Accepted Manuscript

Ocular Involvement Following an Epidemic of *Toxoplasma gondii* Infection in Santa Isabel do Ivaí, Brazil

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PII: S0002-9394(15)00103-8

DOI: 10.1016/j.ajo.2015.02.017

Reference: AJOPHT 9249

To appear in: *American Journal of Ophthalmology*

Received Date: 30 July 2014

Revised Date: 23 February 2015

Accepted Date: 24 February 2015

Please cite this article as: Silveira C, Muccioli C, Holland GN, Jones JL, Yu F, de Paulo A, Belfort Jr. R, Ocular Involvement Following an Epidemic of *Toxoplasma gondii* Infection in Santa Isabel do Ivaí, Brazil, *American Journal of Ophthalmology* (2015), doi: 10.1016/j.ajo.2015.02.017.

ABSTRACT

Purpose: To investigate ocular involvement (prevalence, incidence, lesion characteristics) following post-natally acquired infection with an "atypical" genotype of *Toxoplasma gondii* during a well-characterized 2001 outbreak in Santa Isabel do Ivaí, Brazil, attributed to a contaminated municipal reservoir.
Design: Prospective longitudinal cohort study.
Methods: We performed ophthalmic examinations on 290 of 454 individuals with serologic evidence of *T. gondii* infection during the epidemic (positive IgM antibody tests). Prevalence of ophthalmic findings (intraocular inflammatory reactions [including transient, isolated retinal whitening without clinically apparent retinal necrosis] and necrotizing retinochoroiditis) at initial examination (baseline) and incidence of new findings during 10.5 months of follow-up were calculated. Cumulative risks of ophthalmic events were determined (Kaplan-Meier technique).
Results: Ocular involvement was present in 33 (11.5%) of 288 IgM+ individuals at baseline, including 17 with focal retinal whitening only and 13 with necrotizing retinochoroiditis. Incidence of new ocular involvement was estimated to be 1.73 events per 100 person-months (PM); cumulative risk at 10.5 months was 30.1%. Incident necrotizing retinochoroiditis was more common among those with focal retinal whitening at baseline (6.7/100PM) than among those with no ocular involvement at baseline (1.11/100PM; hazard ratio 6.07 [1.94-19.01]; $p<0.0001$).
Conclusions: Water-borne infection with an atypical genotype of *T. gondii* is associated with substantial risk of ocular involvement. Lesions may continue to develop during the first year after infection. The increased risk of late necrotizing retinochoroiditis associated with isolated focal retinal whitening at presentation suggests the early presence of parasites in the retina, despite initial lack of observable retinal necrosis.

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Short Title: Ocular Disease after an Epidemic of Toxoplasmosis in Brazil

Keywords: epidemic, inflammation, longitudinal, risk factor, toxoplasmosis

Supplemental Material available at ajo.com

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INTRODUCTION

Occasional epidemics of ocular toxoplasmosis provide an opportunity to study clinical features and course of disease in large numbers of patients with known times and routes of infection.[1-8] During October-December 2001, a large cluster of *Toxoplasma gondii* infections was reported in Santa Isabel do Ivaí, a town in Parana, a state in southern Brazil. Epidemiologic investigations implicated a municipal surface water reservoir, believed to be contaminated with cat feces, as the source of infection.[7] The parasite was subsequently found to have an "atypical" genotype, not belonging to one of the three major clonal lineages.[9] Infection with a parasite having an atypical genotype is believed to be a risk factor for severe ocular disease.[10]

Most reports about epidemics of toxoplasmosis have described ocular involvement at the time of infection, but little is known about the subsequent risk of eye disease. Serial ophthalmic examinations were performed on affected individuals following the Santa Isabel do Ivaí outbreak, as a part of the medical response to this public health emergency. Using these prospective data, we determined the incidence and characteristics of ocular involvement during the first year after infection, and identified additional factors that may be related to the severity and course of retinal disease.

METHODS

We performed this prospective longitudinal cohort study as a part of the overall investigation of *T. gondii* infections in Santa Isabel do Ivaí following identification of the outbreak. The study was determined to be exempt from institutional review board (IRB) approval by the Medical Ethics Committee of the Federal University of São Paulo, because data were collected in conjunction with the investigation of a public health emergency. The investigation did adhere to tenants of the Declaration of Helsinki. Analysis of anonymized data was also determined to be exempt by IRBs at UCLA and the United States Centers for Disease Control and Prevention.

In response to the epidemic, a local center was established for the study of ophthalmic disease. Using local media, public health authorities invited citizens to be examined at the center without charge. Everyone who presented for examination was evaluated, whether or not these presenting individuals had systemic or ophthalmic symptoms. Included in this study were those who had serologic evidence of recent infection (positive anti-*T. gondii* IgM antibody tests).

Data Collection

Because of the large number of people who requested examinations, two sessions (January, February 2002) were organized for initial (baseline) ophthalmic evaluations; there were no criteria for assigning individuals to either session, and examinations were performed without knowledge of an individual's IgM antibody status. Some people examined in January chose to return for repeat examination in February, providing 1-month follow-up data. To avoid bias, an attempt was made to re-examine all individuals in June or September 2002, whether or not they had findings on initial examination. To confirm findings, all examinations were performed by at least two of three ophthalmologists (CS, CM, RB). At all visits, fundi were examined by slit lamp biomicroscopy and indirect ophthalmoscopy.

The following demographic and medical data were collected for all individuals: sex; age; anti-*T. gondii* IgM antibody test results (obtained in November or December

2001); the presence of non-ocular signs or symptoms of toxoplasmosis (e.g. sore throat, malaise, lymphadenopathy); and anti-parasitic treatment (either before baseline or during follow-up). For eyes with ocular involvement, the following information was collected at each examination: the presence of signs (redness) and symptoms (visual disturbance, pain, photophobia) of ocular involvement; presence or absence of intraocular inflammatory reactions (anterior chamber cells and flare; vitreous inflammatory reactions; retinal vascular sheathing; or focal retinal whitening without clinically apparent retinal necrosis, presumed to be retinal infiltrates[11] [Figure 1]; and presence or absence of fundus lesions (focal retinal whitening, as described above; foci of necrotizing retinochoroiditis; or retinochoroidal scars consistent with prior episodes of necrotizing retinochoroiditis). Focal retinal whitening was recorded separately from other signs of intraocular inflammation. Lack of retinal necrosis was assumed in areas of retinal whitening if there was no retinal granularity or if choroidal details could be visualized through the retinal haze on fundoscopy. The size (≥1 disc area [DA] vs. <1DA) and location (macular vs. extramacular) were determined for foci of retinal whitening and foci of necrotizing retinochoroiditis. Visual acuity and intraocular pressure were not considered, as not being relevant to the specific purpose of this study.

