Ocular lesions in gerbils (*Meriones unguiculatus*) infected with low larval burden of *Toxocara canis*: observations using indirect binocular ophthalmoscopy

Lesões oculares em gerbils (*Meriones unguiculatus*) infectados com baixo número de larvas do *Toxocara canis*: observações com oftalmoscopia binocular indireta

Ledilma Inês Duarte Colodetti Zanandréa¹, Gabriela Mantovanelli Oliveira¹, Andressa Silva Abreu¹ and Fausto Edmundo Lima Pereira¹

**ABSTRACT**

To study the frequency of ocular lesions in 30 gerbils infected with 100 embryonated eggs of *Toxocara canis*, indirect binocular ophthalmoscopy was performed 3, 10, 17, 24, 31 and 38 days after infection. All the animals presented larvae in the tissues and 80% presented ocular lesions. Hemorrhagic foci in the choroid and retina were present in 92% of the animals with ocular lesions. Retinal exudative lesions, vitreous lesions, vasculitis and retinal detachment were less frequent. Mobile larvae or larval tracks were observed in four (13.3%) animals. Histological examination confirmed the ophthalmoscopic observations, showing that the lesions were focal and sparse. In one animal, there was a larva in the retina, without inflammatory reaction around it. The results demonstrated that gerbils presented frequent ocular lesions after infection with *Toxocara canis*, even when infected with a small number of embryonated eggs. The lesions observed were focal, consisting mainly of hemorrhages with signs of reabsorption or inflammation in different segments of eye, and differing from the granulomatous lesions described in ocular larva migrans in humans.


**RESUMO**

Para verificar a frequência de lesões oculares em 30 gerbils infectados com 100 ovos larvados de *Toxocara canis* foi realizada a oftalmoscopia binocular indireta nos dias 3, 10, 17, 24, 31 e 38 após a infecção. Todos os animais apresentavam larvas nos tecidos e 80% apresentavam lesões oculares, dos quais 92% tinham lesões hemorrágicas focais na coroíde e na retina. Lesões exudativas da retina, lesões do vítreo, vasculite e descolamento da retina foram menos frequentes. Larva móvel ou traços de larva em quatro (13,3%) animais. O estudo histológico confirmou as observações da oftalmoscopia, mostrando que as lesões eram focais e esparsas. Em um animal havia uma larva na retina, sem reação inflamatória em torno dela. Os resultados demonstraram que os gerbils apresentam frequentes lesões oculares após infecção com *Toxocara canis* mesmo quando infectados com pequeno número de ovos larvados. As lesões observadas eram focais, principalmente focos de hemorragia com sinais de reabsorção ou focos de inflamação nos diferentes segmentos do olho, diferentes das lesões granulomatosas descritas na larva migrans ocular humana.


Toxocariasis is an intestinal parasitosis produced by worms belonging to the genus *Toxocara*, frequently in dogs (*Toxocara canis*) and cats (*Toxocara cattii*), in which the adult worms live in the small intestine. Man is a paratenic host in which the ingested eggs deliver larvae into the intestine, from where the larvae migrate to the blood stream, thereby reaching different organs in which they may be killed by granulomatus inflammation around them or may stay in a dormant state for several years¹⁹. Human tissue invasion by *Toxocara* larvae is an anthropozoonosis with universal distribution, but is more prevalent in developing countries¹¹,¹⁸,²⁰. The clinical manifestations of the infection are variable, from asymptomatic infections to fully developed syndromes of visceral or ocular larva migrans¹¹,¹⁸,¹⁹,²⁰. Isolated cases of neurotoxocariasis²², *Toxocara* myocarditis¹ and undefined clinical forms (covert toxocariasis) have been reported²⁹.

¹. Núcleo de Doenças Infecciosas, Centro de Ciências da Saúde, Universidade Federal do Espírito Santo, Vitória, ES.

