A rare case of loiasis clinically manifested 9 years after the last epidemiological exposure

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Summary

A 37-year-old Cameroonian patient, residing in Italy for the past nine years without returning to his home country, showed up at the Emergency Department of Cittadella Hospital with acute hyperemia of the conjunctival tissue, tearing, itching, headache in the right hemisphere and transient edema at ankles and wrists. A foreign body suspected to be a parasite was detected while migrating through his right eye. However, primary identification of the worm was hindered due to partial damage during surgical removal. The laboratory team based on the patient's history and clinical manifestation, suspected blood infection due to microfilariae species and collected a blood sample at 12 pm. *Microfilariae* of *Loa loa* were identified in May Grunwald-Giemsa (MGG) staining (count of 270 *microfilariae*/mL) from a K2-EDTA blood sample. Identification of the *microfilariae* was based on morphological features, patient country of origin, and periodicity of the life cycle of the parasite.

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Informed consent: the patient gave his written consent to use his personal data for the publication of this case report and any accompanying images.

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Introduction

Loiasis is an infection caused by a filarial nematode endemic to West-Central Africa, primarily transmitted to humans through the bite of adult deer flies belonging to the *Chrysops genus*, notably *C. silacea* and *C. dimidiata* [8]. Humans serve as the only known natural reservoir for this parasite. Female worms typically measure 40 to 70 mm in length, while males range from 30 to 34 mm. *Larvae* enter the body through the bite wound and develop into adult worms, migrating through lymphatic vessels to subcutaneous tissues, including the conjunctiva and lymphatic ganglia. Within several months (typically 6 to 12), female worms begin to produce *microfilariae*, which enter the bloodstream from the lymphatic circulation [1,3]. *Microfilariae* measure 230 to 300 µm.

Pathognomonic clinical manifestations of loiasis include transient subcutaneous angioedema known as "Calabar swellings", which may occur in various body areas but are commonly found on the extremities such as forearms, hands, and ankles. Other symptoms include itching, eyelid edema, and irritation of the conjunctiva due to the migration of adult worms within the eyelid [3,4]. While many patients with loiasis remain asymptomatic, eosinophilia is a frequent but non-specific finding [7].

Case Report

A 37-year-old Cameroonian male living in Italy for 9 years without visiting his home country presented to the Emergency Room. Clinical examination revealed no remarkable findings until last year, when the patient started to show acute hyperemia of the conjunctival tissue, tearing, itching, headache in the right hemisphere, and transient edema at ankles and wrists.

Microscopic examination

The Laboratory Team recommended parasitological research in peripheral blood collected in K2-EDTA, with specimen collection scheduled for 12 pm. This timing coincides with the peak *microfilaria* levels, as *Loa loa* exhibits a peculiar diurnal periodicity typically confirmed between 10 am and 2 pm. A complete blood count along with the microscopic examination of both a fresh blood smear and a thin smear stained with May Grunwald-Giemsa (MGG) were performed. *Microfilariae* loads were assessed by calculating the number of *microfilariae*/ml using a measured quantity of blood (3,7µl).

Results

Upon referral to the Ophthalmology Department, the clinical examination of the right eye revealed the presence, under the conjunctival tissue, of a cylindrical foreign body suspected to be a parasite. The worm was surgically removed and sent to our Diagnostic Laboratory for macroscopic identification.

Macroscopic observation in the laboratory revealed a cylindrical segment measuring 2 centimeters in length. However, microscopic examination at 10X magnification showed only a segment of the worm, lacking a tail and buccal rim, which posed challenges for a definitive diagnosis.

Considering the literature suggesting *Loa loa* as the most common parasite migrating in subconjunctival tissue, the laboratory team recommended parasitological research in peripheral blood. The complete blood count revealed no evidence of peripheral eosinophilia or qualitative and quantitative abnormalities (total eosinophil count: 490/µL; relative eosinophil percentage: 7%; total white blood count: 7.1 x 10^9/L). Nevertheless, it is important to note that after years of infection, eosinophilia could be absent, and further assessments cannot be done due to the lack of a complete blood count from previous years.

