Supplementary File

Atypical Clinical Manifestations of Loiasis and their Relevance for Endemic Populations.

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Supplementary Methods

1. Literature Review (Supplementary References)

Below is the full list of papers included in the study following the systematic literature review.

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2. Atypical Ocular Manifestations

From the 32 patients with atypical ocular symptoms, there was no report of co-infections with other agents causing ocular manifestations such as *Onchocerca volvulus* and/or *Chlamydia trachomatis*, nor were onchocerciasis or trachoma mentioned as possible causes of the manifestations observed. Supplementary Table 1 summarizes the information on ocular manifestations; the references are those in Section 1: Literature Review (Supplementary References).

3. Eosinophilia and Co-Infections

There were 199 cases with eosinophilia (defined as a peripheral eosinophil blood count >0.5 x 10^9 cells or a peripheral blood eosinophil count ≥6% [1]. Of these, 182 (91.5%) did not have co-infecting helminths reported, but malaria was recorded at the time of consultation in 5, amoebiasis in 4 (one of whom also had *Trichuris trichiura*, one had hookworm, and one had *Strongyloides stercoralis*), trypanosomiasis in 1 (who also had schistosomiasis) and tuberculosis in 1. Most infectious causes of peripheral blood eosinophilia are parasitic, and particularly helminth infection; protozoa, in general, do not cause eosinophilia [2]. There were 17 (8.5%) cases with co-infecting helminths, 8 with *Mansonella perstans*, 1 with *O. volvulus*, 2 with *Ascaris lumbricoides*, 1 with *Trichuris trichiura*, 1 with hookworm, 1 with hookworm and *Ascaris*, 1 with *Strongyloides stercoralis*, and 2 with *Schistosoma* spp. (The case with *Loa loa* and *O. volvulus* did not have atypical ocular manifestations.)

Supplementary Table 1: Characteristics of Ocular Manifestations in the 32 Patients Identified with Loiasis Atypical Ocular Symptoms

Suppl. Ref.	Sex/Age (years or months)	Ocular Symptoms	Clinical Examination	Diagnosis of *Loa loa* and microfilarial density in blood (mf/ml) when reported	Ocular Symptoms After Treatment or Surgery
S26	M/26 yr	Unilateral loss of vision	Retinal edema, hemorrhages and retinal artery occlusion	*L. loa* microfilariae in blood (unspecified mf/ml)	Not reported
S42	F/60 yr	Unilateral loss of vision, ocular pain	*Loa loa* adult worm visualized in the anterior chamber	*L. loa* adult worm extraction from the anterior chamber	Not reported
S73	F/40 yr	Bilateral reduction in vision	Retinal hemorrhages and exudates, hemorrhages in the vitreous humour	*L. loa* microfilariae in blood (20,000 mf/ml)	Treated with DEC (diethylcarbamazine citrate) and had improvement in ocular symptoms
S78	F/25 yr	Unilateral loss of vision, ocular pain, erythema, photophobia, lacrimation, pruritus, foreign body sensation	*L. loa* adult worm visualized in the anterior chamber, conjunctival hyperemia	*L. loa* adult worm extraction from the anterior chamber	Treatment with albendazole and had progression to full loss of vision in the left eye
S93	M/48 yr	Unilateral blurring of vision, ocular pain, erythema, foreign body sensation, lacrimation, pruritus	*L. loa* adult worm visualized in the anterior chamber, conjunctival hyperemia	*L. loa* adult worm extraction from the anterior chamber	Not reported

S95	F/32 yr	Foreign body sensation, threadlike swelling of the left upper eyelid	Threadlike swelling in the left upper eyelid	*L. loa* adult worm extraction	Not reported
S108	Not reported/7 mo.	Not available due to patient age	Corneal edema, eye was firm to palpation	*L. loa* adult worm extraction from the anterior chamber	48 hr after extraction, the eye was no longer firm to palpation
S115	F/14 mo.	Photophobia, lacrimation	Corneal inflammation and swelling	*L. loa* adult worm extraction	Not reported
S123	M/25 yr	Unilateral reduction in vision with floaters, ocular pain, erythema	Grade one vitreous haze and corneal edema	*L. loa* adult worm extraction from the vitreous humour	Treated with DEC and had improvement in vision, the vitreous had cleared, minimal corneal edema
S130	M/11 yr	Erythema, photophobia, unilateral lacrimation	*L. loa* adult worm visualized in the anterior chamber	*L. loa* adult worm extraction from the anterior chamber	Not reported
S145	F/15 yr	Unilateral reduction in vision, ocular pain, foreign body sensation	*L. loa* adult worm visualized in the anterior chamber, conjunctival injection, hazy cornea	*L. loa* adult worm extraction from the anterior chamber	Eye was surgically removed and pathological examination showed a thick post inflammatory membrane adherent to the iris, hazy anterior chamber, choroidal and retinal detachment