**Address to:** Dr. Fausto E.L. Pereira. Núcleo de Doenças Infecciosas/COE/UFES. Av. Marechal Campos 1468, 29040-001 Vitória, ES.

Fax: 55 27 3335-7294
e-mail felp@ndi.ufes.br

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Human toxocariasis is diagnosed through its clinical manifestations in the classical forms of ocular or visceral larva migrans, and through detection of anti-Toxocara antibodies in serum or, less frequently, through identification of Toxocara larvae in tissues. Classical visceral larva migrans is associated with high eosinophil counts, hepatosplenomegaly, focal pulmonary condensations and high titers of anti-Toxocara antibodies. Computed tomography, magnetic resonance or ultrasonography may demonstrate granulomatous lesions in liver parenchyma. In liver biopsies or in autopsied cases, eosinophil-rich granulomas with Toxocara antigens have been reported, demonstrated by immunohistochemistry.

Ocular larva migrans occurs when Toxocara larvae reach eyeball tissues, where they induce inflammatory reactions, frequently without the signs or symptoms that accompany the visceral larva migrans syndrome. It was first reported 50 years ago, when Wilder reported findings of helminth larvae during histological observations of enucleated eyeballs with tumor-like lesions. Years later, these were confirmed to be Toxocara larvae after morphology studies by Nichols. Since this time, several cases of ocular larva migrans have been reported in different countries, most frequently in children and adolescents, with unilateral lesions. The most frequent lesion types are diffuse endophthalmitis, posterior pole granuloma, peripheral inflammatory mass, diffuse unilateral subacute neuroretinitis and presence of mobile larva in the choriotreinita.

The diagnosis of ocular larva migrans is based on retinoscopic and retinographic studies, and is confirmed by findings of larvae in lesions. Eosinophilia is slight or absent and anti-Toxocara antibodies in serum frequently have low titers. However, searching for anti-Toxocara antibodies or antigens in eye fluids (aqueous or vitreous humors) has been reported to be a sensitive method for detecting ocular infection by Toxocara larvae in experimental and human infections. It is accepted that ocular toxocariasis occurs after migration of small numbers of larvae from the intestine, thereby inducing a weak immune response that favors their ocular localization, with few systemic manifestations.

Experimental studies on ocular lesions induced by Toxocara larvae have been reported using mice, rats, rabbits, guinea pigs, non-human primates and, most recently, in gerbils. Mice are frequently used as experimental animals for Toxocara infection but in these animals, the cornea dries easily and becomes cloudy, making ophthalmoscopic observations difficult.

Gerbils have been described as useful animals for experimentally studying ocular toxocariasis because, apart from their high susceptibility to the infection, they have big eyes with pigmented retina, thus allowing direct examination by means of ophthalmoscopy.

There are few reports on ophthalmic lesions in gerbils infected with Toxocara canis or Toxocara cati, and all of them used heavy infections of more than 1000 embryonated eggs per animal. Since ocular toxocariasis is accepted to be more frequent after acquiring a low larval burden, we decided to study ocular lesions in gerbils infected with a small number of Toxocara eggs, by means of binocular indirect ophthalmoscopy.

MATERIAL AND METHODS

Outbred gerbils (Meriones unguiculatus), five to six months old (15 male and 15 female), were used in the experiments. The animals were purchased from a local breeder and were kept, one or two per cage, at ambient temperature, receiving water and balanced food ad libitum.

The animals were manipulated in accordance with the ethical principles of animal experimentation from the International Union of Animal Protection.

Toxocara eggs were obtained after collecting female adult worms from the intestines of dogs subjected to necropsy at the Center for Zoonosis Control in Vitória, Espírito Santo, Brazil. After washing with tap water, female worms were sectioned at the extremities and pressed with a forceps to release the ovaries, which were chopped with scissors in a Petri dish with saline. The suspension of eggs and ovary fragments was filtered through a nylon mesh and the eggs that were collected were incubated in 2% formaldehyde, at 28°C. After six weeks, the eggs were washed three times in saline and the number of eggs with mobile larvae was counted in a McMaster chamber. The egg suspension was adjusted to a concentration of 200 embryonated eggs with mobile larvae per ml.