The microscopic examination of blood smears revealed a worm exhibiting morphological characteristics consistent with microfilariae of *Loa loa*. These characteristics included a size of 250 µm (Figure 1), large, irregular, and overlapping somatic *nuclei*, a tapered tip of the tail, and a short headspace (Figure 2) [2]. Initially, a differential diagnosis was considered with *Brugia species*; however, the presence of more than two *nuclei* at the tip of the tail led to suspicion of *Loa loa* or *Wuchereria bancrofti* (Figure 3). Subsequently, loiasis was confirmed based on the lack of sheath coloration with MGG staining (Figure 4), terminal *nuclei* extending up to the caudal-end, and the patient's history of residing in an endemic area.

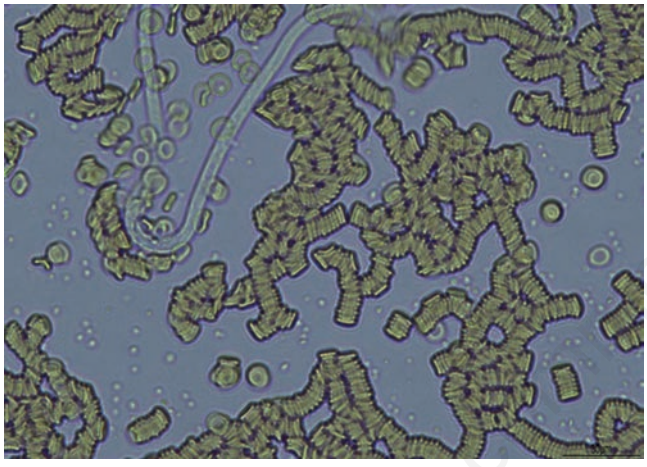

Figure 1. A worm exhibiting morphological characteristics consistent with microfilariae of Loa loa - a size of 250 µm.

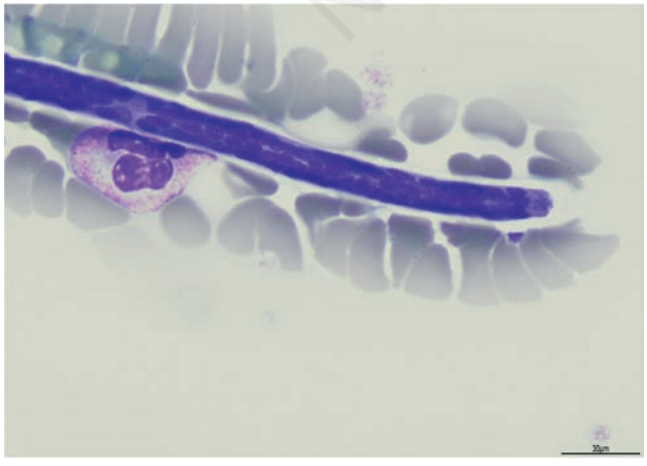

Figure 2. A worm exhibiting morphological characteristics consistent with microfilariae of Loa loa - large, irregular, and overlapping somatic nuclei, a tapered tip of the tail, and a short headspace.

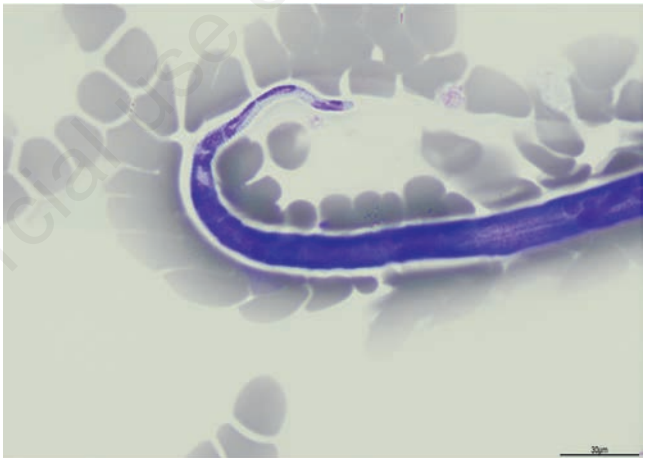

Figure 3. Initially, a differential diagnosis was considered with Brugia species; however, the presence of more than two nuclei at the tip of the tail led to suspicion of Loa loa or Wuchereria bancrofti.