Anti-*T. gondii* IgM antibodies were identified by an ELISA technique (Abbott Laboratories, Abbott Park, IL, USA) at Lacen Laboratory (Curitiba, Brazil). Values ≥0.600 IU/ml were considered to be positive. Test specificity was 95% and sensitivity was 98%, as reported by the laboratory.

Conventions and Definitions

Retinal lesions were described using terms commonly employed by investigators who study ocular toxoplasmosis. Because individuals were examined infrequently, we established standard conventions for inferring disease course, based on findings. These definitions and conventions are listed in the supplemental materials (available at ajo.com).

Data Analysis and Statistic Techniques

We analyzed findings for only one eye per person. For individuals with bilateral disease, the right eye was chosen as the study eye. Only individuals with baseline examinations were included in longitudinal analyses. We chose mid-November 2001 (the mid-point for the interval during which all new infections were reported) as time of infection for all individuals. Incidence was calculated for selected findings on the basis of events per 100 person-months (PM) of follow-up. For individuals with new retinochoroidal scars during follow-up, ocular disease was assumed to have occurred when the scar was identified, for purposes of calculating intervals. For longitudinal subgroup analyses based on the presence or absence of focal retinal whitening, baseline was used as time 0. For all other longitudinal analyses, mid-November 2001 (the assumed time of infection) was used as time 0.

Statistical analyses were performed using SAS software version 9.3 (SAS, Inc., Cary, NC). Cumulative risk of ocular involvement was estimated using the Kaplan-Meier method and compared using the log-rank test. Relative risks were expressed as hazard ratios (HR), estimated from Cox proportional hazards regression models. The Fisher exact test was used to compare proportions between subgroups at baseline.

RESULTS

Santa Isabel do Ivaí had a population of 9147 at the time of the outbreak, approximately 6000 of whom lived in an urban setting (http://tabnet.datasvs.gov.br/tabdata/cadernos/PR/PR_Santa Isabel_do_Ivai_Geral.xs, accessed March 2010). Serologic testing was performed on 3868 volunteers, 460 of whom were found to have anti-*T. gondii* IgM antibodies. A total of 562 people presented for ophthalmic examination, 457 of whom had undergone IgM antibody testing. The 105 individuals without IgM antibody tests were not included in primary analyses, despite the fact that many had systemic symptoms, because infection during the outbreak had not been confirmed. Among the 457 examined individuals who had undergone IgM antibody testing, 454 had positive test results; these individuals constituted the study population.

Characteristics of the study population are shown in Table 1. People of all ages were infected, with men and women involved equally. Symptoms suggestive of systemic toxoplasmosis were present in the majority of those examined. Baseline ophthalmic examinations were performed on 290 of the 454 IgM-positive individuals; they had characteristics similar to those of all IgM-positive individuals. Ophthalmic symptoms were present at baseline in 47% of those for whom data were available (133 of 282 individuals). Ophthalmic involvement at baseline (n=33, Table 2) was similar between individuals with ophthalmic symptoms (n=15 of 133, 11%) and those without symptoms (n=18 of 149, 12%; Fisher exact test, p=0.86). Ophthalmic symptoms were described by some individuals without findings on ophthalmic examination, as were systemic symptoms by some without serologic evidence of infection; these symptoms were attributed by investigators to unrelated disorders or to anxiety about the outbreak.

Results of eye examinations are shown in Table 2. Ophthalmic findings were known for 288 of 290 individuals with baseline examinations. Ocular involvement was present in 33 individuals (11.5%). We re-calculated the prevalence of ophthalmic involvement at baseline in several models that also considered individuals excluded from our primary analyses, based on various assumptions (see supplemental materials, available at ajo.com). Prevalence only varied from 9.6%-12.0% in these models.

There was active disease in both eyes of four individuals at baseline; in each, lesion characteristics (type, size, location) were the same in both eyes. In three, there were foci of retinal whitening only; in the other, there was necrotizing retinochoroiditis in both eyes.

An inactive retinochoroidal scar, without associated retinal or vitreous inflammation, suggestive of healed toxoplasmic retinochoroiditis, was present in an asymptomatic individual at baseline examination in January. We assumed that the scar was the result of prior endemic disease and excluded him from further analysis. He had a relatively low value for anti-*T. gondii* IgM antibodies (2.6 units), and it is known that IgM+ antibodies can persist for 1 year or longer after infection.[12] He had no active disease on each follow-up examination. Two individuals had only intraocular inflammatory reactions at baseline; neither had follow-up examinations. By June 2002, no one was found to have inflammatory reactions only.

Five patients were examined in both January and February. Two had necrotizing retinochoroiditis at both examinations; one had necrotizing retinochoroiditis in January that was resolving with evidence of new scar formation by February; one had focal retinal whitening in January that evolved to a focus of necrotizing retinochoroiditis by

February; and one had only focal retinal whitening in both January and February.

The majority of patients had serial examinations; 236 (81%) of 290 patients with baseline examinations were seen at least twice, and 212 (73%) were seen at baseline and again at least 4 months after baseline. Among 255 individuals with no ocular involvement at baseline, cumulative follow-up was 811 PM. Any ocular involvement during follow-up was seen in 14 individuals (incidence, 1.73/100PM). Foci of retinal whitening were seen during follow-up in five individuals (incidence, 0.62/100PM). Evidence of incident necrotizing retinochoroiditis was seen in nine individuals (one with active necrotizing retinochoroiditis; eight with new retinochoroidal scars; incidence, 1.11/100PM).

When all individuals were considered, cumulative risk of any ocular involvement at 10.5 months after onset of the epidemic was 30.1% (95% confidence interval [CI], 14.0%-57.1%); among those who were untreated, it was 27.4% (95% CI, 11.5%-56.6%; Figure 2). Cumulative risk of necrotizing retinochoroiditis at 10.5 months after onset of the epidemic was 27.2% (95% CI, 12.2%-53.8%); among those who were untreated, it was 26.5% (95% CI, 10.6%-57.2%). Ophthalmic involvement at baseline or during the 10.5 months after onset of the epidemic was not related to age, gender, IgM antibody test values, or presence of symptomatic non-ocular disease (data not shown; all p values ≥0.11).