The gerbils were infected by means of gavaging using an appropriate needle, and each animal received 100 embryonated eggs in a volume of 0.5ml.

Binocular indirect ophthalmoscopy was performed after administering light anesthesia with ether and dilatation of pupils with one drop of 1% tropicamide in each eye. A binocular ophthalmoscope with an OHD-4.2 camera (EYE-TEC Equipamentos) coupled to a high-resolution digital color video system was used. A 30-diopter Volk lens for small eyes was used and all examinations were performed under low illumination in the room. The images were captured and recorded in a PC, using a Dazzle-Pinnacle capture system. All the animals were examined before the inoculum and on days 3, 10, 17, 24, 31 and 38 after infection.

After the last ophthalmoscopic examination (38 days post-infection) all the animals were killed by means of intraperitoneal injection of a lethal dose of pentobarbital. The animals were subjected to necropsy for confirmation of infection by direct examination of liver fragments pressed between two slides or by examination of hematoxylin and eosin-stained paraffin sections of liver, lungs and encephalon. The eyeballs were enucleated, fixed in 10% formaldehyde, sectioned in the sagittal plane and embedded in paraffin, and the sections were stained with hematoxylin and eosin.

RESULTS

Before infection, the gerbil’s eye grounds presented optical discs with or without pigments, sharp blood vessels and reddish retina, allowing excellent visualization, especially in animals with pigmented hair.
The main abnormalities observed before infection were small, multiple white spots in the eyeground (30% of the animals) and areas of rarefaction of pigment epithelium, which were more visible in animals with yellow or white hair. Vitreous asteroid hyalosis and coloboma of the optic disk and a hole in the retina were observed in one animal.

Six animals died within three weeks because of anesthesia.

Twenty-four animals presented ocular lesions observed by means of ophthalmoscopy (five out of the six that accidentally died and 19 out of the 24 that survived until 38 days after infection). The main ocular lesions observed through ophthalmoscopy are shown in Tables 1 and 2, and in Figure 1. There was no gender difference in the frequencies of retinal lesions (13/15 or 86.7% in males and 11/15 or 73.3% in females; p= 0.651).

Ocular lesions started to appear in the first week and, after four weeks, they were present in 80% of the animals. However, they were not observed in six animals (20%) until 38 days post-infection.

The hemorrhagic lesions frequently showed signs of involution one week after they were observed. For this reason, when the animals were examined 38 days after infection, the majority of the hemorrhagic lesions showed a pattern of involution.

The lesions were bilateral in 40% of the animals and were more frequent in the lower areas of the eyeball (inferior temporal and nasal regions; 46 and 42.5%, respectively) and at the posterior pole (46%), while they were less frequent in the superior temporal and nasal regions (23% of the eyes examined).

All the animals were found to be infected, including the six animals without ocular lesions visible during ophthalmoscopy. Infection was demonstrated by findings of *Toxocara* larvae in the liver and nervous tissue, or granulomatous lesions with fragments of larvae in the liver or lungs.

### Table 1 - Results of indirect binocular ophthalmoscopy on 30 gerbils infected with *Toxocara canis*: observations in each infected animal 3, 10, 17, 24, 31 and 38 days after infection.

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N: number of animals, CH: choroidal hemorrhage, DUSN: diffuse unilateral subacute neuroretinitis, EL: retinal exudative lesions, Hwc: hemorrhage with white center, LM: larva migrans, LT: larval track, RH: retinal hemorrhage, RPEL: retinal pigment epithelium lesions, VH: vitreous hemorrhage, VIL: vascular lesions, VIL: non-hemorrhagic vitreous lesions, RD: retinal detachment, †: accidental death due to anesthesia, ind: lesions were not detected, nd: not determined because animal was dead, °lesion in absorption.
These authors observed that lesions were observed in all segments of the eye, consisting especially of hemorrhages. In 10 animals in which two sections per eye were analyzed, the lesions started soon after infection and were similar to focal episcleritis and iridocyclitis, with neutrophils and eosinophils, were observed in three animals. Focal vasculitis and focal dacryoadenitis (both with eosinophils in the exudates) and eosinophilic granuloma in periocular tissues, were observed in one animal, in each lesion. *Toxocara* larvae in the retina were only observed in one animal, but without inflammatory cells around the larvae.

