56:1223–1231.
91. Mimura KK, Tedesco RC, Calabrese KS, et al. The involvement of anti-inflammatory protein, annexin A1, in ocular toxoplasmosis. *Mol Vis.* 2012;18:1583–1593.
92. Hitziger N, Dellacasa I, Albiger B, et al. Dissemination of *Toxoplasma gondii* to immunoprivileged organs and role of Toll/interleukin-1 receptor signalling for host resistance assessed by in vivo bioluminescence imaging. *Cell Microbiol.* 2005;7:837–848.
93. Pissuwan D, Valenzuela SM, Miller CM, et al. A golden bullet? Selective targeting of *Toxoplasma gondii* tachyzoites using anti body-functionalized gold nanorods. *Nano Lett.* 2007;7:3808–3812.
94. Cense B, Gao WH, Brown JM, et al. Retinal imaging with polarization-sensitive optical coherence tomography and adaptive optics. *Opt Express.* 2009;17:21634–21651.
95. Desmettre T, Labalette P, Fortier B, et al. Laser photocoagulation around the foci of *Toxoplasma* retinochoroiditis: a descriptive statistical analysis of 35 patients with long-term follow-up. *Ophthalmologica.* 1996;210:90–94.
96. Orefice JL, Costa RA, Scott IU, et al. Spectral optical coherence tomography findings in patients with ocular toxoplasmosis and active satellite lesions (MINAS Report 1). *Acta Ophthalmol.* 2013;91:e41–e47.
97. Robert-Gangneux F, Darde ML. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin Microbiol Rev.* 2012;25:264–296.
98. Grigg ME, Ganatra J, Boothroyd JC, et al. Unusual abundance of atypical strains associated with human ocular toxoplasmosis. *J Infect Dis.* 2001;184:633–639.
99. Fentress SJ, Steinfeldt T, Howard JC, et al. The arginine-rich N-terminal domain of ROP18 is necessary for vacuole targeting and virulence of *Toxoplasma gondii*. *Cell Microbiol.* 2012;14:1921–1933.
100. Smith JR, Franc DT, Carter NS, et al. Susceptibility of retinal vascular endothelium to infection with *Toxoplasma gondii* tachyzoites. *Invest Ophthalmol Vis Sci.* 2004;45: 1157–1161.
101. Dubremetz JF, Lebrun M. Virulence factors of *Toxoplasma gondii*. *Microbes Infect.* 2012;14:1403–1410.
102. Kong JT, Grigg ME, Uyetake L, et al. Serotyping of *Toxoplasma gondii* infections in humans using synthetic peptides. *J Infect Dis.* 2003;187:1484–1495.
103. McLeod R, Boyer KM, Lee D, et al. Prematurity and severity are associated with *Toxoplasma gondii* alleles (NCCCTS, 1981–2009). *Clin Infect Dis.* 2012;54:1595–1605.