Outcomes differed on the basis of ophthalmic findings at baseline (Figure 3). Among 17 individuals who had only focal retinal whitening at baseline, cumulative follow-up was 75 PM. Evidence of incident necrotizing retinochoroiditis was seen during follow-up in five individuals (one with active necrotizing retinochoroiditis; four with retinochoroidal scars; incidence, 6.7/100PM). The risk of developing necrotizing retinochoroiditis during follow-up was significantly higher for patients with focal retinal whitening at baseline than for individuals with no ocular involvement at baseline (HR 6.07, 95% CI, 1.94-19.01, p<0.0001). At 7 months after baseline, the cumulative risk of incident necrotizing retinochoroiditis (active necrotizing retinochoroiditis or retinochoroidal scars) was 52.4% (95% CI, 20.4%-90.8%) for those with only focal retinal whitening at baseline and was 6.7% (95% CI, 2.6%-16.7%) for those with no ophthalmic involvement at baseline. No patient with focal retinal whitening at baseline had more than 7 months of follow-up. As shown in Figure 3, cumulative risk among those with no ophthalmic involvement increased to 53.1% (95% CI, 11.6%-99.1%) at 8 months, based on the presence of a retinochoroidal scar in one of only three individuals with that length of follow-up; thus, we do not know whether the observed difference between subgroups was sustained.

Among 13 individuals who had necrotizing retinochoroiditis at baseline, cumulative follow-up was 69 PM. Recurrences were seen in three individuals (incidence, 4.3/100PM). The incidence of recurrences was slightly lower among individuals with necrotizing retinochoroiditis at baseline than the incidence of new necrotizing retinochoroiditis lesions during follow-up among those with only focal retinal whitening at baseline, although the difference was not significant (HR, 0.81, 95% CI 0.19-3.40, p=0.76).

Multiple examinations during periods of active disease were not available for most individuals, and as a result, evolution of focal retinal whitening could not be studied in most cases. As noted above, one focus of retinal whitening was known to

evolve directly into a focus of necrotizing retinochoroiditis, with subsequent resolution to a scar. In contrast, we were able to confirm that retinal whitening resolved completely in another individual, leaving a clinically normal fundus, before a retinochoroidal scar was seen in the same area on later examination. Scars were eventually seen during follow-up both in patients who had small foci of retinal whitening at baseline and in those with large foci, but the number of cases was too small to determine the relative risks associated with size or location of retinal whitening for eventual development of retinal necrosis. Three patients with necrotizing retinochoroiditis at baseline were noted to develop independent foci of retinal whitening at a later examination.

Lesion characteristics are shown in Table 3. Lesions that developed during follow-up are similar to those seen at baseline, in terms of size and location, but numbers were too small for meaningful statistical comparisons. The majority of necrotizing retinochoroiditis lesions involved the macula, while the majority of focal retinal whitening lesions involved extramacular locations. Furthermore, macular lesions were more likely to be necrotizing retinochoroiditis; eight (57%) of 14 macular lesions were necrotizing, while only five (19%) of 27 extramacular lesions were necrotizing retinochoroiditis (p=0.017).

The effect of treatment at baseline, if any, could not be evaluated in detail (see supplemental material, available at ajo.com), but outcomes did not appear to differ between treated and untreated individuals (Figure 2). Initial treatment did not prevent development of retinal lesions in some individuals.

DISCUSSION

The reported prevalence of ocular disease among people known to have post-natally acquired *T. gondii* infection has varied markedly. Perkins reported that two (7.7%) of 26 individuals infected in a laboratory accident developed ocular disease.[13] In 1977, an outbreak of *T. gondii* infection in Atlanta, Georgia was attributed to inhalation of infective oöcysts in dust from a riding stable floor.[4] Only one (2.7%) of 37 individuals known to have been infected was found to have eye disease 4 years after the outbreak. In 1995, an epidemic of acquired toxoplasmosis occurred in Victoria, British Columbia, Canada.[5,6] Among 100 individuals known to be infected, 20 (21%) of 97 who underwent ophthalmic examinations were found to have ocular toxoplasmosis; however, these cases were selected, in part, because of known or suspected ocular involvement, and thus, the true prevalence may be substantially lower, based on the number of individuals possibly infected.[14] As with the Santa Isabel do Ivaí outbreak, the Victoria epidemic was thought to be caused by contamination of a surface water reservoir by a parasite with atypical genotype, in this case from cougar feces.[15] These epidemics have been summarized by Holland.[16]

The Santa Isabel do Ivaí outbreak is one of the largest epidemics to have been studied. It supports the concept that individuals infected by parasites with atypical genotypes are at increased risk of ophthalmic disease.[10] In addition, results provide information about the course of ophthalmic involvement in this situation. It is believed that necrotizing retinochoroiditis may first develop long after the initial systemic infection;[17-19] our results confirm that individuals continue to be at risk for ophthalmic involvement for at least many months after infection.

We have previously reported that intraocular inflammatory reactions (anterior chamber cells, vitreous humor cells, retinal vascular sheathing, and focal retinal

whitening without clinically apparent retinal necrosis) may be the only ophthalmic findings when *T. gondii* infection occurs,[11] but that report contained only a small number of selected cases. This study demonstrates the prevalence of such reactions, and provides data regarding the incidence with which they progress to necrotizing retinochoroiditis. The fact that individuals with only focal retinal whitening were at increased risk of eventually developing necrotizing retinochoroiditis supports the concept that the retina is colonized with tissue cysts at the time of infection in more people than would be indicated by the presence of necrotizing retinochoroiditis lesions at time of infection.[11] An alternative explanation is the possibility that localized inflammatory damage to retinal vessels increases the risk of subsequent infection by, or immune reaction to, circulating parasites from reactivation elsewhere.[20] Close follow-up of individuals with intraocular inflammation at the time of *T. gondii* acquisition seems to be warranted. Also, these observations have possible implications for treatment, as it has been shown that secondary prophylaxis with antimicrobial agents can reduce recurrence risk in people presumed to have inactive tissue cysts in the retina.[21]

More than half of necrotizing retinochoroiditis lesions involved the macula, despite the fact that it represents only approximately 5% of the total retina area. In contrast, only 18.5% of focal retinal whitening lesions involved the macula, suggesting that parasites are initially distributed more widely throughout the fundus, but those in the macula are more likely to cause necrotizing infections. A propensity for macular involvement has been described in other studies[22] and has been attributed possibly to unique anatomic or immunologic factors in that microenvironment. No new lesions were seen in the macula during follow-up, but the number of new lesions was small, precluding meaningful comparisons to baseline.