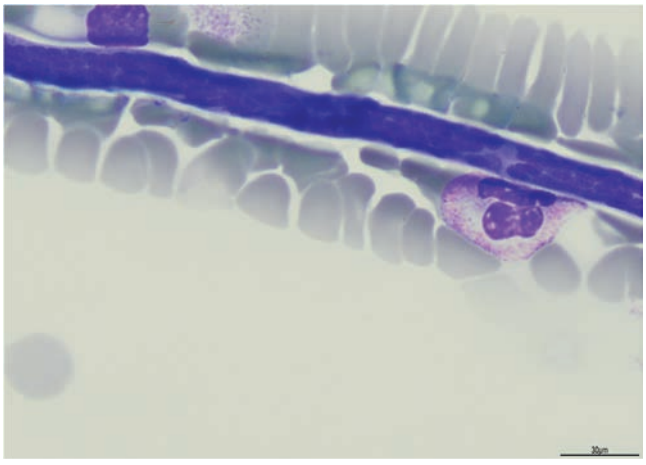

Figure 4. Loiasis was confirmed based on the lack of sheath coloration with May Grunwald-Giemsa (MGG) staining.

Microscopic observation at 10X magnification revealed four *microfilariae* in four blood smears, approximately equating to 270 *microfilariae*/mL. However, the precise parasite load will be definitively determined through thick smears and Quantitative Polymerase Chain Reaction (q-PCR) analysis or also using a rapid flow cytometry method at a specialised centre [5,6].

Further clinical, diagnostic, and serological investigations were recommended to differentiate it from *Onchocerca* worm, which also migrates into subconjunctival tissue. Confirmation via quantitative PCR was also advised for definitive diagnosis.

Discussion

Macroscopic and microscopic findings observed were consistent with loiasis, an infection caused by helminths belonging to the *Nematoda phylum*. According to the literature, the spread of the disease outside the African continent is considered highly improbable due to the absence of the vector *Chrysops* [8]. Therefore, the finding in our patient was unexpected, as he had not returned to Cameroon for nine years and remained asymptomatic throughout this period until 1 year ago. This case report appears to record the longest case of loiasis.

To improve the sensitivity of the diagnosis, it could have been useful to apply a concentration method such as centrifugation of the blood sample lysed in 2% formalin (Knott's technique) or filtration through a Nucleopore® membrane, which were not performed in this clinical case [7]. Loop-Mediated Isothermal Amplification (LAMP) is another alternative to microscopy-based techniques, since it allows a reliable and species-specific determination, helping the non-specialised laboratories where the conventional approaches appear to be challenging [5,6].

Concerning the quantification methods, qPCR for loiasis is a rapid, sensitive, and high-throughput method that can be performed from a small volume of blood both on genomic DNA or RNA with detective limits of 0,1 pg genomic DNA for the *LLMF72* target [5]. Instead, flow cytometry is an even faster technique based on multiple parameters, such as higher Forward Scatter (FSC) and Side Scatter (SSC) of *microfilariae* with respect to leucocytes and double positivity to anti-human immunoglobulin G anti-*Loa loa* and SYBR green for DNA labeling. It has as well a great sensitivity of detection of 10^{-5} [6].

Anyway, as our centre is a first-level analysis laboratory not specialised in parasitic diseases, the patient was referred to the Centre for Infectious and Tropical Diseases of University Hospital of Padua (AOPD) for appropriate diagnostic tests and treatment with the drug of choice, Diethylcarbamazine (DEC). Patients infected with *Loa loa* are recommended to undergo pre-therapy apheresis, depending on the evaluation of the number of *microfilariae*/mL. Patients with a load ≥8000 *microfilariae*/mL in peripheral blood are at risk of fatal encephalopathy caused by microfilariae antigen release when treated with DEC [1].

Anyway, this clinical case has highlighted how collaboration between clinicians, emergency units, and laboratories is essential to ensure the diagnosis and treatment of infectious diseases. In fact, a common analysis such as a blood smear performed by a first-level laboratory guided by a clinical suspicion can have a central role in making a diagnosis even in the presence of a normal blood count.

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