S145	F/25 yr	Unilateral ocular pain, pruritus, foreign body sensation	*L. loa* adult worm visualized in the anterior chamber, discrete opacities in the cornea	*L. loa* adult worm extraction from the anterior chamber	Eye was surgically removed and pathological examination showed a fibrous membrane lining the anterior iris and lens, edematous ciliary body and iris, subcapsular fibrosis
S169	F/15 yr	Unilateral reduction in vision in the left eye	*L. loa* adult worm extending from anterior vitreous chamber to posterior chamber, total retinal detachment leading to rapid opacification of lens	*L. loa* adult worm extraction from the vitreous humour; *L. loa* microfilariae in blood (2,000 mf/ml)	Not reported
S169	F/19 yr	Unilateral reduction in vision	Live microfilariae in the vitreous humour with opacification of the vitreous humour	*L. loa* microfilariae in blood (unspecified mf/ml) and microfilariae from the vitreous cavity	Treatment with DEC decreased microfilarial density
S169	F/21 yr	Unilateral reduction in vision	Microfilariae in the vitreous cavity, panuveitis	*L. loa* microfilariae in blood (300,000 mf/ml) and microfilariae from the vitreous cavity	Not reported
S169	M/22 yr	Unilateral reduction in vision	Macular choroido-retinitis	*L. loa* microfilariae in blood (3,000 mf/ml)	Treatment with DEC and had regression of the retinal lesions with improvement in visual acuity

S169	M/25 yr	Bilateral reduction in vision	Retinal exudates and hemorrhages	*L. loa* microfilariae in blood (20,000 mf/ml)	Treatment with DEC and had a large hemorrhage in the vitreous cavity
S169	M/28 yr	Bilateral reduction in vision	Retinal exudates and hemorrhages, vein thrombosis	*L. loa* microfilariae in blood (50,000 mf/ml)	Treated with DEC and had progressive regression of the retinal lesions without improvement in vision
S169	F/30 yr	Bilateral reduction in vision	Inflammation of the vitreous humour, floating retinal detachments	*L. loa* microfilariae in blood (66,000 mf/ml)	Treatment with DEC and had development of vitreous fibrosis
S169	M/35 yr	Bilateral reduction in vision	Dead microfilariae in the vitreous, grey vitreous humour, opacified lens	*L. loa* microfilariae in blood (unspecified mf/ml) and microfilariae from the vitreous cavity	Treatment with DEC, development of a fibrosis of the vitreous, vision never recovered
S169	F/36 yr	Unilateral reduction in vision	Macular choroido-retinitis	*L. loa* microfilariae in blood (6,000 mf/ml)	Treatment with DEC and had regression of choroido-retinitis
S169	F/40 yr	Bilateral reduction in vision	Irido-cyclitis	*L. loa* microfilariae in blood (15,000 mf/ml)	Treatment with DEC and had resolution of the irido-cyclitis with complete recovery of vision
S169	M/46 yr	Unilateral reduction in vision	Dead microfilariae in the vitreous humour of the right eye, the right vitreous humour appeared grey, left lens opacification	*L. loa* microfilariae in blood (5,000 mf/ml) and microfilariae from the vitreous cavity	Treated with DEC but vision never recovered

S169	M/67 yr	Bilateral reduction in vision	Retinal exudates and hemorrhages, light opacification of lens	*L. loa* microfilariae in blood (10,000 mf/ml)	Treatment with DEC and had resolution of the retinal lesions with improvement in visual acuity
S182	M/12 yr	Bilateral loss of vision	Bilateral retinal haemorrhages with numerous exudates in peripheries, narrowing of arteries, neovascularisation	*L. loa* microfilariae in blood (4,500 mf/ml)	Treated with DEC and had resolution of haemorrhages and neovascularisation, regression of size of exudates with improvement in visual acuity
S187	F/24 yr	Unilateral reduction in vision	Macular degeneration, retinal detachment, peripheral hemorraghes, neovascularisation, scarring. Worm visualized in the sub-conjunctiva	*L. loa* adult worm extraction; *L. loa* microfilariae in blood (unspecified mf/ml)	Underwent surgery for retinal detachment without improvement in vision
S190	F/23 yr	Unilateral (left eye) reduction in vision, decreased visual acuity, ocular pain, pruritus, erythema	Panuveitis, elevated unilateral ocular pressure, hazy cornea. *L. loa* adult worm visualized in the anterior chamber	*L. loa* adult worm extraction from the anterior chamber	Treatment with DEC, topical and systemic steroids, without improvement in vision
S211	M/24 yr	Unilateral loss of vision	Retinal haemorrhages, retinal edema, pseudocystic opacification adjacent to macula	*L. loa* microfilariae in blood (unspecified mf/ml)	Treatment with DEC and had improvement in vision