Epidemics may be associated with unique parasite strains, unusual sources of infection (which determines the stage of the infecting parasite), and variable inocula. As a result, the extent to which information from epidemics of ocular toxoplasmosis is relevant to understanding characteristics of disease in the general population is uncertain. We therefore also compared lesion characteristics and course of infection in this study to those reported for patients with endemic post-natally acquired disease in Erechim, Brazil during an overlapping time-frame[23] and to lesion characteristics described by Dodds and associates for a group of 210 individuals with endemic disease from seven sites in North America, South America, and Europe.[22] Basic findings were similar, as discussed by Arantes and associates;[23] thus, continued study of ocular disease associated with periodic outbreaks of *T. gondii* infection may provide insights that can be applied to the understanding of endemic ocular toxoplasmosis in the general population, if the setting and infecting genotypes are similar.

Cumulative risk of ocular involvement during the first 10.5 months after the outbreak (31%) was higher than the prevalence of toxoplasmic retinochoroidal scars reported for the area of Erechim, also in southern Brazil (point prevalence of 18%, where 85% of the population has serologic evidence of infection; approximate risk calculated as 0.18/0.85=21%).[24] Endemic toxoplasmosis in Erechim is also believed to be caused by parasites with atypical genotypes.[25,26] The reason for this apparent difference in risk is unclear; it may be attributable to different genotypes within the heterogeneous group considered to be atypicals, or to other factors, such as inoculum size or stage of the ingested parasite (oöcysts vs. tissue cysts).[19] These issues are

discussed further by Arantes and associates.[23]

There are several limitations and sources of potential error in this study. Results of a related investigation of parasite genotypes suggested that some individuals in Santa Isabel do Ivaí may actually have had endemic toxoplasmosis unrelated to the epidemic.[9] Possible inclusion of such individuals in this cohort may have led to incorrect estimates of prevalence and incidence of ophthalmic disease associated with the epidemic parasite strain. Ascertainment bias may also have resulted in overestimation of ocular disease prevalence. We cannot rule-out the possibility that ascertainment bias also resulted in confounding factors not considered in our sub-group comparisons. Incidence calculations are based on few events. Furthermore, examinations were not performed immediately; thus, results may underestimate the prevalence of disease at the time of infection, especially for intraocular inflammatory reactions, which can resolve without observable sequelae.[11] Because of the limited number of examinations, we may also have underestimated the number of events during follow-up. We did not document the number, size, or location of scars; thus, we may not have been aware of recurrences that developed and resolved between examinations. We have expressed events in terms of incidence, which will allow comparison to future studies,[27] but this measure may overestimate the occurrence of events, if risk decreases over time.[28] Individuals may have presented for examination differentially, because of symptoms, resulting in artefactually high prevalence and incidence figures; however, presence of symptoms was not a criterion for inclusion, and we made an attempt to examine as many people as possible, regardless of symptoms. We therefore believe that our results are reasonable approximations of the true values. We did not identify the location of necrotizing retinochoroiditis lesions that were first identified as scars during follow-up; those lesions are therefore not represented in Table 3, but the number of such cases was small. We were not able to compare size or other characteristics of scars between those resulting from necrotizing retinochoroiditis at baseline and those that evolved from foci of retinal whitening or from new primary lesions during follow-up. Our distinction between focal retinal whitening and necrotizing retinochoroiditis was based on clinical examination alone; future study of focal retinal whitening lesions with imaging techniques and serial examinations may clarify their histologic nature and the relationship of these lesions to necrotizing retinochoroiditis, if any.

In summary, infection with *T. gondii* that has an atypical genotype is associated with a substantial risk of ocular involvement. Retinal whitening without clinically apparent retinal necrosis may be an early sign of colonization of the retina with tissue cysts that can later reactivate to cause disease. Those tissue cysts in the macula may be more likely to cause necrotizing infections. The study of epidemics may provide information about ocular toxoplasmosis that is applicable to cases of endemic disease in similar settings.

ACKNOWLEDGEMENT

Funding: Supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico and the Fundação de Amparo à Pesquisa do Estado de São Paulo (CNPq/FAPESP), Sao Paulo, Brazil (Drs. Silveira, Muccioli, Belfort); Research to Prevent Blindness (RPB), Inc., New York, NY (Dr. Holland), the Skirball Foundation, New York, NY (Dr. Holland), and the Centers for Disease Control and Prevention, Atlanta, GA (Dr. Jones). Dr. Holland is recipient of an RPB Physician-Scientist Award. Other authors have no financial disclosures. Funding entities had no role in the reporting of study data and the interpretation of the data.

Financial Disclosure: Dr. Holland has served on Advisory Boards for the following companies: Genentech, Incorporated (San Francisco, CA, USA); Novartis International AG (Basel, Switzerland); Santen, Incorporated (Emeryville, CA, USA); and Xoma (US) LLC (Berkeley, CA, USA). Dr. Belfort has served on Advisory Boards or has received grants from Alcon/Novartis International AG (Basel, Switzerland), Allergan, Incorporated (Irvine, CA), Bayer AG (Leverkusen, Germany), Kemin Industries (Des Moines, Iowa USA), NovaBay Pharmaceuticals, Incorporated (Emeryville, CA, USA), and Santen, Incorporated (Emeryville, CA, USA). None of the other authors have financial disclosures.

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Study design: CS, CM, GNH, JLJ, RB
Data collection: CS, CM, ADP, RB
Data management and analysis: GNH, JLJ, FY
Data interpretation: CS, CM, GNH, JLJ, FY, ADP, RB
Preparation of manuscript: GNH
Review and approval of manuscript: CS, CM, GNH, JLJ, FY, ADP, RB
Each author meets the four criteria set by the ICMJE required to claim authorship.

Other Acknowledgements: Anna Elisa Mazzotine, RN (County Department of Health, Santa Isabel do Ivaí, Parana, Brazil) assisted with organization of the study and collection of data.