**DISCUSSION**

Our observations confirm that indirect ophthalmoscopy is easily performed on gerbils, especially among animals with pigmented skin. The ophthalmoscopy patterns confirm the similarity with the human retina that had already been demonstrated by means of optical microscopy.

Spontaneous retinal lesions were observed before infection and probably represented changes due to the aging process. Takayanagy et al. reported only the presence of white spots in three month-old gerbils. The gerbils we used were five to six months old and, for this reason, other spontaneous retinal lesions were observed. In fact, ophthalmoscopy on older gerbils (seven to twelve months old) has demonstrated frequent spontaneous degenerative lesions in the retina, in the form of white spots, retinal epithelial rarefaction and optic disc atrophy (Zanandrea LIDC, Pereira FEL, Oliveira GM, Abreu AS: unpublished data).

All the gerbils infected with 100 embryonated eggs of *Toxocara* presented migrating larvae or eosinophilic granulomas in tissues 38 days after infection, thus confirming the high susceptibility of these animals to *Toxocara* infection. Moreover, 80% of the animals presented retinal lesions, as observed by means of ophthalmoscopy, thus reinforcing other observations that have demonstrated that gerbils are highly susceptible to ocular lesions following *Toxocara* infection.

The frequency of ocular lesions that we have reported here was lower than the 90 to 95% reported by other authors that have reported ocular lesions in gerbils infected with *Toxocara*. These authors continued to perform ophthalmoscopy until 60 to 158 days after infection with large number of infective eggs (1,000 or more embryonated eggs per animal).

The lesions started soon after infection and were similar to what has been reported by other investigators who used gerbils for studies on ocular toxocariasis. These authors observed three to five days after infection and, as we have reported here, hemorrhagic lesions were the most frequent type and presented signs of involution. However, the frequency of migrating larvae in the retina was lower in our observations: five (1.6%) cases presented larvae or tracks of larva: four observed using ophthalmoscopy and one case using microscopy. Takayanagy et al. reported findings of larvae through ophthalmoscopy in 80% of infected gerbils. This high frequency of larvae in the retina would be in relative to the number of inoculated eggs, as demonstrated in mice in which the number of eye invasions due to larvae was in direct relationship with the quantity of inoculum.
As reported by other authors, we did not observe pseudotumoral appearance at the posterior pole or in peripheral granuloma. Vascular abnormalities with a vasculitis pattern were observed in three animals and diffuse unilateral subacute neuroretinitis (DUSN) in one animal. Vasculitis was reported by Alba-Hurtado et al, who accepted immune-mediated mechanisms for the pathogenesis of these lesions. DUSN has not been reported by other authors who studied ocular lesions in gerbils infected with *Toxocara*.

There was no difference in the frequency of lesions between males and females, thus demonstrating that gender did not influence the ocular lesions induced by *Toxocara* in gerbils, as seen in cases of ocular larva migrans among children.

In conclusion, our results demonstrated that ocular lesions are frequent in gerbils infected with *Toxocara canis*, even after infections that result in low larval burden. However, the lesions observed were mainly focal and sparse, and larvae or larval tracks were only observed by means of ophthalmoscopy in four (13.3%) animals. Although ocular lesions were frequent in the eyeballs of the infected gerbils, most of the lesions observed were not similar to what is observed in human cases of ocular larva migrans. Only one animal presented a condition of DUSN, which has been described in cases of ocular larva migrans.

REFERENCES