S236	M/not reported	Blurring of vision and decreased visual acuity	Edema near the optic nerve	*L. loa* adult worm extraction from the conjunctiva	Treatment with DEC and had development of retinal edema that responded to corticosteroids
S250	M/30 yr	Ocular pain, foreign body sensation	Adult *L. loa* worm in the conjunctiva (failed extraction) with a lesion at level of macula to suggest passage of the worm	*L. loa* microfilariae in blood (unspecified mf/ml)	Not reported
S259	M/23 yr	Reduction in vision, pruritus	Anterior uveitis, microfilariae in the anterior chamber	*L. loa* microfilariae in blood (120,000 mf/ml) and microfilariae in the anterior chamber	Not reported
S266	M/38 yr	Bilateral reduction in vision, photophobia	Adult *L. loa* worm in the left upper eyelid, retinal hemorrhages and exudates, obstructed retinal vessels	Microfilariae in the Cerebrospinal fluid (CSF) and positive skin snip* (not due to onchocerciasis)	Not reported

* *Loa loa* microfilariae can be found in bloodless skin snips [3].

4. Statistical Analyses

4.1 Chi-Squared Test

Pearson's chi-squared test was used to test whether there was a statistically significant association between blood microfilarial density (MFD) levels and atypical presentation; where MFD levels had been categorised as: Zero (amicrofilaraemic, no mf detectable in blood smear), Low (greater than 0 but < 8,000 mf/ml) and High (≥ 8,000 mf/ml). All individuals for whom information on manifestation (either typical or atypical) and MFD were included. There are, therefore, more individuals included in this test than in the subsequent regression-based analyses, where data on a wider range of factors, specifically an individual's age, sex, residency status and MFD level (and eosinophilia status for Model 2) were required for them to be included.

Supplementary Table 2: Distribution of Individual Patients According to Presentation of Manifestations and Blood Microfilarial Density (MFD) Levels

	MFD Levels			
Manifestations	**Zero**	**Low**	**High**	**Total**
Typical	62	30	3	95
Atypical	40	21	16	77
Total	102	51	19	172

Pearson's chi-squared test using these data gave a chi-squared value of 13.492, which, with 2 degrees of freedom, yielded a p-value of 0.001176.

4.2 Multivariate Logistic Regression

Model 1: Atypical Presentation ~ Age + Sex + Residency Status + MFD Level (n = 154 individuals)

Model 2: Atypical Presentation ~ Age + Sex + Residency Status + MFD Level + Eosinophilia Status (n = 114 individuals)

We defined two multivariate logistic regression models (Model 1 and Model 2, the results of which are detailed in Figure 3 of the main text) that were fitted to the IPD collated through the systematic literature review. For both models, age was included as a continuous variable. Sex was defined as a categorical variable, with a reference of 0 for women and 1 for men. Residency status was included as a categorical variable; in almost all instances, residency status could easily be determined from the article text, but in a small number of cases (n = 3 individuals), it was impossible to ascertain whether an individual was living abroad or in an endemic country at the time of the consultation detailed in the reference, and thus that individual was excluded from the analyses presented here. MFD levels were included as a categorical variable, with an individual's MFD being either "Zero", "Low" or "High" (as defined in the section above). Eosinophilia status was also included as a

categorical variable, with a reference value of 0 if the individual was not eosinophilic, and a value of 1 if the patient presented with eosinophilia (with being eosinophilic defined as having a peripheral eosinophil blood count > 0.5 x 10^9 cells or a peripheral blood eosinophil count ≥ 6% as described in the main text). The number of individuals in each category for the categorical variables is detailed in Supplementary Table 3.

Supplementary Table 3: The Distribution of Individuals Across Each of the Categories Used in the Multivariate Logistic Regression Models 1 and 2

	Model 1 MFD Levels Only (n = 154 Individuals)	**Model 2** MFD Levels and Eosinophilia (n = 114 Individuals)
Factor	**Number of Individuals**	**Number of Individuals**
Males (median age)	76 (36)	53 (35)
Females (median age)	78 (29)	61 (28)
Locals living in non-endemic country	43	31
Expatriates	73	57
Locals living in endemic country	38	26
Zero mf	90	70
Low mf	48	32
High mf	16	12
Eosinophilia	Not included	98

In addition to Models 1 and 2, we also explored a second set of models, defined here as Models 3 and 4. Although Models 3 and 4 share the same structure as Models 1 and 2, they differ in their categorisation of MFD levels. Instead of three categories (Zero, Low and High), individuals were assigned to one of four categories based on the MFD recorded. These categories were: Zero (amicrofilaraemic, no mf detectable in blood smear), Low (> 0 but < 8,000 mf/ml), High (≥ 8,000 but < 30,000 mf/ml) or Very High (≥ 30,000 mf/ml). The categorisations were chosen based upon their relationship with the risk of marked or severe adverse events (SAEs) following ivermectin treatment, with ≥ 8,000 mf/ml and ≥ 30,000 mf/ml associated, respectively, with an increased risk of marked adverse events (reversible functional impairment for several days), and permanent neurological sequelae including fatal encephalopathy [4].