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FIGURE LEGENDS

Figure 1: Two individuals with discrete foci of retinal whitening, but no clinically-apparent retinal necrosis. Both had IgM antibodies against *Toxoplasma gondii*, attributable to infection during a 2001 outbreak in Santa Isabel do Ivaí, Parana state, Brazil. Left: This individual had a small focus (<1 disc area [DA]) in the right anatomic macula. Right: This individual had a large (>1 DA) focus in the extramacular retina. Such lesions were noted to follow one of two courses in affected individuals: complete resolution without clinically apparent retinal lesions; or progression to necrotizing retinochoroiditis. Those with resolution could eventually develop a necrotizing retinochoroiditis lesion in the same area, suggesting the presence and persistence of parasites at the site of infiltration.

Figure 2: Ocular Involvement following an outbreak of *Toxoplasma gondii* infection in Santa Isabel do Ivaí, Parana state, Brazil. Top: Kaplan-Meier plot showing the proportion of all individuals who remained free of any retinal involvement (dotted line) or remained free of necrotizing retinochoroiditis (solid line). Bottom: Kaplan-Meier plot showing the percentage of individuals who were never treated with antimicrobial agents and who remained free of any retinal involvement (dotted line) or remained free of necrotizing retinochoroiditis (solid line). Time 0 is mid-November 2001, the mid-point in the period during which positive anti-*T. gondii* IgM antibody tests were reported, and used for study purposes as the time of infection for all individuals. Each line on the two graphs refers to a distinct outcome for the entire population being studied, which allows comparison of the time courses for those outcomes; the two lines do not represent subgroups.

Figure 3. Kaplan-Meier plots showing the proportion of individuals examined during an outbreak of *Toxoplasma gondii* infection in Santa Isabel do Ivaí, Parana state, Brazil, who remained free of necrotizing retinochoroiditis. The dotted line corresponds to those who had no ocular involvement at baseline; the solid line corresponds to those who had retinal whitening, but no clinically apparent retinal necrosis, at baseline. The latter group was statistically more likely to develop necrotizing lesions during follow-up (hazard ratio, 6.07; 95% confidence interval, 1.94-19.01, $p<0.0001$). Time 0 was the baseline examination for each individual.

TABLE 1. Demographic and Medical Data for 454 Individuals with Serologic Evidence of Recent *Toxoplasma gondii* infection in Santa Isabel do Ivaí, Brazil.

Characteristic	All Individuals (n=454)	Individuals with Baseline Examinations (n=290)
Sex (n [percentage])		
Male	222 (49%)	141 (49%)
Female	232 (51%)	149 (51%)
Age (years)	n=442[a]	N=279[a]
Mean ± SD	26.1±17.7	27.0±16.8
Median (range)	22 (0 – 84)	25 (0-84)
IgM antibody test result[b]	n=269[a]	n=269[a]
Median (range)	3.5 (0.6 – 80.9)	3.5 (0.6-80.9)
Clinically apparent non-ocular toxoplasmosis[c] (n [percentage])	395 (88%) (n=450[a])	260 (91%) (n=286[a])
Ophthalmic symptoms[d] (n [percentage])	227 (51%) (n=445[a])	133 (47%) (n=282[a])
Antiparasitic treatment (n [percentage])	14 (3%)	10 (3%)
Received before initial eye examination	5 (1%)	3 (1%)
Received after initial eye examination	9 (2%)	7 (2%)

SD=standard deviation

a. Number of individuals for whom values were known.
b. Performed by ELISA technique. Values <0.6 are considered negative.
c. Arthralgias, fatigue, fever, malaise, lymphadenopathy, sore throat, or a combination of these disorders.
d. Blurring or other visual disturbance, redness, pain, or photophobia.

TABLE 2. Ophthalmic Findings for 454 Individuals with Serologic Evidence of Recent *Toxoplasma gondii* infection in Santa Isabel do Ivaí, Brazil, Based on Date of Examination

Characteristic	January 2002	February 2002		June 2002		September 2002		
							Follow-up	
	Baseline[a]	Baseline[a]	Follow-up[b]	Initial[c]	Follow-up[d]	Initial[c]	From Baseline	From June 2002 only
Patients examined (n)	74[e]	216	27	161	211	3	29	17
Patients whose findings were available (n)	72[e]	214[e]	25[e]	160	210	3	29	17
Active ocular involvement (n)	8	24	5	4	7	0	3	1
Inflammatory reaction only[f]	1	1	0	0	0	--	0	0
Retinal lesions	7	23	5	4	7	--	3	1
Necrotizing retinochoroiditis[g]	5	8	3	0	2	--	1	1
New	5	8	1[h]	--	1	--	0	0
Recurrent	NA	NA	2	--	1	--	1	1
Focal retinal whitening only[i]	2	15	2	4	5[j]	--	2	0
Retinochoroidal scars only (n)	1	0	2	13	13[j]	--	9	5

NA = not applicable.

a Initial examination, if performed in January or February 2002, was considered baseline examination for longitudinal analyses.
b Individuals whose initial examination was in January 2002.
c Individuals who were not examined in January or February 2002. They were not included in longitudinal analyses.
d Individuals who had baseline examinations in either January or February 2002.
e The two individuals examined in January whose findings were not available were seen in follow-up in February; their findings in February were considered baseline findings for purposes of analysis; thus, the total number of individuals for whom baseline findings were available was 288.
f Anterior chamber cells, vitreous humor cells or haze, retinal vascular sheathing, or a combination of these findings in the absence of focal retinal whitening or necrotizing retinochoroiditis.
g Descrete foci of dense retinal opacification with thickening and overlying inflammatory material.
h The patient had progression to necrotizing retinochoroiditis from a focus of retinal whitening only (seen in January 2002).
i Descrete foci of retinal whitening without clinically apparent retinal necrosis.
j. One person had both a retinochoroidal scar and an unrelated focus of retinal whitening; the case was categorized with those having retinal whitening.

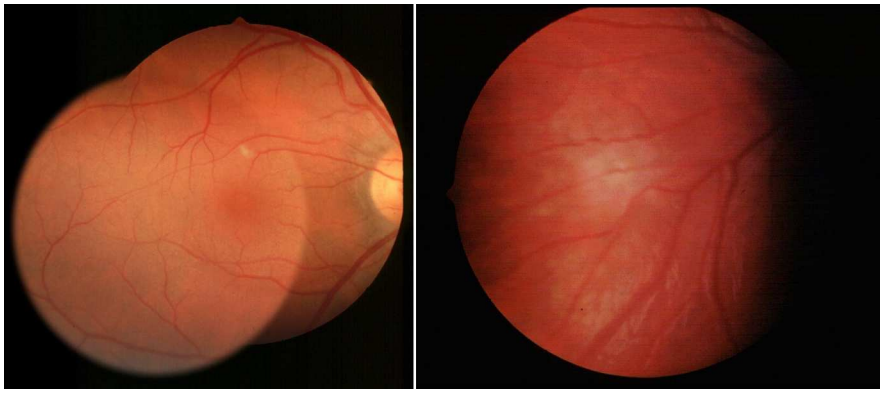
Table 3. Characteristics of Retinal Lesions Associated with an Outbreak of *Toxoplasma gondii* Infection in Santa Isabel do Ivaí, Brazil.

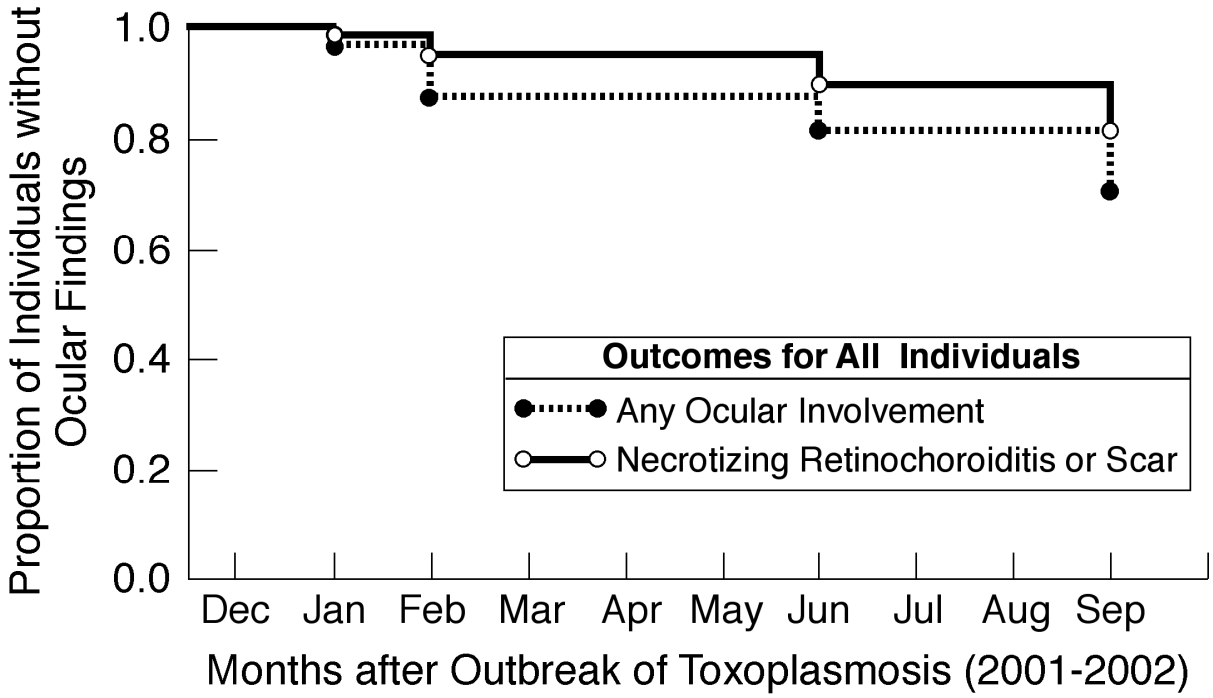
Characteristic	All lesions[a] N=454	Lesions Present at Baseline[b]	New Lesions Identified During Follow-up Only[c]
Individuals with necrotizing retinochoroiditis lesions	16	13	2
Location (n [percentage])			
Macular	8 (57%)	7 (64%)	0
Extramacular	6 (43%)	4 (36%)	2
Size (n [percentage])			
>1DA	2 (17%)	2 (2%)	0
≤1DA	10 (83%)	7 (78%)	2
Individuals with focal retinal whitening only	28	18	6
Location (n [percentage])			
Macular	5 (19%)	2 (12%)	1
Extramacular	22 (81%)	15 (88%)	5
Size (n [percentage])			
>1DA	4 (15%)	3 (18%)	0
≤1DA	23 (85%)	14 (82%)	5