Analysis of these models revealed similar results to those of the models presented in the main text, although the additional granularity granted through subdivision of the MFD categories allowed resolution of a potential dose-response relationship between MFD levels and the odds of presenting atypically. Specifically, for Model 3, individuals with high MFD levels had a significantly increased risk of atypical manifestations (OR 8.27, 95% CI 1.27–53.71, p = 0.03), and individuals with very high MFD levels had an even greater risk (OR 10.84, 95% CI 1.04–113.07, p = 0.05) (Supplementary Table 4, Model 3). Neither zero nor low MFD levels were associated with an increased risk of atypical manifestations.

Similar results were observed for Model 4 (including eosinophilia status), although the significance associated with high MFD level was lost (Supplementary Table 4, Model 4), a feature likely attributable to the substantial drop in sample size that accompanied further conditioning an individual's inclusion if their eosinophilia status was reported. However, individuals with very high MFD level were still at a (marginally) significantly higher risk of atypical manifestations (OR 12.72, 95% CI 0.97-167.25, p = 0.05). The stratification of MFD into a larger number of levels led to significant reductions in the average size of each group, particularly for the high and very high categories (Supplementary Table 4), leading to substantial uncertainty in these estimates. Nevertheless, these results support the possibility of a dose-response relationship between atypical presentation and the intensity of microfilarial infection an individual harbours. The number of individuals in each category for the categorical variables considered in the analyses is provided in Supplementary Table 4 and the results of the models in Supplementary Table 5.

Supplementary Table 4: Number of individuals in each category for Models 3 and 4.

	Model 3 MFD Levels Only (n = 154 Individuals)	**Model 4** MFD Levels and Eosinophilia (n = 114 Individuals)
Factor	**Number of Individuals**	**Number of Individuals**
Males	76 (36)	53 (35)
Females	78 (29)	61 (28)
Locals living in non-endemic country	43	31
Expatriates	73	57
Locals living in endemic country	38	26
Zero mf	90	70
Low mf	48	32
High mf	9	6
Very High mf	7	6
Eosinophilia	Not included	98

Supplementary Table 5: Results from Multivariate Logistic Regression Models 3 and 4. Individuals are categorised into one of four categories based on their MFD levels; either Zero (amicrofilaraemic, no mf detected in blood smear), Low (> 0 but < 8,000 mf/ml blood), High (≥ 8,000 but < 30,000 mf/ml blood) or Very High (≥ 30,000 mf/ml blood).

	Model 1 MFD Levels Only (n = 154 Individuals)		**Model 2** MFD Levels and Eosinophilia (n = 114 Individuals)	
Factor	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Age	1.02 (0.99–1.05)	0.16	1.03 (0.99–1.07)	0.10
Sex	1.94 (0.89–4.20)	0.10	2.97 (1.09–8.07)	0.03*
Demographic				
Local living in non-endemic country	Ref	NA	Ref	NA
Expatriate	1.93 (0.73–5.09)	0.18	2.50 (0.74–8.39)	0.14
Local living in endemic country	9.17 (3.07-27.37)	<0.001*	22.62 (4.91-104.12)	<0.001*
MF Densities				
Zero mf	Ref	NA	Ref	NA
Low mf	1.46 (0.63–3.34)	0.38	1.62 (0.56–4.69)	0.37
High mf	8.27 (1.27–53.71)	0.03*	13.21 (0.81–215.05)	0.07
Very high mf	10.84 (1.04-113.07)	0.05*	12.72 (0.97-167.25)	0.05*
Eosinophilia	Not included	NA	0.47 (0.12–1.86)	0.28

4.3 Confidence Interval Calculation: Non-Parametric Bootstrap

In order to calculate the 95% confidence intervals (CIs) for the mean MFD of each residency status group shown in Figure 4 of the main text), an ordinary non-parametric re-sampling technique [5] was used. This process involved the following steps:

1. For each residency status group of size *n*, resampling (at random) with replacement of *n* patient MFD was conducted.
2. The mean of this newly generated sample was calculated.
3. Steps 1 and 2 were repeated 10,000 times to yield an empirical sampling distribution of the mean MFD for each different residency status group.

4. The lower and upper 95% CIs were calculated as the 2.5% and 97.5% percentiles of this sampling distribution.

Supplementary References:

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