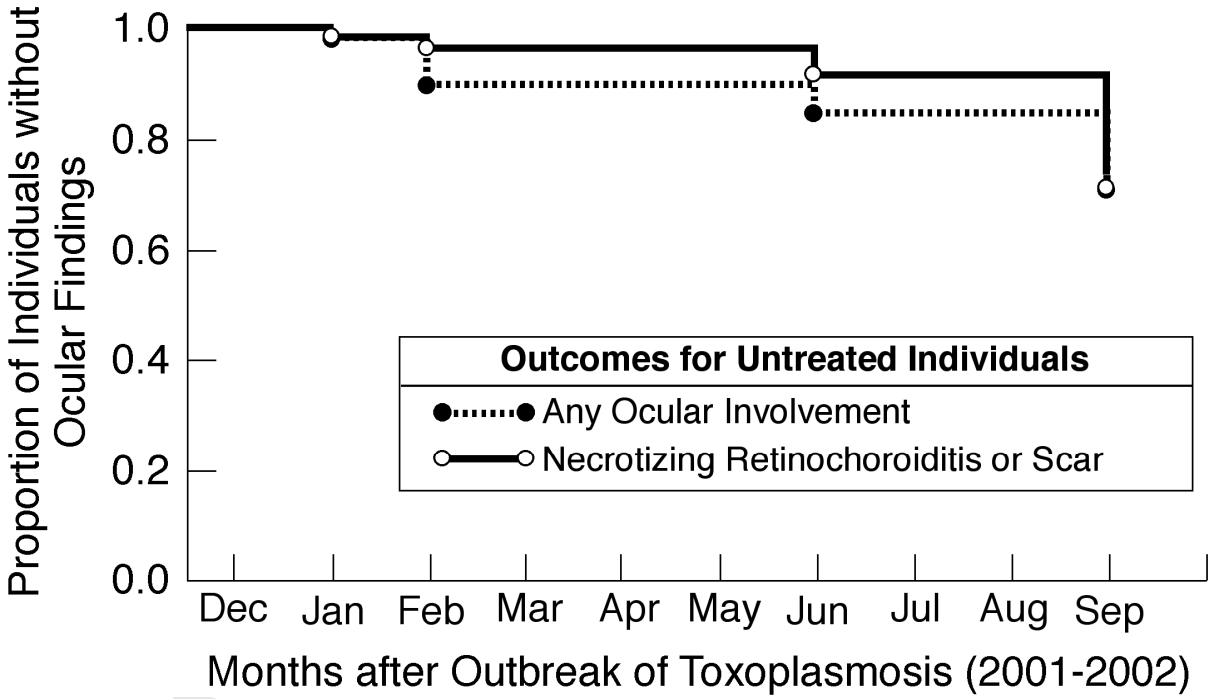
DA=disc area

a. Includes lesions seen in patients without baseline examinations.
b. Includes those individuals whose ophthalmic findings were known in January or February 2002.
c. Includes only those individuals whose ophthalmic findings were known in June or September 2002, and who were known not to have focal retinal lesions in January or

February 2002. Reactivations are not included. No new lesions were seen on follow-up examinations in February 2002 among patients whose baseline examinations had been in January 2002.




Proportion of Individuals without Ocular Findings
1.0
0.8
0.6
0.4
0.2
0.0
Outcomes for All Individuals
Any Ocular Involvement
Necrotizing Retinochoroiditis or Scar
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Months after Outbreak of Toxoplasmosis (2001-2002)


Proportion of Individuals without Ocular Findings
1.0
0.8
0.6
0.4
0.2
0.0
Outcomes for Untreated Individuals
Any Ocular Involvement
Necrotizing Retinochoroiditis or Scar
Dec
Jan
Feb
Mar
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May
Jun
Jul
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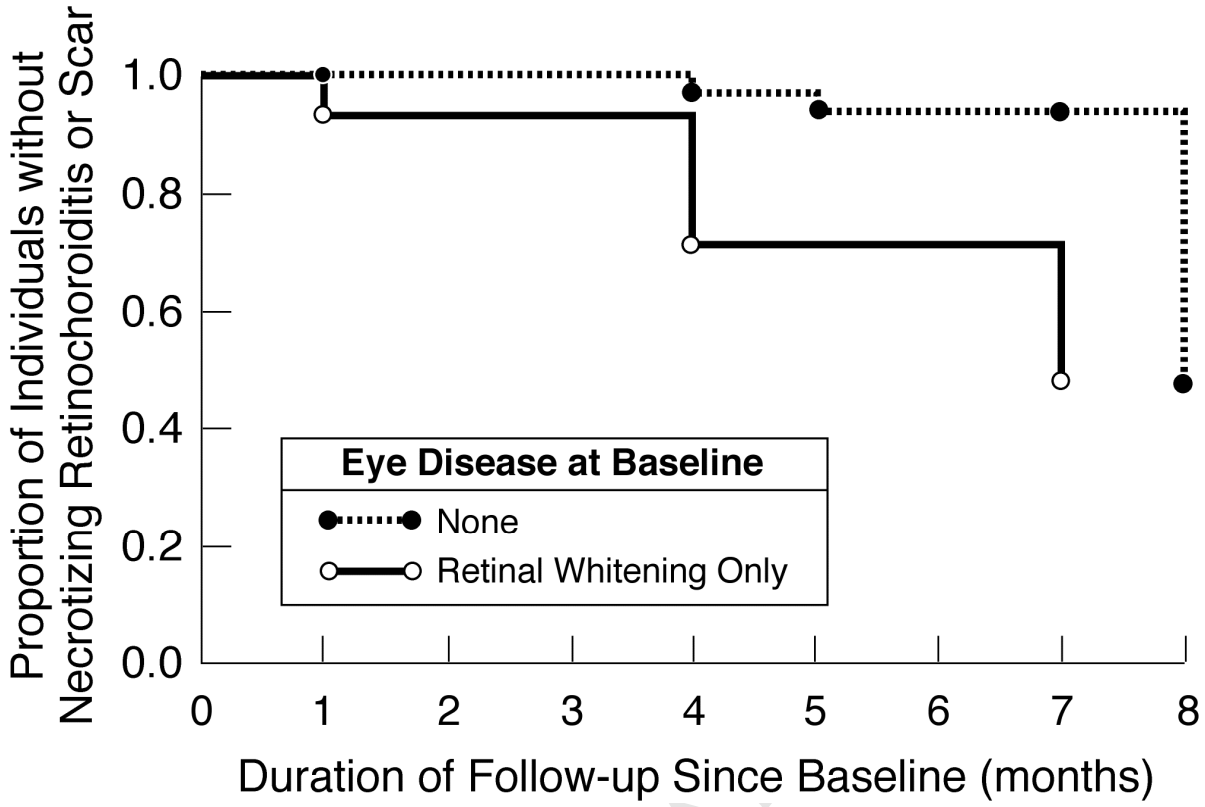
Months after Outbreak of Toxoplasmosis (2001-2002)



BIOSKETCH

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Ocular Involvement Following an Epidemic of *Toxoplasma gondii* Infection in Santa Isabel do Ivaí, Brazil

SUPPLEMENTAL MATERIAL

Definition of Terms used for this Study

1. **Active** disease was defined as the presence of intraocular inflammation involving the retina, choroid, or intraocular fluids.
2. The term **necrotizing retinochoroiditis** referred to one or more descrete foci of dense, creamy-white retinal and choroidal inflammation with overlying vitreous inflammatory reactions, whether or not they was an associated retinochoroidal scar. All such lesions among study participants were assumed to be caused by *T. gondii* infection.
3. Retinochoroidal scars were assumed to be related to healed areas of retinal necrosis caused by *T. gondii* infection, if they were discrete, round or oval in shape, and had hyperpigmented borders with an atrophic center.
4. **Isolated intraocular inflammatory reaction** was defined as the presence of anterior chamber cells and flare; vitreous inflammatory reaction; retinal vasculitis; or focal retinal whitening without the presence of retinal necrosis, as determined by fundoscopy.
5. A **primary** retinal lesion was defined as a focus of disease not arising at or near the border of a retinochoroidal scar.
6. **Satellite lesions** were defined as foci of retinal inflammation at or near the border of a previous retinochoroidal scar.
7. An **initial** lesion was defined as one seen in the absence of retinochoroidal scars anywhere in either eye. By definition, all initial necrotizing retinochoroiditis lesions were primary lesions.
8. A **recurrent lesion** was defined as one seen in the presence of one or more retinochoroidal scars anywhere in either eye. Recurrent necrotizing retinochoroiditis lesions could be primary (i.e. not arising at or near the border of a retinochoiroidal scar) or a satellite **(**arising at or near the border of a retinochoiroidal scar**).**
9. **Immediate ocular disease** was defined as (1) the presence of an initial necrotizing retinochoroiditis lesion or isolated intraocular inflammation at baseline, if the first eye examination was prior to or at the same time as the first positive anti-*T. gondii* IgM antibody test; or (2) the presence of an initial necrotizing retinochoroiditis lesion or isolated intraocular inflammation on a first eye examination performed within 3-months after the first positive IgM test, based on the assumption that an episode of necrotizing retinochoroiditis could have persisted for as long as 3 months.
10. **Incident ocular disease** was defined as (1) an initial necrotizing retinochoroiditis lesion or isolated intraocular inflammation on any examination after the first positive anti-*T. gondii* IgM antibody test, if previous ophthalmic examinations had been performed since the first positive test and revealed no evidence of intraocular disease; or (2) an initial necrotizing retinochoroiditis lesion identified more than 6 months after the first positive anti-*T. gondii* IgM antibody test, whether or not there had been prior ophthalmic examinations. The latter definition assumed, for purposes

of this study, that the course of active disease will be less than 6 months, such that active disease detected after this period must not have been present since initial infection.

Conventions used for this Study

The presence of lymphadenopathy, sore throat, malaise, or a combination of these factors in the setting of a positive anti-*T. gondii* IgM antibody test was assumed to be the manifestation of systemic toxoplasmosis in this study population.

Consistent with the above definitions, we assumed that the presence of retinochoroidal scars in June or September 2002 among individuals without lesions at baseline examination in January or February 2002 was the result of necrotizing retinochoroiditis that developed and resolved between examinations. Furthermore, necrotizing retinochoroiditis or a retinochoroidal scar in June or September 2002 was considered a recurrence if it met one of the following criteria: (1) presence of necrotizing retinochoroiditis in an eye that had had at least two prior examinations, the first of which had no lesions, only intraocular inflammatory reactions, or a primary necrotizing retinochoroiditis lesion, and the second of which had only a retinochoroidal scar; or (2) the presence of a new retinochoroidal scar in an eye without retinal lesions on at least one prior examination. If patients had only intraocular inflammatory reactions, including retinal whitening, at baseline and were found to have retinochoroidal scars on a second examination in June or September 2002, it was not possible to tell whether the baseline lesions had progressed directly to retinal necrosis or had resolved without scarring, followed later by development of necrotizing retinochoroiditis that resolved with scar formation; these patients were not analyzed as having had recurrences.

If patients were examined in both January and February 2002, findings in January were considered the baseline findings. Foci of necrotizing retinochoroiditis seen during both January and February in the same eye were considered to represent the same episode of active disease, rather than considering the lesion in February to be a recurrence, because lesion activity typically persists for at least 4-6 weeks.[1,2] If a patient had only retinal whitening in January 2002 and a necrotizing retinochoroiditis lesion in February 2002, it was considered progression of the retinal whitening to a focus of necrotizing retinochoroiditis, rather than a recurrence.

Prevalence of Ophthalmic Involvement

We considered how prevalence of ophthalmic involvement would vary if we included more of the examined individuals than only those with positive anti-*T. gondii* IgM antibody tests. Ophthalmic findings were also known at baseline for 87 of the 105 individuals without IgM antibody tests; two had ophthalmic disease (both with intraocular inflammatory reactions, one of whom had focal retinal whitening) and one had retinochoroidal scars. Of the three individuals with negative IgM antibody tests, two had ophthalmic disease at baseline (both with intraocular inflammatory reactions, one of whom had focal retinal whitening). Thus, when one considers the 90 excluded individuals with baseline examinations, five had ocular involvement (4.4%). Had the individuals with negative IgM tests been included in our analyses (assuming false-negative results), the prevalence of ocular involvement at baseline would have been

12.1% (35 of 290 individuals). Had each of the 87 individuals without tests at baseline been included in our analyses (assuming all were infected recently), the prevalence of ocular involvement at baseline would have been 9.6% (36 of 375 individuals). If we had assumed that each individual with active ophthalmic disease (n=38) and each individual without ophthalmic disease, but with systemic symptoms or a positive IgM antibody test, had been infected during the epidemic, and we based our analyses on these individuals, the prevalence of ophthalmic involvement would have been 10.3% (38 of 369 individuals [288 individuals with positive IgM antibody tests, five additional individuals with ophthalmic disease, and 76 additional individuals without ophthalmic disease and no IgM antibody tests, but with systemic symptoms]). Had we included all 90 of the excluded individuals in our analyses, the prevalence of ocular involvement at baseline would have been 10.1% (38 of 378 individuals).

Consideration of Treatment Effect

Treated patients received sulfadiazine and pyrimethamine plus folinic acid. All but one of 14 treated individuals had ocular involvement at some point during the course of disease (five treated patients did not receive baseline examinations). Treatment was started before initial ophthalmic examinations for one individual without eye disease; for all other individuals, treatment was started before identification of retinal lesions or in response to the identification of such lesions. Among four individuals with only intraocular inflammatory reactions (focal retinal whitening) at baseline, three were seen in follow-up; two developed necrotizing retinochoroiditis, despite treatment. One of three treated patients with necrotizing retinochoroiditis at baseline was found to have disease recurrence during follow-up (a focus of retinal whitening).

Information available to us did not allow conclusions regarding the effect of treatment on ocular disease. For individuals who had retinal lesions on initial examinations after the start of treatment, the temporal relationship between the onset of ocular involvement and start of treatment could not be determined. We noted that treatment did not prevent recurrences or progression of disease in all cases, and did not appear to alter the cumulative risk of ocular involvement. The apparent lack of treatment effect may reflect the possibility that some parasite genotypes are more resistant to antimicrobial drugs than others.[3]

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3. Holland GN. Ocular toxoplasmosis: a global reassessment. Part II: disease manifestations and management. *Am J Ophthalmol* 2004;137(1):1-17.